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Infectious diseases in forest trees caused by viruses, mycoplasma-like organisms and primitive bacteria

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Key words. Viruses; mycoplasma-like organisms; Rickettsia-like bacteria; forest tree-diseases.

The spread of forest decline in Europe and other parts of the northern hemisphere in the last decade is associated with the complex interaction of a number of abiotic and biotic factors that lead to stress and to gradual or sudden degeneration of the trees. According to Manion[24] a decline syndrome may be caused by three or more sets of interchangeable factors of predisposing, inciting, and contributing categories interacting in a decline disease spiral (fig. 1).

Predisposing factors such as genetic potential, old age of the tree, and environmental conditions (climate change, wrong soil type or site conditions, poor fertility, air pollu-

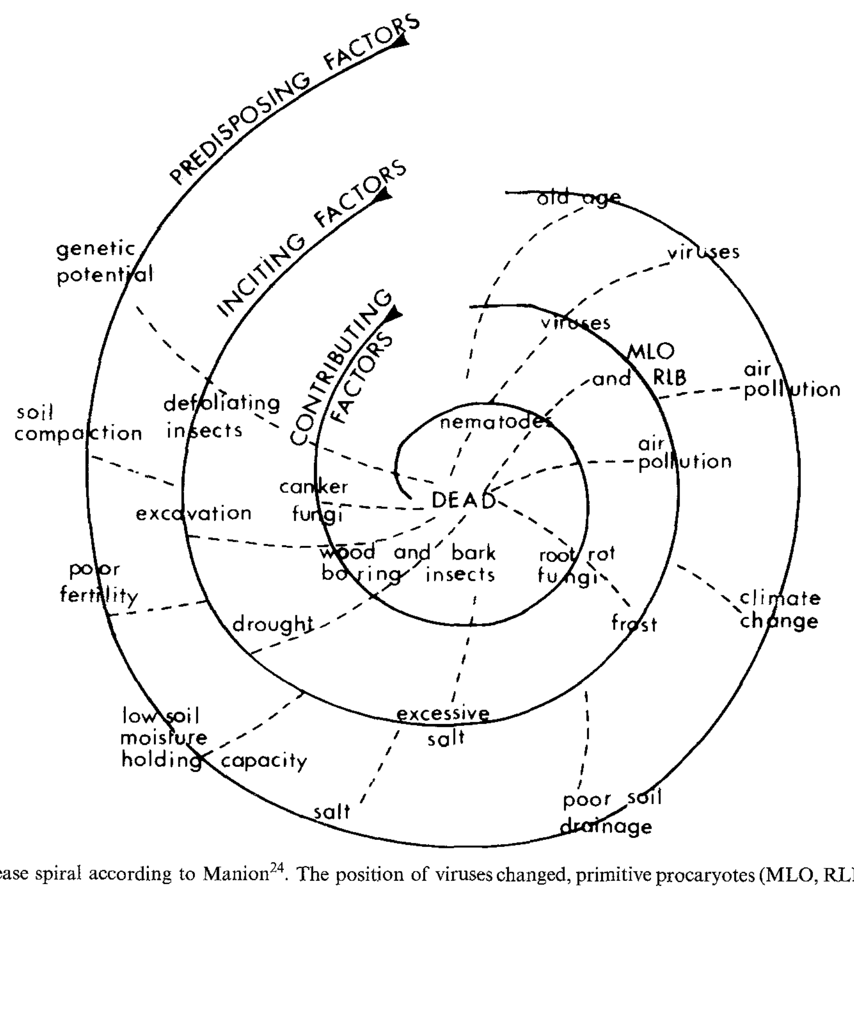

Figure 1. Decline disease spiral according to Manion[24]. The position of viruses changed, primitive procaryotes (MLO, RLB) added by the author.

tants leading to soil acidification and direct action on the leaves, low soil moisture or poor soil drainage, soil compaction) exert long term and permanent stress on the plant and predispose it to damage due to the action of other biotic factors.

Inciting factors of short duration produce a severe injury, especially on trees with reduced capacity for regeneration caused by predisposing stress. Incitants may be air pollutants, drought periods, frost, and defoliating insects or pathogens.

Contributing factors join the decline spiral and may finally lead to a serious degeneration or death of the weakened tree. In this category Manion puts bark beetles, root-rotting fungi, canker fungi, viruses, and mycoplasmas as indicators of weakened hosts.

