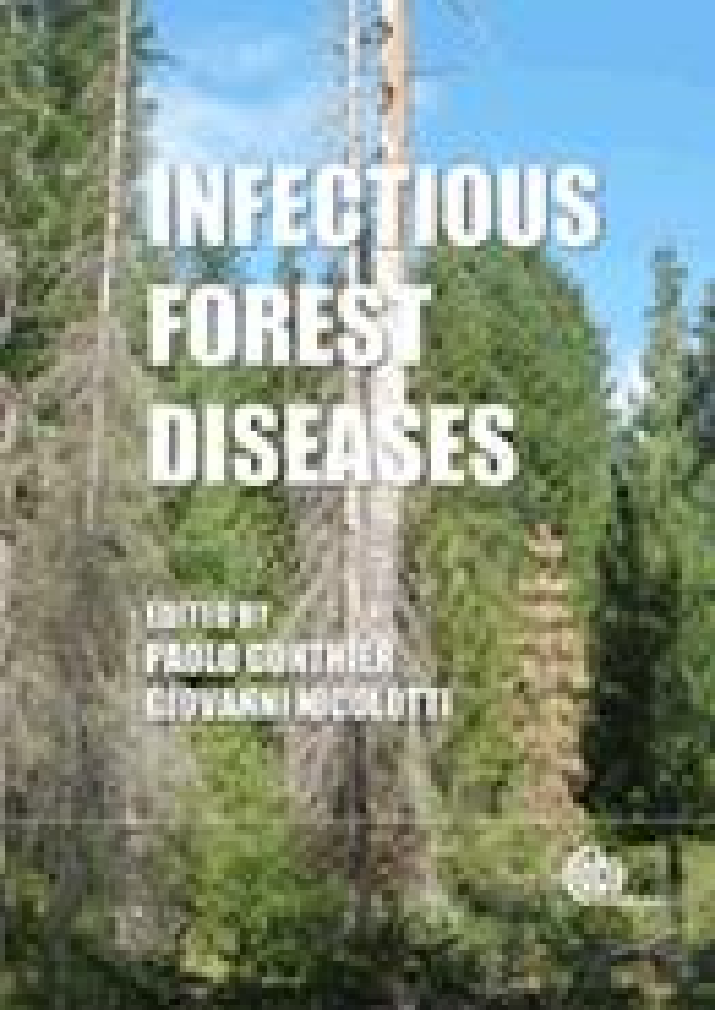
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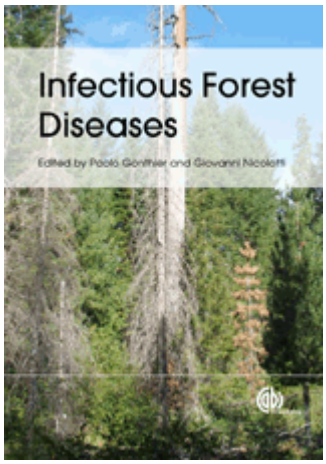
Infectious Forest Diseases
Edited by Paolo Gonthier and Giovanni Nicolotti

3 Forest Diseases Caused by Viruses

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3.1 Pathogens, Significance and Distribution

A lack of knowledge of the presence and frequency of occurrence of viral diseases in forest trees leads to the impression that they are rare and therefore not important. The opposite is true. Viruses are responsible for far greater economic losses than generally recognized. This is because the symptoms are very different from those attributed to bacteria and fungi, and the losses are often more insidious and less conspicuous, so go unnoticed and untreated.

Plant viruses play a central role in the plant health status of forest trees because the high degree of disease that they cause leads to extensive tissue damage (Nienhaus, 1985). Viral pathogens are present in plants in every ecosystem and induce substantial losses worldwide in agriculture, horticulture and forestry (King *et al*., 2011). There is an urgent need for reliable methods for virus detection and identification in forest trees – as well as a need for tools for disease management. Knowledge on virus characteristics followed by knowledge of their epidemiology are the first steps in developing appropriate phytosanitary strategies to produce virus-free plants and to keep tree seedlings free of plant viruses (Jones, 2004). The mode of transmission has to be considered as an important factor affecting the spread and impact of a virus infection within a forest.

Viruses have been recovered and identified from many deciduous fruit trees and a scattered number of broadleaved and coniferous forest trees. Conversely, other viruses, such as maple mosaic virus and oak ringspot virus are still not classified. In this chapter we focus on important plant genera cultivated in forests, park areas and on roadsides (Table 3.1). To date, viruses from 17 different genera have been identified. The latest, *European mountain ash ringspot-associated virus* (EMARaV) has recently been classified by the International Committee on Taxonomy of Viruses (ICTV) (Mühlbach and Mielke-Ehret, 2011). Interestingly, some plant genera, such as *Carpinus*, are susceptible to only one or two virus species while others, such as *Betula* and *Fraxinus*, are hosts to multiple virus species. Because of the economic and ecological importance of forest trees for industry, for fuel and in the future (to offset the impact of climate change), there is a need to increase research on plant viruses.

Obviously, viruses had infected trees and caused diseases for centuries before they were

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Table 3.1. Economically important viruses detected in forest and roadside trees.

Host genus	Virus species	Particle morphology[a]	Genus[b]	References
Abies	Not identified	Isometric		Flachmann *et al.*, 1990
Acer	*Arabis mosaic virus* (ArMV)		*Nepovirus*	Thomas, 1970
	Maple mosaic virus	Not visualized		Szirmai, 1972
	Maple leaf perforation virus	Not visualized		Šubíková, 1973
	Not identified	Rod-shaped		Lana *et al.*, 1980; Führling and Büttner, 1998
	ArMV		*Nepovirus*	Erdiller, 1986
	Cucumber mosaic virus (CMV)		*Cucumovirus*	
	Sowbane mosaic virus (SoMV)		*Sobemovirus*	
Aesculus	*Apple chlorotic leaf spot virus* (ACLSV)		*Trichovirus*	Hentsch *et al.*, 1997
	Strawberry latent ringspot virus (SLRSV)		*Nepovirus*	
	Apple mosaic virus (ApMV)		*Ilarvirus*	Sweet and Barbara, 1979; Polák and Zieglerová, 1997
	Cherry leaf roll virus (CLRV)		*Nepovirus*	Sweet and Campbell, 1976
Betula	ArMV, CLRV, *Tomato ringspot virus* (ToRSV)		*Nepovirus*	Cooper and Massalski, 1984
	Tobacco necrosis virus (TNV)		*Necrovirus*	
	Prunus necrotic ringspot virus (PNRSV)		*Ilarvirus*	
	Prune dwarf virus (PDV)		*Ilarvirus*	
	CLRV		*Nepovirus*	Cooper and Atkinson, 1975; Jones *et al.*, 1990; Jalkanen *et al.*, 2007; von Bargen *et al.*, 2009; Büttner *et al.*, 2011
	ApMV		*Ilarvirus*	Gotlieb and Berbee, 1973; Hardcastle and Gotlieb, 1980; Polák and Procházková, 1996; Polák and Zieglerová, 1997
Carpinus	ApMV		*Ilarvirus*	Polák and Zieglerová, 1997
Fagus	CLRV		*Nepovirus*	Nienhaus *et al.*, 1985; Winter and Nienhaus, 1989
	Brome mosaic virus (BMV)		*Bromovirus*	
	Bean yellow mosaic virus (BYMV)		*Potyvirus*	
	Not identified	Filamentous	–	
	CLRV		*Nepovirus*	Jones *et al.*, 1990
	Tomato black ring virus (ToBRV)		*Nepovirus*	Schmelzer *et al.*, 1966
	TNV		*Necrovirus*	Büttner and Nienhaus, 1989a
Fraxinus	ArMV		*Nepovirus*	Cooper, 1975; Cooper and Sweet, 1976; Cooper *et al.*, 1983

Continued

Table 3.1. Continued.

Host genus	Virus species	Particle morphology[a]	Genus[b]	References
	CLRV		*Nepovirus*	Hamacher and Quadt, 1991; Giersiepen, 1993
	TNV		*Necrovirus*	Ciferri *et al.*, 1961; Casalicchio, 1965
	Tobacco mosaic virus (TMV)		*Tobamovirus*	Lana and Agrios, 1974a,b
	TMV		*Tobamovirus*	Ferris *et al.*, 1989
	Tobacco rattle virus (TRV)		*Tobravirus*	
	ToRSV		*Nepovirus*	
	TMV		*Tobamovirus*	Castello *et al.*, 1984; Shiel and Castello, 1985
	Tobacco ringspot virus (TRSV)		*Nepovirus*	
	Not identified	Isometric		Bertioli *et al.*, 1993
	TRSV		*Nepovirus*	Hibben and Walker, 1971; Hibben and Bozarth, 1972
Picea	Not identified	Rod-shaped		Cech *et al.*, 1961
	Tomato mosaic virus (ToMV)		*Tobamovirus*	Jacobi and Castello, 1992; Castello *et al.*, 1995; Bachand and Castello, 1998, 2001
	Not identified	Filamentous		Castello *et al.*, 2000
	Not identified	Rod-shaped		Biddle and Tinsley, 1968
	Not identified	Rod-shaped		Soikkeli, 1983
Pinus	Not identified		Unassigned genus within *Partitiviridae*	Veliceasa *et al.*, 2006
	Not identified	Rod-shaped		Soikkeli, 1983
	Scots pine mosaic virus	Not visualized		Schmelzer *et al.*, 1966
	Scots pine bushy stunt virus	Not visualized		Jančářík and Blattný, 1966
	Not identified	Rod shaped		Biddle and Tinsley, 1968
	TNV		*Necrovirus*	Büttner and Nienhaus, 1989a
Populus	*Poplar mosaic virus* (PopMV)		*Carlavirus*	Schmelzer, 1966; Navrátil and Boyer, 1968; Cooper and Edwards, 1981; Cooper *et al.*, 1986; Smith and Campbell, 2004; Smith *et al.*, 2004
	ArMV, ToBRV		*Nepovirus*	Cooper and Sweet, 1976
	TRV		*Tobravirus*	
	TNV		*Necrovirus*	Hibben *et al.*, 1979
Prunus	*Plum pox virus* (PPV)		*Potyvirus*	Schimanski and Funk, 1968;
	Prune dwarf virus (PDV)		*Ilarvirus*	Schimanski *et al.*, 1975; Németh *et al.*, 2010
	CLRV		*Nepovirus*	
	Apple stem pitting virus (ASPV)		*Foveavirus*	
	CLRV		*Nepovirus*	Löw, 1995