In this contribution the role of viruses and primitive procaryotic organisms, such as mycoplasma-like organisms (MLO) and Rickettsia-like bacteria (RLB), not seriously considered by Manion, will be discussed in more detail in order to elucidate their contribution to the decline syndrome of forest trees.

Viruses are widespread pathogens in agricultural crops and weeds; the latter are considered important reservoirs of viruses. Therefore, it can be expected that they are also present in forest ecological systems. Viruses can only be replicated in living cells, because they lack their own metabolic activities. They can be soil- and airborne, and are transmitted from one plant to the other by mechanical means through wounds, arthropods, nematodes or fungal vectors, and a considerable number by seed and pollen. The international transfer of contaminated plant material is another important means of virus distribution.

The primitive procaryotes (MLO, RLB) are obligate par-

Virus and similar diseases of forest trees (MLO: Mycoplasma-like organisms, RLB: Rickettsia-like bacteria, EM: electron microscopy)

Symptoms	Cause	Demonstration/transmission	Location	References
Elm (*Ulmus* spp.)				
Mottling	Elm mottle virus	EM/mechanical, seed, pollen	Europe	20, 39
Mosaic	Cherry leafroll virus	EM/mechanical, seed, pollen	Europe, USA	11, 41
Mottle/mosaic	Tomato ringspot virus	mechanical	USA	45
Chlor./necrotic striping	Potyvirus group	EM	Europe	42
Phloem necrosis	MLO	EM/leaf hoppers	USA	12, 46
Leaf scorch	RLB	EM	USA	14
Witches'broom	MLO	EM/grafting	Europe	6
Ash (*Fraxinus* spp.)				
Degeneration	Tobacco ringspot virus	EM/mechanical, nematodes	USA	15, 17, 18
Mosaic	Tobacco mosaic virus	EM/mechanical	USA	23
Chlor./necrotic flecking or leaf curl	Tobacco necrosis virus and unknown viruses	EM/mechanical, grafting	Europe	7,9
Birch (*Betula* spp.)				
Mottling, decline	Cherry leafroll virus	EM/mechanical, seed, pollen	Europe	10, 40
Mottling, little leaf	Thread-like and/or spherical particles (ringspot type)	EM/mechanical	FRG	Nienhaus (unpublished)
Poplar (*Populus* spp.)				
Mosaic	Carlavirus group	EM/mechanical, aphids	Europe	3, 38
Decline	Potyvirus group	EM/mechanical	USA	26
Degeneration	Tobacco necrosis virus	EM/mechanical	USA	16
Oak (*Quercus* spp.)				
Mottling, spotting, mosaic, or leaf deformation	Tobacco mosaic virus	EM/mechanical	FRG, GDR Hungary	19, 29, 42
Latent or mottling	Tobacco mosaic virus	EM/oak powdery mildew	USA	28, 34
Spotting, mottling	?	grafting, aphids	ČSSR	4, 5
Ringspotting, decline	Thread-like particles	EM/grafting	USA	1, 21
Ringspotting	Spherical particles	EM	FRG	Nienhaus (unpublished) 29
Leaf scorch	RLB	EM	USA	14
Beech (*Fagus sylvatica*)				
Mottling, spotting	Tomato black ring virus	EM, mechanical	UK	cit. 42
Degeneration, little leaf, mottle	Potyvirus group or spherical particles	EM, mechanical	FRG	Nienhaus (unpublished)
Bark necrosis	MLO	EM	FRG	35
Larch (*Larix* spp.)				
Witches'broom, degeneration	RLB	EM/soil-borne	FRG	30, 31
Degeneration	MLO	EM	FRG	Nienhaus (unpublished)
Spruce (*Picea* spp.)				
Growth asymmetry, needle fall	Rod-shaped particles	EM/aphids	ČSSR	4, 8
Discoloration, needle fall	Rod-shaped particles	EM	UK, FRG	2, Frenzel (pers. comm.)
Pine (*Pinus* spp.)				
Mottling, needle fall	Rod-shaped particles	EM	Europe	1, 38

asites, generally invading the vascular system of the plant (MLO restricted to the phloem tissue, RLB mainly to the phloem or xylem). Like viruses they are transmissible by insect vectors. Both viruses and these organisms can be the cause of severe diseases of nonwoody and woody plants. Thousands of virus diseases, 250 diseases associated with MLO, and at least 20 diseases associated with RLB have been described worldwide. For further information see selected literature[13, 22, 25, 27, 33, 36, 44].

A considerable number of viruses and some MLO have caused epidemics in agricultural crops when environmental conditions, host range and other factors were favorable for the parasites and their vectors[36]. A typical example is the quick decline of citrus crops caused by citrus tristeza virus and the elimination of millions of orange trees in all citrus producing areas: 7 million trees within 10 years in Brasilia, 10 million within 20 years in Argentina. Once introduced the pathogen quickly spreads by its vector to susceptible trees growing on hypersensitively reacting root stocks. Only one other example will be mentioned; the decline of coconut palms in the Caribbean and in Africa. Again, millions of palms died within a few decades. We observed a complete degeneration of bearing palms, and a dieback of 2–4-year-old palms within few months[32, 43].

One is tempted to speculate that epidemics of a similar kind may also be an important incitant of our forest decline. Controversial public discussions force research workers to make critical analyses: are viruses and/or primitive organisms (MLO, RLB) inciting factors of forest decline?

Information about viruses and MLO- and RLB-diseases of forest trees is rather rare. One reason may be that, until recently, the interest of research in forest pathology was traditionally focused on insect damage and fungal diseases. A second reason is the technical difficulty of demonstrating, isolating and transmitting viruses and obligate prokaryotic pathogens in woody plants. And, last but not least, the economic importance of the loss of a relatively small number of forest trees has been considered as negligible compared with agricultural crops such as fruit trees. Now we are confronted with widespread decline in our forests, research has been initiated in order to elucidate the importance of parasitic effects in forest trees. The table summarizes observations about diseases associated with viruses, MLO and RLB in a number of species.

Elm diseases (*Ulmus* spp.) have been described since 1899 in North America. A phloem necrosis leads to yellows disease and decline of different elm species. A widespread dying of trees was reported from Kentucky, Indiana and Illinois during the late 1800s. Thousands of trees died in Ohio and other midwestern states during the 1930s and 1940s. The disease was still increasing during the 1970s in the eastern half of the United States. It is caused by MLO which are transmitted by the leaf hopper *Scaphoideus luteolus*[12, 46]. A regional restricted leaf scorch associated with RLB was observed in Washington DC[14]. A mosaic symptom and decline in North America can be caused by a strain of the cherry leafroll virus (nepovirus group) transmissible by seed and pollen[11]. In Europe another seed and pollen transmissible virus, the elm mottle virus, which has been transmitted experimentally to 66 herbaceous plant species, and causes mottle symptoms in *Ulmus glabra, U. carpinifolia* and *U. vulgaris*, has been found in Germany and Scotland[39]. A chlorotic/necrotic stripe symptom of *U. glabra* was associated with a virus of the potyvirus group in Czechoslovakia and the German Democratic Republic[42]. A witches' broom probably due to MLO infection was observed on *U. carpinifolia* in Czechoslovakia[6].

Ash trees (*Fraxinus* spp.) degenerated by infection of a strain of tobacco ringspot virus (nepovirus group) in North America. It is transmissible by nematodes[15, 17, 18]. Another disease with mosaic and ringspotting can be caused by a strain of tobacco mosaic virus described in the USA[23]. Different graft-transmissible diseases of ash trees have been found in Europe causing different types of leaf discoloration and necrosis. One of these viruses was identified as tobacco necrosis virus[7, 9, 37].

Birch trees (*Betula* spp.) suffer from infection by the cherry leafroll virus in England and Central Europe (*Betula pendula, B. verrucosa*). The virus is seed and pollen transmissible[10, 40]. Leaf discoloration and little leaf (fig. 2a) are associated with thread-like or, sometimes, with spherical virus particles in the Rhineland area. The first may belong to the potex- or potyvirus group (fig. 3).

Poplar trees (*Populus* spp.). Decline in the USA is caused by a virus belonging to the potyvirus group. It could be transmitted by mechanical means[26]. In deteriorating clones of aspen, tobacco necrosis virus was identified in USA[16]. A mosaic in Europe can be induced by flexible thread-like particles belonging to the carlavirus group transmissible by aphids and mechanical means[42].