	Tomato bushy stunt virus (TBSV)		*Tombusvirus*	Allen and Davidson, 1967
	ACLSV		*Trichovirus*	Rana *et al*., 2008
Quercus	TMV		*Tobamovirus*	Yarwood and Hecht-Poinar, 1970; Yarwood, 1971; Nienhaus and Yarwood, 1972; Yarwood and Hecht-Poinar, 1973; Horvath *et al*., 1975; Nienhaus, 1975
	TNV		*Necrovirus*	Büttner and Nienhaus, 1989a
	Not identified			Nienhaus, 1985
	Not identified	Not visualized		Kim and Fulton, 1973; Büttner and Führling, 1993, 1996
Robinia	*Peanut stunt virus,* PSV (formerly *Robinia mosaic virus*)		*Cucumovirus*	Atanasoff, 1935
	SLRSV		*Nepovirus*	Bang *et al*., 2006; Borodynko *et al*., 2007
Salix	ToMV		*Tobamovirus*	Kopp *et al*., 1999
	TNV		*Necrovirus*	
	BMV		*Bromovirus*	
Sambucus	CLRV (*Golden elderberry virus*, GEV)		*Nepovirus*	Hansen and Stace-Smith, 1971; Ellis *et al*., 1992
	Tomato black ring virus (TBRV)		*Nepovirus*	Pospieszny *et al*., 2004
	Elderberry latent virus (EILDV)		*Carmovirus*	Jones, 1972, 1974; Ellis *et al*., 1992
Sorbus	ApMV		*Nepovirus*	Polák and Zieglerová, 1997
	CLRV		*Nepovirus*	Rebenstorf *et al*., 2006
	ACLSV		*Trichovirus*	Sweet, 1980
	European mountain ash ringspot-associated virus (EMARaV)		*Emaravirus*	Ebrahim-Nesbat and Izadpanah, 1992; Mielke *et al*., 2008; Kallinen *et al*., 2009; Mielke-Ehret *et al*., 2010; Valkonen and Rännäli, 2010; Mühlbach and Mielke-Ehret, 2011
Ulmus	*Elm mottle virus* (EMoV)		*Ilarvirus*	Schmelzer *et al*., 1966; Schmelzer, 1969; Jones and Mayo, 1973; Jones, 1974
	CLRV (formerly *Elm mosaic virus*)		*Nepovirus*	Swingle *et al*., 1941, 1943; Ford *et al*., 1972; Schmelzer, 1972
	ToRSV		*Nepovirus*	Varney and Moore, 1952
	TBSV		*Tombusvirus*	Novák and Lanzová, 1980
	Not identified	Filamentous		Bandte *et al*., 2004

[a]Particle morphology is only accounted for viruses that are not yet identified; those that are not visualized in the electron microscope are termed 'not visualized'.
[b]The virus genus is given for all classified virus species, which are written in italic letters, as is the genus in which it is classified.

first detected and demonstrated to be the causal agents of those diseases. As stated above, virus symptoms often go unrecognized; this is because they can produce visible symptoms in some varieties but remain latent in others. Conversely, some viruses produce distinctive symptoms, whereas others are more difficult to detect visually. As an example, a wide distribution of *Cherry leaf roll virus* (CLRV) has been confirmed in Finnish birch species (*Betula* spp.) (Jalkanen *et al*., 2007; von Bargen *et al*., 2009) where they cause significant reductions in tree vitality and yield, whereas CLRV infection in olive trees is symptomless and does not cause significant damage (Langer *et al*., 2010). Furthermore, symptoms caused by mineral deficiency, ozone or drought can be confused with chlorotic and necrotic discolorations induced by fungal or viral pathogens and sucking insects. Grafting and budding failures, which still occur frequently in fruit and ornamental trees, were often considered to be the result of incompatibility problems associated with a particular rootstock/scion cultivar combination. In reality, viruses are frequently responsible for these complete or partial incompatibilities and bud-take problems. This problem is often observed in fruit trees affected by viruses (Németh, 1986).

It is known that CLRV in birch leads to degeneration and in many other tree species to decline (Table 3.2). Similar effects are known from decline-affected European mountain ash (*Sorbus aucuparia* L.) infected with EMARaV, which produces chlorotic ringspots, mottling and staghead (Table 3.3, Plate 1(H)). Other symptoms, such as characteristic virus-like symptoms in oak, associated with degenerated twigs and a distinct loss of vigour have not yet been correlated with a viral pathogen (Table 3.4).

Losses from virus-diseased forest trees are difficult to measure unless trees are visibly damaged or deficiencies in wood quality are noticed. Furthermore, variations in damage by one particular pathogen or by interacting pathogens can, over time, cause different degrees of losses between growing regions or even forest stands. In addition, different management practices, cultivar and species differences, differences in virulence of the virus isolate or strain, and climate conditions can hamper an accurate assessment of virus-induced losses. However, knowledge and information on losses are essential in order to determine economic thresholds for effective control measures.

Viruses are pathogens of small size. They can only be observed by electron microscopy. The structure of a virus is given by its coat proteins, which surround the viral genome. Over 50% of known plant viruses are rod shaped and between 300 and 500 nm in length and 15 and 20 nm in diameter. The second most common structure is formed by isometric particles 40–50 nm in diameter. In addition, a very small number of plant viruses have a lipid envelope around the viral capsid. Viruses are obligate parasites and have no energy metabolism of their own; consequently they require living cells to replicate. They may induce metabolic and structural disarrays in plant cells to varying degrees depending on the viral species and plant susceptibility. Once entry into the cell is achieved, nucleic acids, amino acids and enzymes of the host are taken over by viruses for their replication, placing additional demands on host metabolism. This host–pathogen interaction shows how viruses alter plant vitality.

As shown in fruit trees, virus infections can affect the size, shape and quality of wood and seeds, and can induce coloured patterns on and malformations of leaves. For instance, *Citrus tristeza virus* (CTV), which represents one of the major threats to citrus production worldwide, can cause three different syndromes, depending on the virus strain and on scion cultivar rootstock combinations. These are: (i) decline; (ii) yellowing and stunting; and (iii) stem pitting (Moreno *et al*., 2008). The stem pitting is accompanied by lower yield and diminished fruit quality, independently of the rootstock. Another devastating viral disease, Little cherry disease (LChD), caused by members of the *Closteroviridae* family, is reported in *Prunus* spp. (Ludvíková and Suchá, 2011). Fruits of sensitive cultivars do not mature up to picking time, and therefore most cherries of diseased trees are pointed in shape, imperfectly coloured, small and insipid in taste; they are

Table 3.2. Main characteristics of *Cherry leaf roll virus* (CLRV).

Significance and distribution	Numerous plant species have been recorded that are affected by CLRV (Jones, 1985). Natural host range comprises at least 18 genera of broadleaved trees and shrubs as well as a variety of herbaceous plants. The virus is widespread in forest tree species, for example throughout Europe and North America (Büttner *et al*., 2011). Incidence of CLRV has been reported mostly from tree species of the temperate regions, but it was shown by von Bargen *et al*. (2009) that the virus was also detectable in broadleaved trees north of the Arctic Circle. CLRV is most commonly detected in birch (*Betula* spp.), black elderberry (*Sambucus nigra* L.), European mountain ash (*Sorbus aucuparia* L.) and European ash (*Fraxinus excelsior* L.) (Cooper and Massalski, 1984; Führling and Büttner, 1997; Rebenstorf *et al*., 2006; Jalkanen *et al*., 2007).
Diagnosis	Most commonly, CLRV induces leaf roll, chlorotic ringspots and vein-banding symptoms in leaves. However, symptoms differ depending on plant species, virus strain, location and season. Usually, CLRV can be detected by mechanical transmission to herbaceous indicator plants. Another possibility is transmission by grafting to woody plants in their early physiological stages, which has been shown for diverse birch species, sweet cherry (*Prunus avium* (L.) L.) and blackberry (*Rubus* spp.). However, a long latent period has to be expected until the appearance of symptoms, as summarized in Büttner *et al*. (2011). Electron microscopy has been applied for detection of CLRV-like particles in infected birch species (Hamacher and Giersiepen, 1989). Detection and identification of viruses in infected plants by immunosorbent electron microscopy (ISEM) was reported to be as sensitive as ELISA (Lesemann, 1982). However, the most sensitive detection of CLRV from woody hosts is by immunocapture-reverse transcription-PCR (IC-RT-PCR), as established by Werner *et al*. (1997). This technique has been refined in order to allow the routine testing of large sample numbers of woody hosts (Gentkow *et al*., 2007) as well as the characterization of CLRV variants by restriction fragment length polymorphism (RFLP) analyses (Buchhop *et al*., 2009).
Biology, epidemiology and management	The genome of CLRV contains two (+) single-stranded (ss)RNA segments, which are separately encapsidated in isometric particles, with a diameter of approximately 28 nm (Büttner *et al*., 2011; von Bargen *et al*., 2012). Both particles are required to establish an infection in host plants (Jones and Duncan, 1980). CLRV is a seed- and pollen-borne virus (Mink, 1993; Johansen *et al*., 1994) that infects seedlings of woody plants. Dissemination of CLRV by infected pollen is considered to be an important natural mode of transmission in forest tree species (Card *et al*., 2007). The virus can be horizontally transmitted between adjacent trees, as well as transmitted vertically from mother plants to progenies by fertilization with CLRV-carrying pollen. CLRV is also readily transmitted by water (Bandte *et al*., 2007) and root connections, and might therefore be soil transmittable (Büttner *et al*., 2011). It is not considered to be transmitted by nematodes. Insects may contribute to the infection of plants with CLRV in an indirect manner through wounds via virus-contaminated pollen, or pollinating insects may transfer infected pollen to healthy plants. It is of major importance to eliminate CLRV-infected trees as main virus reservoirs. However, large scale eradication is not advisable owing to ecological and economic constraints. As the impact of insect vectors in the dissemination of CLRV within and between host plant species is still unknown, combating putative vectors in forest stands cannot be recommended either. The lack of knowledge of key epidemiological aspects of virus dispersal illustrates that effective control of CLRV in forested areas is not possible. Hence, an integrated strategy relying on prophylactic control measures has to be established to prevent the spread of CLRV in forested areas. This must include the use of virus-free certified planting material, monitoring and early detection of the virus in symptomatic trees, followed by eradication of infected plants.