Oak trees (*Quercus* spp.) are infected by strains of tobacco mosaic virus (TMV). They could be transmitted mechanically in Germany[29], or by the oak powdery mildew *Sphaerotheca lanestris* in California[28, 34, 47]. The infection was found to be associated with mottling, mosaic and leaf malformation, or often latent. TMV particles were also observed by electron microscopy in the German Democratic Republic[42] and in Hungary[19]. Similar diseases have been reported from Czechoslovakia which were seed, aphid and graft transmissible[5]. A disease with chlorotic ringspot symptoms, veinbanding and die-back of branches in *Q. marilandica* and *Q. velutina* could be transmitted by grafting. The virus has been described as flexuous, thread-like particles observed by electron microscopy[1, 21]. In the Rhineland area a characteristic ringspotting of *Q. sessiliflora* was found to be associated with spherical particles, but could not be transmitted by mechanical means, so far[29] (Nienhaus 1978, unpublished; fig. 2b). A leaf scorch of oak trees found in USA was associated with RLB[14].

In beech trees (*Fagus sylvatica*) with mottling and spotting, tomato black ring virus was isolated in the United Kingdom by Cadman in 1961[42]. This virus belongs to the nepovirus group transmissible by nematodes. Recently we found a widespread disease of old beech trees in the Rhineland area showing little leaf and chlorosis followed by necrosis and dieback of branches (fig. 2c). 95% of the leaf samples were contaminated with flexuous virus particles belonging, most probably, to the potyvirus group (fig. 3b). The virus could be transmitted to herbaceous plants such as *Chenopodium quinoa* and *Nicotiana benthamiana*. In some sites of the same area another virus was isolated from degenerating beech trees which caused

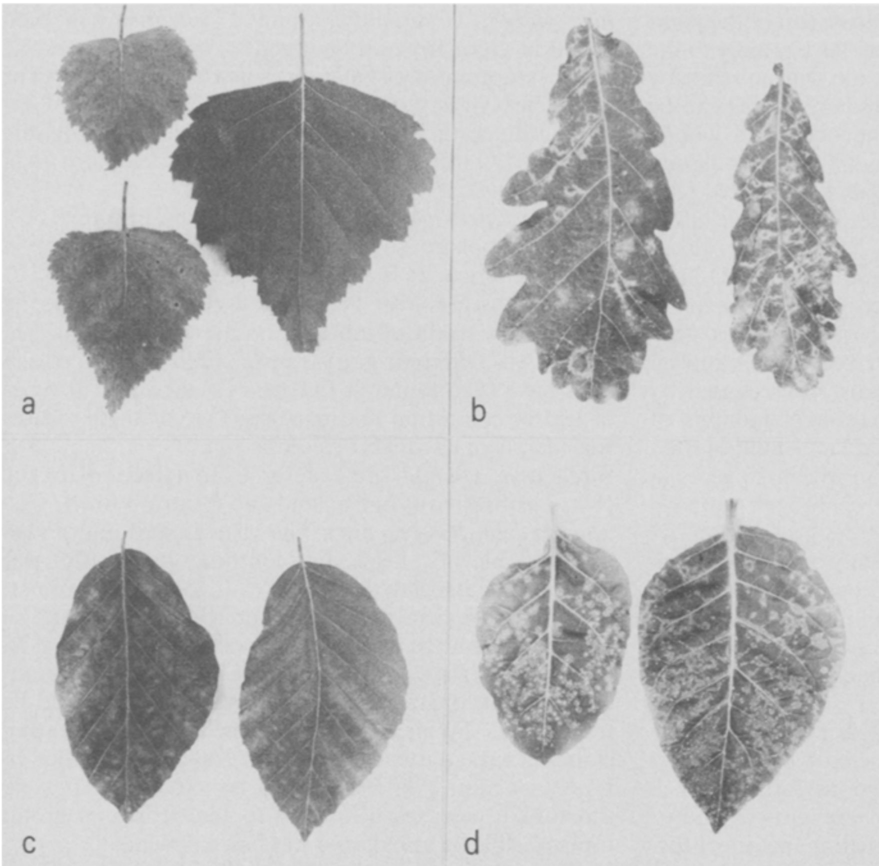

Figure 2. Leaf symptoms associated with virus infections. *a* Little leaf and leaf chlorosis of birch trees (left), leaf of a healthy tree (right); *b* ringspotting of oak trees; *c* chlorotic mottle of beech trees; *d* ringspotting of tobacco plants caused by virus extracted from beech trees (left: inoculated leaf, right: symptoms by systemic infection).

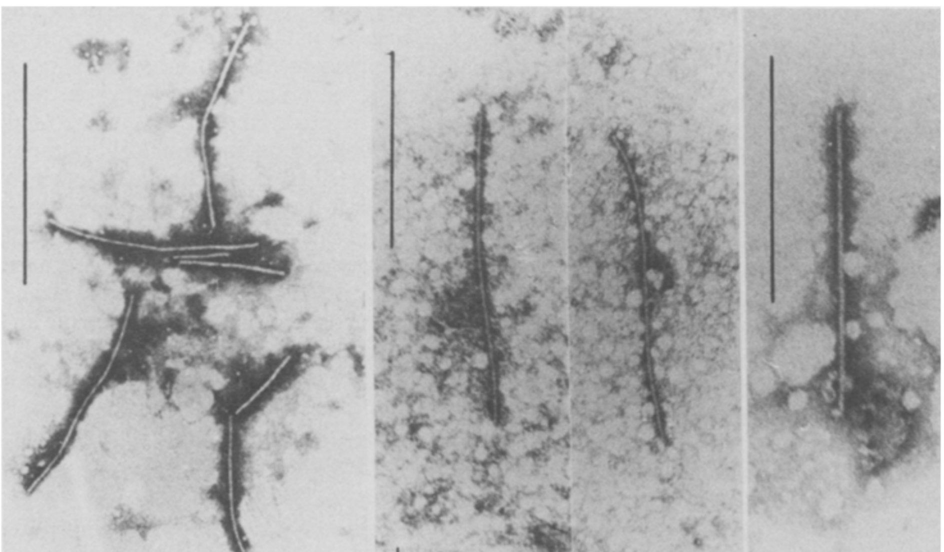

Figure 3. Electron microscopical demonstration (negative staining) of virus particles in extracts of virus diseased forest trees. *a* Flexuous threads of 550 nm normal length (potexvirus group; birch and beech trees); *b* flexuous threads of 780 nm normal length (potyvirus group; birch, beech and spruce trees); *c* rod-shaped particle of 600 nm normal length (unknown group; pine tree). Bar 500 nm.

ringspot symptoms in *Nicotiana tabacum* (fig. 2d). In other parts of Germany Parameswaran (1980) demonstrated MLO structures in beech trees with bark necrosis, by electron microscopy[35].

In conifers reports on virus infections or MLO and RLB contamination are rare. The demonstration and isolation of such pathogens is more difficult than with those in broad-leaved trees because of tannins and other cell compounds.

Larch trees (*Larix* spp.) degenerate with witches' broom symptoms in different sites in West Germany owing to infection by Rickettsia-like bacteria (RLB) (figs 4, 5). The disease does not occur in regions higher than 500 m above sea level where climatic conditions are unfavorable for the pathogen. It seems to be soil-borne transmissible[30, 31].

We also found mycoplasma-like organisms (MLO) in a few young declining larch trees from a nursery in northern Germany in 1982 (fig. 6).

Figure 4. 50-year-old larch tree with witches' broom symptoms (left) and healthy tree (right).

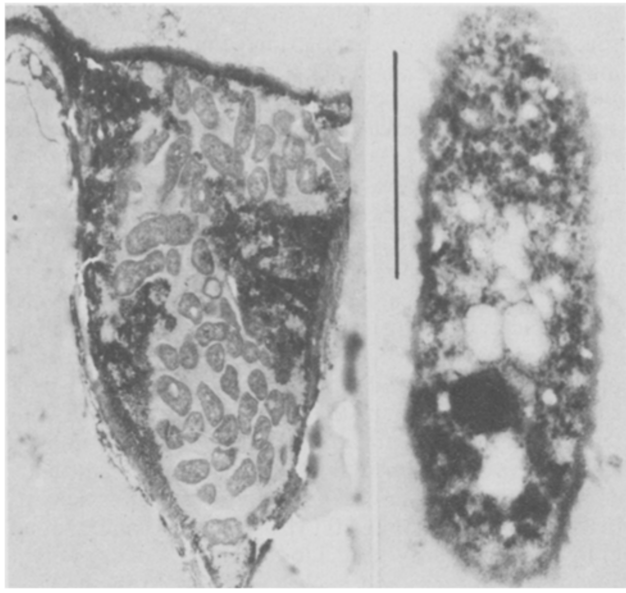

Figure 5. Rickettsia-like bacteria in a phloem cell of a witches' broom diseased larch tree (left), Rickettsia-like bacterium with a typical ridged cell wall (right). Bar 500 nm.