Table 3.3. Main characteristics of *European mountain ash ringspot-associated virus* (EMARaV).

Significance and distribution	EMARaV is a novel plant RNA virus with a multipartite genome of negative polarity, which infects European mountain ash (*Sorbus aucuparia* L.) in many parts of Europe, from the mountainous areas of Austria (Tyrol) in the south to Finland and Russia in northern Europe (Benthack *et al.*, 2005; Mielke *et al.*, 2008; Kallinen *et al.*, 2009).
Diagnosis	The characteristic symptoms are chlorotic ringspots and chlorotic mottling on leaves. In addition, affected trees often show reduced growth and gradual decay over years. The serological detection of EMARaV would require undertaking the laborious procedure of preparing nucleocapsid fractions from European mountain ash leaf extracts, followed by protein analyses by polyacrylamide gel electrophoresis and Western blotting. This technique is not well suited to the routine processing of large quantities of samples. In contrast, RNA preparation for reverse transcription-PCR (RT-PCR) is done by quick and simple standard procedures (i.e. methods based on the silica binding of RNA). Leaves, inner bark and vegetative buds can all be used as source of RNA, which allows diagnosis through all seasons of the year.
Biology, epidemiology and management	The means of natural transmission of EMARaV are still unclear. The agent has been shown to be transmissible by grafting (Führling and Büttner, 1995), but the first efforts to transmit the virus mechanically on to healthy European mountain ash seedlings or indicator plants by means of abrasion were only partially successful. Furthermore, the vertical distribution of EMARaV via seeds or pollen has not yet been reported. However, recent studies have revealed that an eriophyid mite, *Phytoptus pyri* Pagenstecher, might be a candidate vector of EMARaV (Mielke-Ehret *et al.*, 2010). Whenever European mountain ash seedlings produced in nurseries are used for new plantations, it is mandatory to test them for EMARaV infection before planting. Only virus-free seedlings, carefully tested by RT-PCR, must be used. However, trees in the wild are naturally dispersed via seeds or by the suckers of older trees, and while the transmission of EMARaV via seed has not yet been reported, vegetative transmission by suckers – a very common means of natural propagation of trees – is possible. In the case that the putative vector transmission of EMARaV by the eriophyid mite *P. pyri* is confirmed, the population density of these gall mites must be controlled. However, adult-stage gall mites escape from the galls and are distributed by the wind, therefore can cover large distances (Schliesske, 1995). This feature would make any forest phytosanitary measures that use chemical or mechanical treatments an extremely difficult task.

not fit for the fresh fruit market (Bajet *et al.*, 2008). Severe damage has occurred in particular in North America. Thus, in the Kooltenay region (British Columbia, Canada), where the disease was first observed in 1933, sweet cherry (*Prunus avium* (L.) L.) production dropped by roughly 90% within 30 years (1949–1979). Just recently, *Little sweet cherry virus* was detected in flowering and sweet cherry trees in China (Rao *et al.*, 2011).

Baur (1907) was the first to publish observations of an infectious chlorosis on *Fraxinus* spp. and *Sorbus* spp., though the nature of the causal agent was not known and even remained disputed for another century. In the case of *Sorbus* spp., Mielke and Mühlbach (2007) described the new virus EMARaV (Table 3.3). Kegler (1960) and Schmelzer (1966) carried out initial studies on virus infection by visually observing symptoms on the leaves and habit of forest trees. Overall, various investigations have significantly expanded our knowledge of viruses in forest ecosystems, and these are thoroughly summarized in an extensive review by Nienhaus and Castello (1989). Continuing surveys of forested areas, public greens and young seedlings in nurseries seem to confirm,

Table 3.4. Main characteristics of the graft-transmissible chlorotic ringspots of *Quercus robur* associated with a virus.

Significance and distribution	Surveys in nurseries, forest stands, park areas and roadsides have led to the registration of many English oaks (*Quercus robur* L.). In Northern Europe, seedlings and trees with characteristic virus-like symptoms such as chlorotic ringspots, chlorotic spots and mottling were observed (Büttner and Führling, 1996). Some of these plants also exhibited twig degeneration and suffered from a distinct loss of vigour. Over time, the proportion of those diseased oak seedlings in the total number of cultivated oak seedlings increases (Bandte *et al.*, 2002). Similar observations have been made in an oak gene conservation seed orchard established in 1992. Earlier studies have also observed these virus-like symptoms (Nienhaus, 1975; Nienhaus and Castello, 1989), but neither the aetiology nor the epidemiology of a viral agent has been described so far.
Diagnosis	Although mechanical transmission to indicator plants failed, the graft-transmissible properties of the causal agent give a prominent pointer to an infectious disease (Büttner and Führling, 1996). Infections with *Tobacco mosaic virus* (TMV), *Tobacco necrosis virus* (TNV), *Cherry leaf roll virus* (CLRV) and *Brome mosaic virus* (BMV) were ruled out by applying serological and molecular assays. The difficulty in purifying and visualizing virus particles prompted an attempt at double-stranded (ds)RNA detection.
Biology and epidemiology	Small dsRNA could be isolated from leaf, bud and bark tissue of trees independent of symptom development, geographic location and sampling date (Büttner and Führling, 1996). The physical characterization of these fragments suggested an infection with a non-symptomatic latent cryptic virus. This assumption is supported by the partial characterization of dsRNA fragments via reverse transcription-degenerate oligonucleotide primed-PCR (RT-DOP-PCR) and complementary (c)DNA cloning (Hahn, 2006). Sequence analysis revealed strong similarities to the RNA-dependent RNA polymerase (RdRp) associated with the family *Partitiviridae*. Additionally, a fragment of approximately 10 kb appeared in some samples. The size and its occurrence in different tissues and developmental stages pointed to the *Endornaviridae*, a new family of plant RNA viruses. These viruses share common properties, such as symptom-free infection of their hosts, transmission only via ovaries and pollen (vertical transmission), and the lack of formation of typical virions. In particular, they are not known to have an effect on their hosts. The observed characteristic chlorotic ringspots and mottling may neither be related to the presence of cryptic plant viruses nor to the presence of endornaviruses.

step by step, the dispersal of viruses in many deciduous trees.

In the Ninth Report of the ICTV, some 2285 virus and viroid species have now been listed (King *et al.*, 2011). Most of them are well-characterized viruses, but only a few have been detected in trees and shrubs. Investigations on viruses in the forest ecosystem are extremely rare compared with those on viruses of the agricultural and horticultural environments.

3.2 Diagnosis

The diagnosis of plant viruses involves more than just the detection of a disease, because detection is also the basis of the major means of control, i.e. effective quarantine, and thereby the means of prevention of disease establishment. The early and accurate diagnosis of plant diseases is a crucial component of any tree management system. Virus diseases can be managed most effectively when

control measures are introduced in the early stages of plant growth. For example, there are effective tests for virus detection in seeds and mother plants, which are conducted before the initiation of cuttings. Further tools for control are mentioned later in the chapter in Section 3.4 on management strategies.

Many of the symptoms observable in virus-diseased trees suggest that alterations of endogenous levels of hormones or alterations in the metabolism of plant growth substances may be involved. Changes in endogenous hormone levels are the cause of symptoms associated with virus infection such as growth inhibition, tumour formation, and hyperplastic and hypoplastic changes in leaves. Virus infections can usually be recognized by mosaic-like leaf patterns, ringspots, lines and mottling of light and dark green. The infection often spreads over the whole leaf blade, having begun at one focal point. Young growing leaves infected during early development are often deformed or involute. Leaf areas that are yellow or slightly faded are considered chlorotic and develop around the primary site of infection. Areas with brown or dead tissue are considered necrotic. Chlorosis is caused by a breakdown of chlorophyll, resulting in a decreased rate of photosynthesis. Heavy infections are characterized by complete loss of chlorophyll in infected tissues, which are typically yellow-like due to the presence of only carotenoids.

Interactions between a host and a virus may result in visible symptoms. Infection causes changes in choloroplast morphology and cell/tissue metabolism, which adversely affect photosynthesis and, consequently, plant growth (Hamacher, 1994). Chloroplast abnormalities occur, such as changes in external morphology and size, disorganization or impaired development of lamellae, membrane vesiculation, increases in size and number of plastoglobuli, and an accumulation of phytoferritin and starch grains. Not all abnormalities are necessarily observed in one situation. They are also dependent on the severity of the symptoms induced by a particular strain of virus and on the type of tissue affected.

Virus infection also leads to a reduction in transpiration rate, which is often correlated with reduced leaf stomatal aperture. It has also been shown that the turgidity of a leaf both on and immediately after inoculation can influence the creation or development of infection sites and the apparent susceptibility of a plant to virus infection.

Reliance on symptoms alone is not adequate for proper identification of viruses. However, a thorough knowledge of symptomatology is absolutely essential to recognize virus-induced symptoms in the field (Plate 1).

Virus infections are often confused with symptoms caused by other biotic and abiotic factors, and these have to be distinguished by reliable diagnostic methods (Büttner and Führling, 1993). Advances in the development of modern methods for virus detection provide specific and sensitive tools that are needed for the detection of viruses in early stages of infection. However, it has to be understood that the detection of viruses in woody plants is much more difficult than in herbaceous hosts. The high amount of phenolic compounds, the irregular distribution of viruses in trees and the often low virus concentrations make detection complicated. These factors demand the development of techniques and methods that are adaptable to woody plants. In addition, complications in detection can develop when the trees are infected simultaneously by several viruses, a common phenomenon in nature. The complex nature of virus infections in trees requires development in the future of more refined diagnostic techniques for both the determination of known viruses and the identification of possibly unknown viruses.

As a general tool to detect viruses in forest trees is not available, we suggest a variety of techniques that have to be carried out in combination (Fig. 3.1). The methods suggested include: biological indexing, electron microscopic observations, antibody-based methods, double-stranded (ds)RNA-analysis, molecular hybridization and PCR. DNA microarrays have to be discussed as possible future tools. When using these diagnostic tools, it is important to have standard controls whenever tissue from healthy plants and cultivars is available.

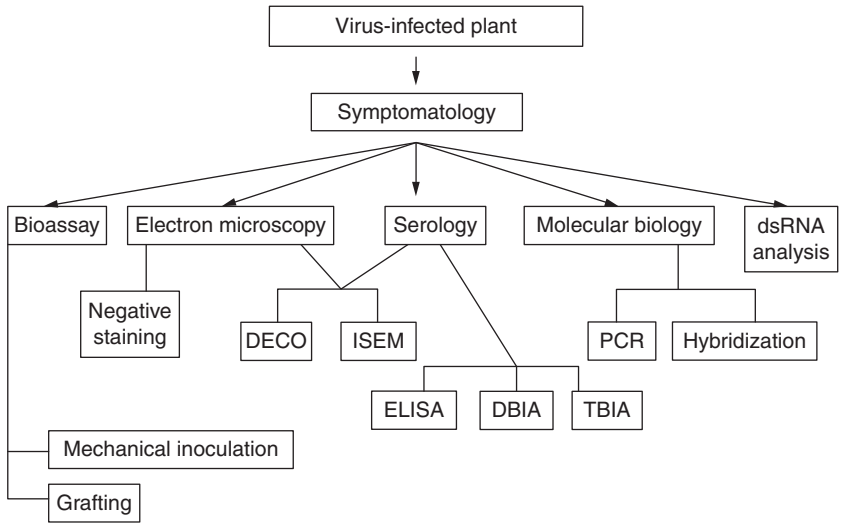

Fig. 3.1. Flow-chart of diagnostic methods and tools for the detection of viruses in trees. DBIA, dot-blot immunoassay; DECO, method of decoration of virus particles by antibodies (antibody binding); dsRNA, double-stranded RNA; ISEM, immunosorbent electron microscopy; TBIA, tissue-blot immunoassay.

3.2.1 Biological indexing

Biological indexing is a time-consuming, labour-intensive technique that requires greenhouse or orchard space. It is the method of choice for the detection of graft-transmissible pathogens for which no precise information is available. It enables the transmission of viruses to healthy plants and the demonstration of both known and unknown or uncharacterized agents, and confirms their infectious character. Biological indexing comprises two general methodologies: (i) the herbaceous host bioassay, which involves inoculation to a range of sensitive indicator cultivars; and (ii) the woody host bioassay, which relies on budding and grafting techniques. The first method requires that sample tissue is macerated in suitable buffer and rubbed on to the leaves of herbaceous plants, which are then incubated and observed for symptom development over time. Efforts to transmit a virus from a woody to a herbaceous plant can often be difficult due to phenolic compounds in the tissue. Nepoviruses such as CLRV can be reliably transmitted (Table 3.2), but other virus genera might be more difficult to transmit or may fail to induce symptoms and thus can escape detection. The techniques used in the woody host bioassay are well known from traditional techniques used to propagate cultivars. All viruses can be transmitted by grafting.

3.2.2 Electron microscopy

Electron microscopy is used to identify particle morphology as a complement to biological indexing or to confirm the viral origin. The sensitivity of this method is poor for detection purposes. It is not applicable to large-scale analyses and is not a suitable tool for detecting viruses directly in plant material from trees, because of the irregular distribution and the low concentration of the pathogens. Some improvement has been made to the method based on the use of antibodies. Combination of the high resolution of the electron microscopy with the specificity of the antibodies has a significant advantage in the detection process. The principle is the selective trapping of plant virus particles on grids that are pre-coated with specific antibodies (Roberts and Harrison, 1979; Russo *et al.*, 1980). The technique has also been coupled with the use of protein A or colloidal gold

for a better decoration of the virus particles (Kalashjan and Lipartia, 1986). Immune electron microscopy is highly reliable when samples contain virus particles, but its application remains limited by the low number of samples that can be processed. These methods have been used to detect/locate CLRV on and in pollen from birch and walnut (*Juglans* spp.) trees (Massalski and Cooper, 1984; Massalski *et al*., 1988).

3.2.3 ELISA

ELISA, first established by Clark and Adams (1977), is still an inexpensive and reliable assay for routine testing for the presence of viruses in leaves, stems, bark, flowers and roots. However, the quality of the test results depends on the quality and availability of the pathogen-specific polyclonal and monoclonal antibodies. Various formats have been developed and assayed based on the use of specific antibodies, as reported by Koenig and Paul (1982) and Cambra *et al*. (1991, 1997). Grüntzig *et al*. (1994) evaluated a sampling schedule for the best virus detection by ELISA considering their irregular distribution within the sample tree and demonstrated that the sampling point in the tree canopy has an influence on the reliability of the diagnosis.

The main versions of ELISA used are the double antibody sandwich (DAS) and triple antibody sandwich (TAS). Lateral flow devices and sticks have also been developed and applied. Their use is limited to the rapid diagnosis of materials showing virus-typical associated symptoms. According to Cambra *et al*. (2006), ELISA will remain the primary method of choice for the universal detection and routine screening of viruses in woody plants, using either a single monoclonal antibody or mixtures of monoclonal antibodies. However, the assays lack the sensitivity required for the detection of woody plant viruses, which are usually present in low titres in their hosts.

3.2.4 Hybridization and PCR

Hybridization and PCR are sensitive diagnostic techniques and enable virus detection in the smallest amounts. Specific DNA or complementary (c)DNA sequences are amplified *in vitro* from trace amounts in a complex mixture of templates. RNA viruses require the additional step of reverse transcription (RT) to convert their sequences from RNA to DNA before the amplification process begins.

The dot–blot hybridization is the most common molecular hybridization. Nucleic acid solution is directly applied on to a membrane such as nitrocellulose or nylon, followed by specific probes (Mühlbach *et al*., 2003). The technique is based on the specific interactions between complementary purine and pyrimidine bases forming A–T and G–C base pairs. This interaction results in a stable hybrid formed by part of the nucleic acid sequence of the target molecule and the labelled complementary sequence. The dot–blot hybridization can be applied to detect particular plant RNA viruses in those cases where a genome specific probe is available. By varying the hybridization conditions, a sequence identity of 60–70% between the specific probe and the target RNA is sufficient for the detection. The dot–blot hybridization is well qualified for routine diagnostic testing of large-scale samples.

Werner *et al*. (1997) evaluated a method for detecting CLRV in seeds of birch and concluded that the use of specific antibodies for immune capture (IC), followed by RT-PCR, is the most sensitive technique for detecting viral RNAs. The method provides a cheaper and more reliable system for routine use. PCR is more specific than the hybridization and, moreover, enables the differentiation of virus strains.

3.2.5 DsRNAs detection

The tools for detecting dsRNAs led to a step forward when searching for an unknown single-stranded (ss)RNA virus. DsRNAs are formed during virus replication, and therefore consist of full-length genomes. After separation on gels, the size and patterns of these virus-specific dsRNAs can be useful in

virus characterization and provide useful information on the type of virus (Valverde *et al*., 1990). This technique is based on the premise that healthy plants not infected with a virus do not contain dsRNAs. Multiple dsRNA bands on gels after gel electrophoresis may indicate the detection of a monopartite virus that produces sub-genomic RNAs during its replication cycle. Such multiple bands can also indicate that the plant is infected by at least two viruses or by a replicating multipartite virus (Jelkmann, 1995). The method described by Morris and Dodds (1979) involves total nucleic acid extraction, followed by binding of dsRNA to cellulose and then its elution. The protocol of Tzanetakis and Martin (2008) has been adapted to woody plant material and been further modified to isolate dsRNA successfully from leaves of diseased English oaks (*Quercus robur* L.). The leaves show chlorotic ringspots caused by an unknown graft-transmissible agent (Table 3.4). The quality of extracted dsRNA is dependent on the amount of plant compounds such as glycoside, polysaccharides and polyphenols that are present and can interfere with electrophoretic mobility of nucleic acids. Alternatively, monoclonal antibodies to dsRNA are applicable to detect infection in plants by RNA viruses in crude nucleic acids (Lukács, 1994).

3.2.6 Tissue print

Tissue printing of plant material directly on to nitrocellulose membranes or filter paper is a suitable procedure for sample collection and storage off-site, prior to subsequent processing in the laboratory. Direct immunoprinting ELISA using monoclonal or recombinant antibodies without the need for extract preparation is the officially recommended protocol in the European Union (EU) for the detection of CTV (López *et al*., 2003). Tissue print can also be coupled with amplification methods based on RT-PCR. The so-called print-capture procedure described by Olmos *et al*. (1996) for the detection of *Plum pox virus* (PPV) in peach and apricot seedlings provides similar sensitivity to that of immune capture-RT (IC-RT-PCR) applying PPV-specific antibodies.