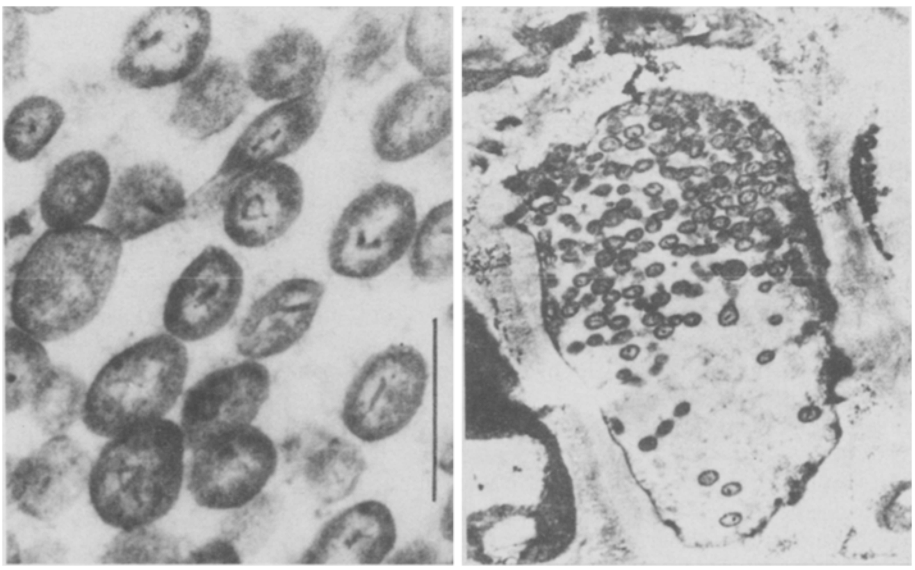

Figure 6. Mycoplasma-like organisms in a phloem cell of degenerating young larch trees (left), higher magnification of the pleomorphic organisms without cell wall (right). Bar 500 nm.

In spruce trees (*Picea* spp.) which, besides *Abies alba*, are suffering severely in many parts of Europe, virus-like particles of different lenghts and diameters could be demonstrated by electron microscopy (United Kingdom[2], Czechoslovakia[4,8], Federal Republic of Germany, Black Forest: Frenzel, pers. communication). The decline of spruce with needle discoloration and growth abnormalities reported from Czechoslovakia could be transmitted by grafting and by aphids. Recently we found similar particles in a young spruce tree with heavy chlorosis in Bavaria. According to morphological characteristics they may belong to the potyvirus group (fig. 3b).

With pine trees (*Pinus* spp.) there are a few reports on virus-like particles demonstrated by electron microscopy. They were rod-shaped, 300 and 600 nm in length, and found in the United Kingdom, the German Democratic Republic and southern Europe[2,42]. In a few samples of degenerating pine trees *(Pinus silvestris)* in the Rhineland area we found some rod-shaped particles of 600 nm in length (fig. 3c).

Results from soil and root samples in spring and summer 1984 in our laboratory indicate that, at least in several sites of North-Rhine-Westfalia, different species of forest trees are contaminated with a number of different viruses not yet determined. In soils under beech, oak and coniferous trees a heavy contamination of nematodes belonging to the genus *Longidorus* has been detected (Sikora, Büttner, Nienhaus, unpublished). Species of this genus are well known vectors of nepoviruses in agricultural crops.

Summarizing these observations it can be stated that a number of viruses, and in some cases also mycoplasma-like organisms, and Rickettsia-like bacteria (MLO, RLB) are inciting factors for decline of forest trees. According to most observations, however, I rate viruses as predisposing factors leading to early senescence of trees. Senescence reduces the regeneration capacity of the host plants, and the juvenile metabolic vigor is lost. Under abiotic stress conditions the infected trees have less potential for recovery from inciting factors than non-infected trees. Experimental research with annual plants demonstrates for instance that with tobacco mosaic virus in tobacco plants, about 10^{12} virus particles can be found in 1 g of tobacco leaves, i.e. more than 10% of dry weight are foreign proteins and nucleic acids coded by the viral genome, not available for plant metabolism. Virus infection leads to reduction of the photosynthetic system, and to disturbances in the transporting system, in the storage parenchyma, and in absorbing roots. Similar changes can be induced by many other biotic and abiotic stress factors that are generally unspecific. They result in degeneration of roots and mycorrhizae, reduction in stored food reserves, reduced growth, premature yellowing of the leaves and needles, reduced size of foliage, dying branches and other symptoms of degeneration.