3.2.7 Multiplex RT-PCR

A multiplex RT-PCR was reported by Saade *et al*. (2000), enabling the detection of *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple mosaic virus* (ApMV), which are the three most important ilarviruses affecting stone fruits and forest trees (Table 3.1). Similarly, Menzel *et al*. (2002) established a multiplex RT-PCR for the reliable detection of four economically important apple viruses, including an internal control by co-amplification of the mitochondrial *nad*5 gene from plant mRNA. The assay allowed the reliable detection of different virus isolates from different geographic regions all year round. Coupling the amplification of multiple RNA target sequences with a colorimetric detection system called RT-PCR-ELISA (Menzel *et al*., 2003) revealed that this technique was more reliable for the detection of *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV) and ApMV than indexing by woody indicators. Sánchez-Navarro *et al*. (2008) pushed multiplexing by RT-PCR to its limits by the development of an assay for the simultaneous identification of eight important viruses affecting stone fruit trees. The method included the detection of ApMV, PNRSV, PDV and ACLSV, which are also frequently found in important forest tree species (Table 3.1).

Apart from PCR, a protocol based on the loop-mediated isothermal amplification (LAMP) has been developed as a very sensitive diagnostic tool for the reliable detection of different strains of PPV in nectarines and peach trees (Varga and James, 2006). The major advantage of the recently reported one-step RT-LAMP for the fast detection of PPV in woody hosts is that it has the same sensitivity as RT-PCR paired with very high specificity and low artefact susceptibility, especially when combined with a simplified and standardized virus extraction method from woody plants (Hadersdorfer *et al*., 2011).

Although this technique is not applied for the detection of viruses that are relevant in forest trees, it may be a promising low-cost alternative to the widely applied PCR in such instances.

3.2.8 Microarrays

There are only few published reports describing the use of microarrays for the detection of viruses relevant to woody hosts. The detection and genotyping of PPV using an oligonucleotide array has been employed by Pasquini *et al*. (2008), while Lenz *et al*. (2008) described an array for simultaneous detection of RT-PCR-amplified target sequences of several viruses from fruit tree samples. Abdullahi and Rott (2009) developed a microarray immunoassay by immobilizing specific antibodies on to glass slides followed by colorimetric detection of target antigens following the DAS procedure. The antibody microarray was suitable for the detection of several grapevine and fruit tree viruses, with sensitivities and specificities comparable to those of microtitre plate-based ELISA. Recently, Engel *et al*. (2010) and Abdullahi *et al*. (2011) reported the development of diagnostic oligonucleotide microarrays. These were designed for the simultaneous detection of up to 13 different grapevine viruses, including the nepoviruses *Arabis mosaic virus* (ArMV), and *Tomato ringspot virus* (ToRSV), which have also been reported in forest trees (Table 3.1). Although there are no DNA arrays available for the detection of the all-important viruses of forest trees, the multiplexing capacity of microarrays for use with samples from woody plants has been demonstrated by the abovementioned examples. The method has to be considered as a powerful tool for the simultaneous detection of viruses in woody host species suitable, for instance, for high throughput plant certification purposes.

3.3 Infection Biology and Epidemiology

Plant viruses are distributed worldwide and they have been detected in different environments, such as woody and herbaceous plants, soil, surface waters, glacier ice, seawater and clouds. The mode of transmission has to be considered as an important factor influencing spread and impact of a virus infection, regardless of the plant involved (Büttner and Bandte, 2001). Vector or non-vector transmission, however, has to be determined. The great majority of plant viruses are dependent for their spread upon efficient transmission from infected to healthy plants by vectors. Prominent specific vector organisms for plant viruses are plant-feeding arthropods, nematodes and plant parasitic fungi. About 94% of known plant virus vectors are arthropods, with 55% of them being aphids (Ng and Falk, 2006). Whiteflies, thrips, mealy bugs, plant hoppers, grasshoppers, scales and a few beetles also serve as vectors for certain viruses. Some viruses may persist for weeks or months and even replicate in their insect vectors; others are carried for less than an hour. The vector transmission is a very specific process. A specific virus can only be transmitted by a single vector type. Conversely, each vector species can transmit one or more different viruses. Furthermore, some viruses are transmitted by seeds and pollen, and/or by soil and water. Mechanical transmission has to be regarded as an important manner, which often occurs in the form of human handling of plant material during production, or by animals carrying the virus on their body parts and/or mouthparts, and even after passage through the human alimentary tract (Tomlinson *et al*., 1982).

3.3.1 Mechanical transmission

Most viruses of forest trees and urban greens spread from diseased plants by contact with each other, by infected propagative material or through natural root grafting to neighbouring trees. They are distributed inadvertently by man on a massive scale because of vegetative propagation via rooting cuttings during propagation, e.g. *Poplar mosaic virus* (PopMV) in poplar (*Populus* spp.). Budding and grafting, in which infected mother plants perpetuate the viruses, are not common for these types of trees. Viruses can be spread by direct transfer

of sap through the contact of sap of the wounded plant with a healthy plant (Nienhaus *et al*., 1990). Such contact may occur during normal forestry management, such as damage caused by contact with human tools, or naturally by animals feeding on the plant.

3.3.2 Transmission through water and soil

Büttner and Nienhaus (1989b) detected plant viruses in water samples collected from forest districts in Germany. In this study, mechanically transmissible tobamoviruses, potexviruses and tombusviruses were isolated from water samples taken from creeks, ponds and drainage ditches. Several studies on the water transmission of viruses in different parts of the world have confirmed a relatively high concentration of several plant viruses in surface waters such as ditches, lakes, rivers and streams (Koenig, 1986; Rohwer *et al*., 2009). The significance of water transmission on the epidemiology and ecology of plant viruses has not been well studied. Our own investigations and those of other scientists indicate that water transmission of viruses occurs more frequently than was once thought (Büttner and Koenig, 2013). During a survey of plant viruses in small ponds and creeks in a forested area in Germany, several viruses were isolated. The *Carnation Italian ringspot virus* (CIRV) was identified; this had previously been isolated only twice from carnations originating from Italy and USA, and infected carnations only with difficulty (Büttner *et al*., 1987). The role of this tombusvirus in the forest ecosystem is still unknown.

In the early 1980s, *Tomato bushy stunt virus* (TBSV) was used to demonstrate that humans can act as carriers of plant pathogens by consuming infected plants and shedding infective viral particles in their faeces (Tomlinson *et al*., 1982). It was suggested that plant viruses with no known vectors, such as most tobamoviruses and tombusviruses, may have a certain 'alimentary resistance' and stay intact after passing through the alimentary tract (Tomlinson *et al*., 1982; Zhang *et al*., 2006), which enables humans and other animals to act as carriers of these viruses in all ecosystems, including forested areas. In a metagenomic study of the RNA viral communities in human faeces, sequence portions of more than 30 different plant viruses, especially of tobamoviruses, tymoviruses, marafiviruses, maculaviruses, carmoviruses and necroviruses were detected (Rohwer *et al*., 2009). These findings are important for those working on virus epidemiology and involved in forest tree health because this form of transmission has an impact on plant protection services, quarantine stations, breeders, nurserymen and research laboratories.

Analysis of soils from forest stands in Germany using the bait-plant technique confirmed the presence of potexviruses, tobamoviruses, necroviruses and potyviruses (Büttner and Nienhaus, 1989a).

The need and use of water and soil for crop production makes it necessary to be fully aware of the importance that these media have as a source and vehicle of virus movement. The viruses of highest significance in the waterborne and soil-borne transmission of infectious diseases are essentially those that have the ability to maintain their infectivity in this environment even though viruses cannot replicate outside the cells of hosts. Most viruses that are passively transmitted in this manner are released from disturbed plant roots, are very stable, reach high concentrations in plants, and infect a wide range of plant species and genera. Virus particles are known to be absorbed to clay particles and organic plant debris (Kegler *et al*., 1995). The stability of the coat protein explains their longevity in water and soils, and their stability in extreme environments (Koenig, 1986; Tomlinson, 1987). Viruses can be transported by water and soil from infected to healthy plants, where they can cause an infection after entering the plant passively through their roots (Schwarz *et al*., 2010).

Furthermore, it is remarkable that viruses have been found in ancient glacial ice (Castello *et al*., 1999). *Tomato mosaic virus* (ToMV), a very stable plant virus with a wide host range that includes spruce, was detected in this environment (Castello *et al*., 1995; Bachand and Castello, 1998). ToMV-infected red spruce (*Picea rubens* Sarg.) seedlings are affected by a reduction in seedling height, weight, root volume, bud break and mean shoot length when

compared with control seedlings (Bachand *et al*., 1996). Surprisingly, needles of infected seedlings were less susceptible to freezing damage than those of control seedlings. Fillhart *et al*. (1997) postulated that the atmospheric spread of infectious plant viruses without invertebrate vectors represents a potentially long-distance transport mechanism for stable plant viruses like ToMV. The authors detected ToMV in more than half of the investigated cloud samples collected from the summit of a mountain in New York and in collection sites along the coast of Maine, USA. Perhaps the virus becomes airborne as a result of ToMV contaminated soil particles that serve as cloud condensation nuclei.

3.3.3 Transmission through vectors

Vectors are of major importance in the dynamics of virus populations and they have a strong influence on virus aetiology. Many species of invertebrates, nematodes, fungi and fungal-like organisms are involved in virus transmission.

Insects

When all known plant viruses are considered, approximately 70% are insect transmitted. Aphids constitute the most important group of vectors, because of their natural abundance in the ecosystem and due to their feeding behaviour. Vectors are classified based on the properties of the relationship established, including length of time required for acquisition, latency and retention. Two major categories of transmission are distinguished, circulative and non-circulative, based on the sites of retention and routes of movement through the vector (Matthews, 1991). Circulative transmission can be classified into: (i) non-propagative, when the virus does not replicate in the vector although it reaches the digestive tract and haemolymph, and from there the salivary glands, from which it is inoculated during the subsequent feeding; and (ii) propagative, when the virus replicates inside cells of the vector and has a long-term association with the vector. These categories of plant viruses encode genes that are differentially expressed in their infection cycle during the infection of plants or insects. They belong to the families *Bunyaviridae*, *Reoviridae* and *Rhabdoviridae*, and to the genera *Marafivirus* and *Tenuivirus*.

Numerous experiments on vector transmission of the above-mentioned viruses have been carried out with herbaceous plants or fruit trees and grapevines, but not with common forest trees. Therefore, any information obtained on the potential transmission of viruses within forest trees is more a logical consequence than a proof of experiment. There are only a few studies on vectors within the forest context.

Nematodes

Only a small number of genera of nematodes are involved in plant virus transmission, but they are important and widespread. Some soil-borne viruses are transmitted by these plant parasitic soil-borne nematodes to horticultural plants (Brown *et al*., 1995). Their movement is limited, and their activity greatly depends on the texture and water content of the soil. ArMV, a member of the nepoviruses and a well-known pathogen of a number of woody hosts, is transmitted by species in the two genera of *Xiphinema* and *Longidorus*. Members of the tobraviruses are transmitted by species of *Trichodorus*. Once acquired, viruses may persist in transmissible form in nematodes for up to a year or more, depending on the species. The viruses do not replicate in the nematode vector. They are bound reversibly and in a specific manner to the upper regions in the digestive tract of the nematodes, from which they are released when the nematode's saliva is pumped into the host cells during the feeding process. Tobraviruses, as well as nepoviruses, have wide host ranges, which aids their survival in a given region.

Fungi and fungal-like organisms

Some plant viruses in several genera are transmitted by plant pathogenic fungi, the majority to herbaceous host plants such as grain crops and sugarbeet. A successful transmission is dependent on the way in which the fungus carries the virus (Adams, 1991).

Furoviruses, Pecluviruses, Pomoviruses, Benyviruses and bymoviruses are transmitted by species of *Polymyxa* or *Spongospora*, which were previously classified as fungi but have now been assigned to the protozoa. Viruses are carried in zoospores, which are released into the surrounding aqueous medium and carry the viruses inside the protoplast. These organisms deposit viruses into root cells when infecting the roots of a new host plant. The virus may also remain infectious in resting spores in air-dried soil for many years. Viruses transmitted by *Olpidium brassicae* (Woronin) P.A. Dang and *O. bornovanus* (Sahtiy.) Karling, such as *Tobacco necrosis virus* (TNV), are carried on the surface of the spores. Viruses transmitted by *Olpidium* spp. do not remain infectious in air-dried soil and are characteristic in having a wide host range.

Knowledge of the importance of the fungal transmission of viruses to forest tree species is for the most part non-existent and urgently requires research input. About 40 years ago, particles resembling *Tobacco mosaic virus* (TMV) were found in preparations of five out of 12 rust (*Pucciniales*) and two out of seven powdery mildew (*Erysiphales*) species (Yarwood and Hecht-Poinar, 1973). The strains were transmissible when asexual spores (conidia) were dusted on to leaves of *Chenopodium quinoa* Willd. The recovery of infective virus from oaks was reported formerly by Yarwood and Hecht-Poinar (1970). Nienhaus (1971) confirmed the hypothesis of fungal transmissibility by isolating TMV from oaks (*Quercus agrifolia* Née, *Q. phellos* L.) as well as from powdery mildew (*Sphaerotheca lanestis* Harkn.) from the same diseased oaks.

3.3.4 Seed transmission

Forest viruses may be responsible for a wide range of economic losses due to their impact on tree seed survival and growth, and also because of the long distance spread of seeds. For example, the germination of seeds from CLRV-infected birch trees is strongly reduced (Cooper and Atkinson, 1975). Presumably the embryo is invaded during the transmission of CLRV by seeds.

Two general types of seed transmission can be distinguished. First, transmission may be the result of contamination of the seed coat with virus, resulting in subsequent infection of the germinating seedling by mechanical means. The external virus can be inactivated by certain treatments, thus eliminating almost all seed-borne infection. In the second and more common type of seed transmission, the virus is found within the tissues of the embryo, which may become infected through the ovary or via the pollen. Most seed-transmitted viruses are also transmitted through the pollen from infected plants, and all known pollen-transmitted viruses are seed transmissible. The mechanisms by which pollen transmits viruses are either through their presence within the sperm cell nucleus or cytoplasm or, alternatively, their presence on the exine of the pollen grains. The germ tubes growing from such pollen may then pick up virus particles and actively carry them to the ovule.

Viral infection of the embryo axis has been associated with the transmission by seed of numerous viruses that are important to forest trees and shrubs, such as Ilarviruses and Nepoviruses, e.g. ArMV, *Strawberry latent ringspot virus* (SLRSV) and *Tobacco ringspot virus* (TRSV). Virus replication within the embryo is presumably reinitiated when the metabolism of infected cells increases at germination (Johansen *et al*., 1994). Cooper *et al*. (1986) detected Ilarviruses in sweet cherry seed, and PopMV in large-scale surveys of poplar germplasm. Cucumoviruses are transmitted through seeds and are very commonly found in cultivated plants and also in woody hosts, e.g. *cucumber mosaic virus* (CMV) in *Berberis thunbergii* DC. (Cooper, 1993). Tobamoviruses are known to be transmitted by contaminated seed coats and thereby infect the seedling at germination; these viruses have been detected in English oak and maple (*Acer* spp.) trees (Führling and Büttner, 1998).

3.4 Management Strategies and Tactics

Once infected with a virus, perennial woody trees cannot be cured. Therefore, forest tree

virology plays a major role in conserving the economic value of a forest stand (Hubbes, 1993). There is substantial evidence that shows the potential impact of virus disease on forest trees.

The two requirements for effective control of viruses are the identification of the causal pathogen and the determination of the possible mechanisms of transmission; this was shown for CLRV by Hamacher and Quadt (1994). A strong basic knowledge of virus characteristics, experience in symptomatology and access to reliable and sensitive virus detection are prerequisites for the proper diagnosis of forest tree viruses and for their effective management. Virus control in forests is based on prevention, and so strategies have to be adapted to the origin of the planting material. Knowledge of the status of gene banks, nurseries, forest stands and trees in urban areas, such as roadside trees, adds supporting information for developing control plans.

With the exception of the elimination of infected and unthrifty trees, it is usually impractical, if not impossible, to prevent the spread of viruses between trees in the field once infected trees have been transplanted into the field or are growing under natural conditions. Attention, inevitably, has to be focused on the nursery production of planting stock, where critical examination and assessment of vigour can be practised routinely. Prevention is only possible when the pathogen is detected and its general properties, including its mechanisms of natural dispersal, are known. Further promising approaches to prevent the transmission of viruses within nurseries are the use of stringent hygienic measures, the use of surfactants to avoid the spread of viruses by tools and containers or in set-up areas, and the control of potential water or vector transmission of viruses. Plant nurseries are typically placed on open-textured soil that might potentially harbour soil-borne vectors and stable viruses. Healthy plants growing in this soil are commonly infected with viruses (Cooper, 1993). So it is recommended to leave wide headlands and to avoid planting seedlings in recently cleared areas that contained infected plants for at least 3 years, unless the soil is partially sterilized. Alternatively, trees should be propagated in steam-sterilized horticultural soil mixtures.

Virus-infected trees in forest stands cannot be eliminated on a large scale. Moreover, they have to be registered and the cause of virus emergence has to be analysed. A programme for the choice of tree species for future planting has to be developed, taking into consideration not only seedling stage but also plant health over a tree's lifetime, as well as the complication of natural succession forests. As mentioned previously, management strategies are prevention tools. They can be classified in three categories: (i) removal or avoidance of source of infection; (ii) control or avoidance of vectors; and (iii) protection of the plant from systemic disease. This requires breeding for resistance, the propagation of virus-free plants, the use of virus-free seeds and practices designed to reduce virus spread by vectors.

A continuous effort to breed virus-resistant plant varieties, especially for renewable resources (e.g. poplar species) should be a major future concern. Breeding, however, does not provide an advantage in all cases. One has to be aware that viruses continually mutate in the field with respect to both virulence and the range of plants they can infect. For instance Atreya *et al*. (1990) were the first to confirm the hypothesis that a coat protein mutation affects the aphid transmissibility of a potyvirus, and thus plays a role in determining aphid transmissibility. Evolutionary constraints on the emergence of plant RNA viruses are summarized and reviewed by Elena *et al*. (2011). Thereby, viral emergence results from an evolutionary process in which the main players are ecological factors, virus genetic plasticity and host factors. Hence, breeding for resistance or the development of transgenic plants are unlikely to give a permanent reliable solution.

When testing seeds, there are two protocols for the preparation of the samples: (i) direct preparation of the seeds, when possible; and (ii) using leaf tissue after the seeds have germinated. In all cases, the seed coat has to be removed before sample preparation. When viruses are transmitted through seed, major efforts should be made to control stock plants and seeds from nurseries and breeding

programmes. Certification schemes would lead to access to virus-free seeds and mother plants, as has been achieved in the successful certification schemes in fruit crop production. It should be remembered that the development of high-quality certification in fruit trees took over 20 years within the EU. The time invested in developing certification for trees is a secondary consideration when fighting for a sustainable virus-free and healthy forest.

Using fruit trees grown in temperate areas that are affected by many viruses as an example, it should be possible to develop efficient strategies for virus elimination in forest trees over time. This can be achieved using a number of different techniques: (i) meristem tissue culture; (ii) thermotherapy *in vivo* or *in vitro*; (iii) a combination of *in vitro* thermotherapy and meristem tissue culture; and (iv) *in vitro* micrografting (Barba *et al*., 1992). In all cases, pathogen elimination is a host–pathogen dependent process. Simplification of recommendations for certification should be avoided. However, tools are available that allow logical steps to be taken to obtain virus-free propagation material in forest trees. Worldwide plant health is a major concern to forestry phytosanitary agencies, simply because, as already noted, no effective cure exists to treat trees with established infections.