Manion's concept (1981) of describing viruses as contributing factors within the same category as bark beetles and root rot fungi attacking weakened trees is questionable. Viruses and obligate primitive microorganisms need living tissue with high metabolic capacities for optimal multiplication. Once established they lead to early senescence, predisposing plants for all types of stress factors. Our recent knowledge urgently requires the following measures:

- drastic reduction of environmental stress factors, such as air pollutants as the main decline-inducing factors,
- improvement of planting sites by measures discussed in several other papers,
- selection of tree species and varieties to be replanted on suitable sites,
- use of high quality of seed and individual young plants of specific origin for replanting purposes.

We have to live with viruses and microorganisms. Most probably they have more or less slowly invaded our forests during hundreds of years without causing serious damage by their own, except in a few regionally restricted cases. There is no indication that they are the actual cause of the sudden epidemic-like spread of the new decline of our forests. We have to realize, however, that they play their role as an important predisposing factor in the decline disease spiral.

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Short Communications

Electron diffraction of the metallic elements in the pineal organ of a freshwater fish, *Mystus vittatus* (Bloch.)

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Summary. By electron diffraction pattern the presence of metallic elements, particularly chromium-nickel, chromium phosphide, copper, aluminum-copper and zinc has been shown in the pineal organ of a freshwater teleost, *M. vittatus*. It is likely that their occurrence within the pineal is due to binding with the neurosecretory material fractions/ligands.
Key words. Mystus vittatus; pineal organ; electron diffraction; metallic elements.

The ultrastructure of the teleostean pineal organ has been extensively studied, but the existence of metallic components in the pineal organ of fish has not been investigated so far. There are, however, reports of several investigations where metallic elements were discovered in mammalian pineals [2–8], which motivated the present study.

Over 100 adult *M. vittatus*, which were procured from local ponds at and around Gyanpur (India) were sacrificed in the present study. The pineal organ was carefully taken out with the help of the binocular dissecting microscope (× 20). A 2% glutaraldehyde and 1.3% paraformaldehyde solution[9] in 0.1 M cacodylate buffer (pH 7.2) was used as fixative at 4°C. Quickly-dissected pineal organs were left in fixative for 1.5 h then washed in 0.1 M cacodylate buffer containing 7% sucrose solution. All pineal specimens were post fixed in ice-cold 0.5% osmium tetraoxide solution in the same buffer for 2 h. The fixed pineal was rinsed in four changes of ice-cold double distilled water for 1 h and dehydrated in a graded series of ethanols. After passing through acetone, the whole pineal sac was used as a thin flake and examined with a Philips EM-200 Electron Microscope under transmission by employing the diffraction contrast images (DCI) and selected area diffraction (SAD) modes. Detailed investigation leading to identification of crystalline phases was done by calculating the d-spacing of the diffraction phases through a camera constant by formula: $d = \frac{\text{camera constant}}{r}$ (where d and r denote interplaner spacing and distance of the diffraction lines or spots from the center of transmitted beam position respectively). This constant was obtained by employing spec pure silver (99.999%) thin film as the standard material. The structural data from the ASTM index card were collected and compared against the data calculated from the diffraction experiments. The interplanar d-spacings were also checked and compared.

The DCI mode often revealed the presence of agglomerate which appeared to have a crystalline metallic character. A representative example of this is shown in figure 1. The SAD mode was then utilized to obtain a diffraction pattern (DP) of these agglomerates, which invariably revealed the presence of crystalline components represented by sharp Bragg diffraction rings and/or spots. DP from the randomly oriented tiny agglomerates gave the ring pattern (figs 2–5) and regions of large agglomerates (fig. 6) exhibited a single crystal (see figs 7 and 8); the figures show typical examples. The most dominant metallic elements found in the fish pineal were chromium-nickel and chromium-phosphide. The next most often encountered element corresponded to copper. In other cases, as well as these metallic elements others were found, which were identified as