Virus testing in nurseries requires good diagnosis, assisted by information on symptoms, mode of transmission and mode of dispersal and host range. The detection of the progress of the epidemic has to be determined using data from: (i) plant material; (ii) age of the plant; (iii) season when samples are taken; and (iv) information on virus species. From our experience, leaves, stems and bark should always be tested in parallel to achieve optimum test results in a survey. The application of indicator plants for grafting is also recommended to confirm replication of a viral agent after transmission from diseased to healthy plants. This type of indexing is a common tool for the confirmation of virus detection for some viruses in fruit trees, such as ApMV, ACLSV and PPV.

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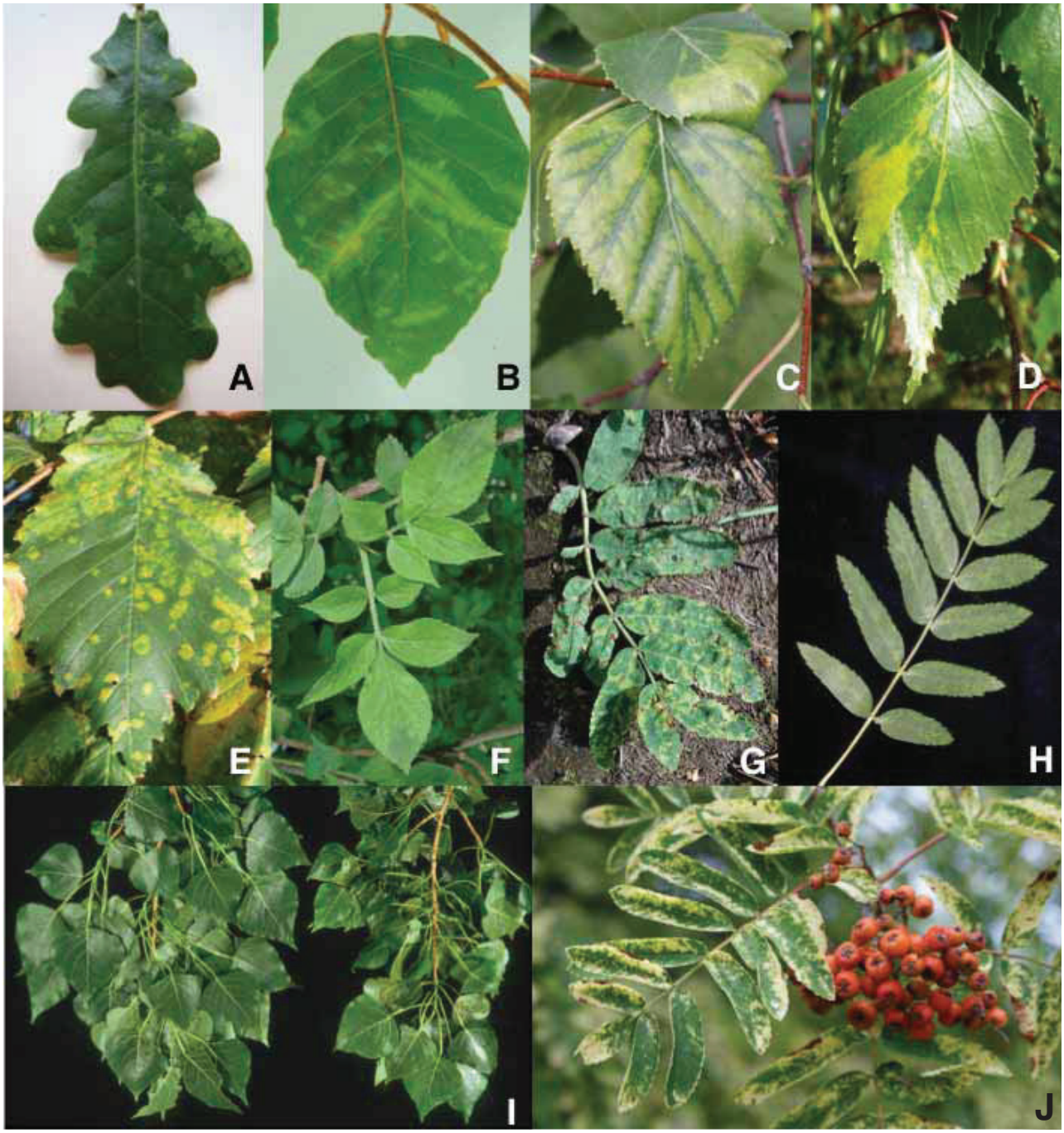

Plate 1. Virus-induced leaf symptoms: (**A**) chlorotic ringspots in *Quercus robur*, (**B**) chlorotic line pattern in CLRV-infected *Fagus sylvatica*, (**C**) extensive intercostal chlorosis of CLRV-infected *Betula pubescens*, (**D**) chloroses of ArMV-infected *Betula pendula*, (**E**) chlorotic ringspots in *Ulmus laevis*, (**F**) chlorotic leaf pattern of CLRV-infected *Sambucus nigra*, (**G**) chlorotic and necrotic spots in CLRV-infected *Sorbus aucuparia*, (**H**) chlorotic ringspots in EMARaV-infected *S. aucuparia*, (**I**) little leaf and staghead induced by PopMV in *Populus nigra*, and (**J**) virus-like symptoms in *S. aucuparia* associated with ApMV.

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Plate 2. Douglas-fir dwarf mistletoe (*Arceuthobium douglasii*) causes witches' brooms. These witches' brooms have been found to be important nesting structures for wildlife. Credits: David C. Shaw.
Plate 3. Prescribed fire is an important control for dwarf mistletoes in western North America. Credits: Robert L. Mathiasen.

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Plate 4. The Japanese pine sawyer *Monochamus alternatus*, a major insect vector of the pine wood nematode *Bursaphelenchus xylophilus* in Asia. Credits: Naoto Kamata.
Plate 5. A *Pinus thunbergii* stand suffering from the pine wilt disease caused by the pine wood nematode *Bursaphelenchus xylophilus.* Credits: Kazuyoshi Futai.

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Plate 6. Manual stump treatment with urea solution against *Heterobasidion annosum s.l.* on a *Picea abies* stump. Credits: Paolo Gonthier.
Plate 7. Single-grip harvester performing stump treatment against *Heterobasidion annosum s.l.* in thinning. Credits: Stefan Örtenblad.

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Plate 8. *Armillaria ostoyae* mycelial fans. Credits: Philippe Legrand.
Plate 9. *Armillaria gallica* sub-cortical rhizomorphs. Credits: Jean-Jacques Guillaumin.

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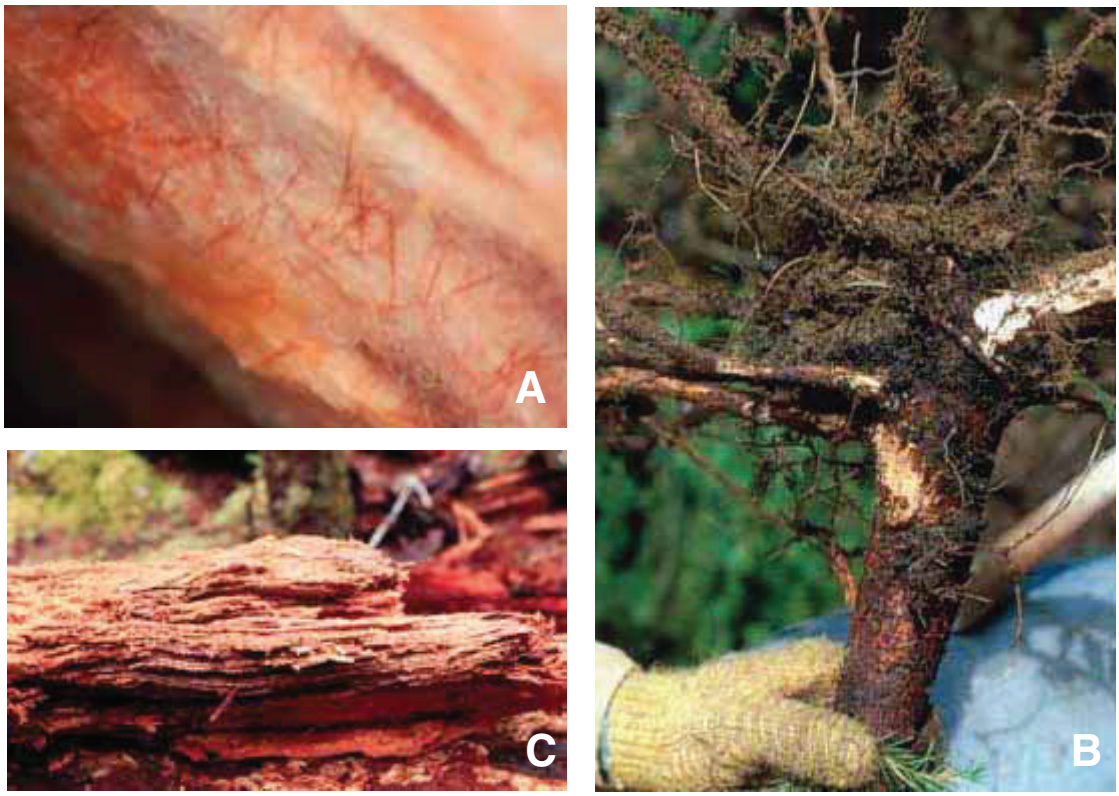

Plate 10. Typical appearance of upturned trees whose root systems have been infected with (**A**) *Phellinus sulphurascens* and (**B**) *Onnia tomentosa*. Note that *P. sulphurascens* causes more extensive root decay resulting in the 'root ball' appearance. Credits: (**A**) Canadian Forestry Service/Natural Resources Canada, (**B**) Kathy J. Lewis.

Plate 11. (**A**) Dark brown setal hyphae in wood colonized by *Phellinus sulphurascens*, (**B**) yellow white mycelium of *Onnia tomentosa* in the bark of colonized spruce root, and (**C**) laminated decay pattern caused by *P. sulphurascens*. Credits: (**A, C**) Canadian Forestry Service/Natural Resources Canada, (**B**) Kathy J. Lewis.

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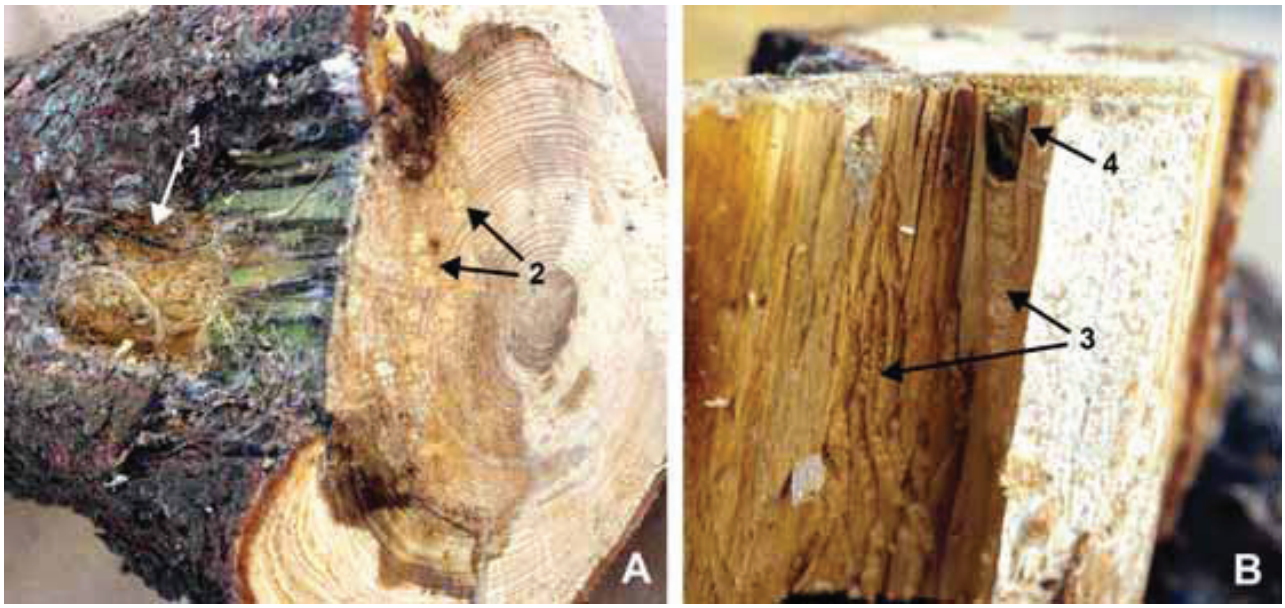

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Plate 12. Cross-section (**A**) and longitudinal section (**B**) of wound heart rot columns caused by *Amylostereum areolatum* in stems of *Picea abies*. Arrows indicate: (**1**) *Rhyssa* sp., a parasitic wasp (*Ichneumonidae*), looking for larvae of *Sirex juvencus* inside the wound to put the egg in (note woodpecker activity on a surface of the wound, also in a hunt for the larvae); (**2**) and (**3**) larval tunnels of *S. juvencus* filled with a fine frass; (**4**) dead adult of *S. juvencus* in the pupal chamber. Note that larval tunnels are always confined within a heart rot column. Credits: Vytautas Bardauskas.

Plate 13. *Platanus hybrida* infected by *Ceratocystis platani*. Sawdust generated during felling is collected in tarps. Credits: Giovanni Nicolotti.

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Plate 14. Vibratory plow used in Minnesota to sever root grafts between healthy oaks and trees with oak wilt. The blade is inserted into the ground and vibrates as it is pulled through the forest floor with minimal soil disturbance. Credits: Charles Evenson.
Plate 15. Storage of *Picea abies* logs in stacks constantly sprinkled with water to protect them against insect infestations and infections by blue-stain and other fungi.

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Plate 16. Wrapping and airtight sealing of fresh logs of high quality in a double layer of UV-resistant polyethylene foil is a relatively new long-term storage method to protect logs against insect infestations and infections by blue-stain and other fungi.
Plate 17. Resinous lesion associated with blackstain root disease on *Pinus Ponderosa*. Credits: Paolo Gonthier.

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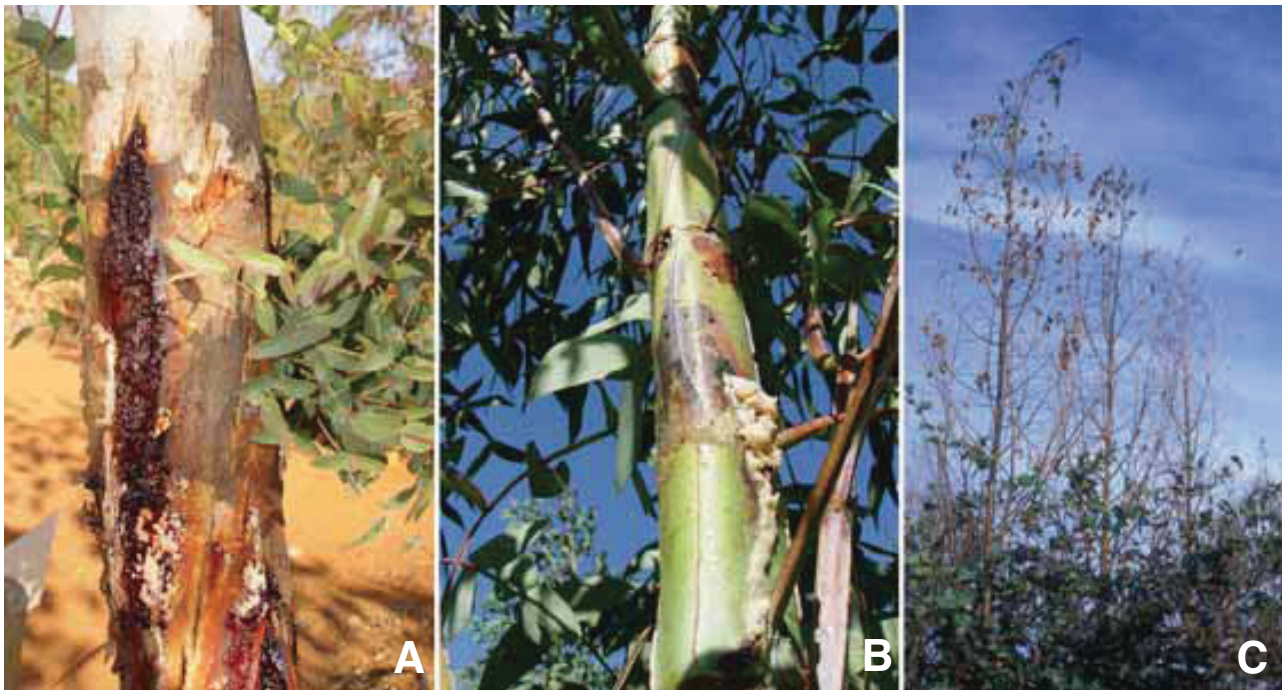

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Plate 18. Examples of canker (**A**, **B**) and dieback (**C**) of eucalyptus trees caused by species of the *Botryosphaeriaceae*.

Plate 19. Bark necrosis (cankers) on chestnut trees caused by virulent strains (**A**) and hypovirulent strains (**B**) of *Cryphonectria parasitica*. Credits: Phytopathology WSL.

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Plate 20. Biological control of chestnut blight by inoculation of virulent cankers with hypovirus-infected *Cryphonectria parasitica* strains. Credits: Phytopathology WSL.
Plate 21. Development of the necrotic process on young *Cupressus sempervirens* stems 30 days (**A**), 60 days (**B**) and 90 days (**C**) after the artificial inoculation with *Seiridium cardinale*.

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Plate 22. Crown symptoms caused by *Seiridium cardinale* on *Cupressus sempervirens*.
Plate 23. Canopy dieback in *Pinus radiata* due to pitch canker. Each dead branch corresponds to at least one infection by *Gibberella circinata*.

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Plate 24. Discolored tissue corresponds to a lesion produced by *Gibberella circinata*, which was revealed by removing the bark at an infection site on a branch of *Pinus radiata*.
Plate 25. Stand of *Larix decidua* declining as a consequence of stems girdled by *Lachnellula willkommii*. Murau, Styria, Austria. Credits: Thomas L. Cech, BFW.

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Plate 26. Stand of *Larix decidua* sanitary thinned resulting in efficient reduction of larch canker. Neckenmarkt, Burgenland, Austria. Credits: Hannes Schönauer, BFW.
Plate 27. Stem breakage of a *Populus tremuloides* tree at a Hypoxylon canker.
Plate 28. Young Hypoxylon stem canker on *Populus tremuloides* that originated in a branch gall resulting from the oviposition of a poplar gall saperda (*Saperda inornata*).

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Plate 29. An aerial spray trial with copper fungicides against Dothistroma needle blight on *Pinus radiata* in Kaingaroa Forest, central North Island, New Zealand. Compartment 904B. (**A**) Unsprayed treatment and (**B**) single spray treatment (see van der Pas *et al*., 1984).
Plate 30. *Lithocarpus densiflorus* mortality caused by *Phytophthora ramorum* at Shell Beach, Tomales Bay State Park, Marin County, California. Credits: Doug Schmidt, Garbelotto Laboratory, UC Berkeley.

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Plate 31. An oak injected with phosphonates to control *Phytophthora ramorum*. Credits: Doug Schmidt, Garbelotto Laboratory, UC Berkeley.
Plate 32. Fungicide treatment against birch rust in a nursery. Credits: Erkki Oksanen, METLA.