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Übersichtsartikel zu einem bedeutenden Virus in Laubgehölzen – Kirschenblattrollvirus: Auftreten, Übertragung und Diagnose

A review of an important virus of deciduous trees – cherry leaf roll virus: occurrence, transmission and diagnosis

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Zusammenfassung

Das Kirschenblattrollvirus (cherry leafroll nepovirus, CLRV) ist in Laubgehölzen – und häufig beobachtet im Forst – weit verbreitet und verursacht schwere Schäden an verschiedenen Baumarten. Die Übertragung durch Nematoden, Samen und Pollen sowie die weite Verbreitung machen diesen viralen Erreger zu einem bedeutenden Pathogen. Viruserkrankungen an Forstgehölzen sind bisher wenig exakt untersucht und dokumentiert. Es besteht von daher ein großer Informationsbedarf für Forstleute und Baumschuler, über diese Gefahr zu informieren. Mit diesem Artikel soll eine Zusammenfassung der bisherigen Forschungsergebnisse zur Verbreitung, Übertragung und Diagnose des Kirschenblattrollvirus in Laubgehölzen gegeben werden. Probleme mit virusinfiziertem Pflanzenmaterial, die in Genbanken, Baumschulen, öffentlichem Grün und dem Forst auftreten können, werden diskutiert. Die frühe Diagnose ist wichtig um der Ausbreitung der Erkrankung vorzubeugen und somit das ökonomische Potential und die Vitalität des Bestandes zu erhalten. Aufforstungen und sonstige Neupflanzungen sollten mit CLRV-freien Sämlingen vorgenommen werden.

Stichwörter: Kirschenblattrollvirus, Laubgehölze, Auftreten, Übertragung, Diagnose.

Summary

Cherry leaf roll nepovirus (CLRV) is wide-spread in various deciduous trees – and often observed in forested areas. The virus causes severe damage in several tree species. Its transmission by nematodes, seed and pollen as well as the frequency of its occurrence makes the virus an important pathogen. Virus diseases of forest trees are rarely investigated and documented. There is a definite requirement to inform forest rangers and nurseryman of this danger. This paper summarizes investigations on the occurrence, transmission and diagnosis of cherry leaf roll nepovirus in deciduous trees. Problems with virus-infected plant material that might develop in gene banks, nurseries, public gardens and forest stands are discussed. The early detection of CLRV is important to prevent the spread of the disease and to preserve the potential economic value and vitality of forest stands. New stands have to be established with CLRV-free seedlings.

Key words: cherry leaf roll virus, deciduous trees, occurrence, transmission, diagnosis.

Symptoms and occurrence in deciduous forest trees

CLRV is known to infect a wide host range of deciduous forest trees and shrubs including species of *Betula, Fagus, Fraxinus, Juglans, Ulmus*, as well as *Prunus avium, Rhamnus alnus* and *Sambucus nigra* (BANDTE and BÜTTNER, 2000)(Fig. 1 a-f). Due to different symptoms shown by the host plants, a variety of names for the disease exist (SCHMELZER, 1977).

A first report was given by SCHUSTER and MILLER (1933), who described in particular the symptoms of walnut blackline disease, now known to be induced by CLRV. CLRV-infected birch trees showing yellow vein netting, chlorotic ringspots and mottling were observed in Germany (SCHMELZER 1972 a) and the United Kingdom (COOPER and ATKINSON, 1975) (Fig. 1a). MAYHEW and EPSTEIN (1971) as well as FORD et al. (1972) reported on the discovery and characterization of the virus infecting *Ulmus americana* in the United States of America, where the pathogen induces chlorotic ringspots and mosaic and often leads to dieback. In the United Kingdom (COOPER, 1980), in Bulgaria (LAZAROVA-TOPCHIISKA, 1990) and in the United States of America (MIRCETICH et al., 1980) CLRV-infected walnut develops a chlorotic leaf pattern and blackline. Leaf rolling and death were observed with infected *Prunus avium* (CROPLEY, 1961). The pathogen was recovered from declining beech with chlorotic leaf mottling and spotting in Germany (WINTER and NIENHAUS, 1990)(Fig. 1b). Also, POLAK (1995) found the virus to be associated with the syndrome of decline of European beech in the Czech Republic. NIENHAUS and HAMACHER (1989) transmitted a CLRV isolate to white ash seedlings, which developed chlorotic spots, ringspots and line patterns similar to foliar symptoms on diseased European ash in Europe (SCHMELZER et al., 1966; NIENHAUS and CASTELLO, 1989)(Fig. 1c). The yellow net on leaves of *Sambucus nigra* was observed by MILICIC et al. (1987) in Yugoslavia, where transmission of the pathogen to test plants and serological investigations confirmed CLRV to be the causal agent of the disease (Fig. 1d). WERNER et al. (1997 a) detected CLRV in diseased *Rhamnus frangula* from forest stands and nurseries in northern Germany (Fig. 1e).

FÜHRLING and BÜTTNER (1997) found cherry leaf roll virus widely spread in nurseries, public gardens and forest stands in northern Germany. They often detected the pathogen in *Betula* spp., *Rhamnus frangula, Sambucus nigra* and *Fraxinus excelsior*. In the United Kingdom CLRV was found in about 18% of the plant material sampled in amenity birch trees (COOPER and MASSALSKI, 1984). Similar results were obtained by GRÜNTZIG et al. (1996) who made a survey in nurseries and forest stands in eastern Germany (Saxony and Lower Saxony). The authors found about 14% of the 1045 investigated birch trees to be infected by the pathogen. These infection rates ranged from 7% in nurseries to 26% in public gardens and avenue trees. It is obvious that most diseased trees concentrate and form infection foci within stands. Rarely are single infected trees distributed regularly in the stand, thus allowing the percentage of infection to be determined. According to our experience 60 to 80 % of the trees in such foci can be infected.

Transmission

Mechanical transmission, grafting and contact

CLRV can be transmitted by mechanical inoculation and by grafting (JONES, 1986). SCHMELZER (1966) proved CLRV isolated from *Sambucus racemosa* to be pathogenic to 62 species in 24 families. The author found most of the virus-susceptible plants in the families *Chenopodiaceae, Compositae* and *Solanaceae*. Plants known to be virophilous, such as *Amaranthus caudatus, Datura stramonium* and *Lycopersicum esculentum*, were shown to be resistant to the pathogen. HORVATH (1979) added 34 other plant species susceptible to the virus and 23 plants of eight families which seemed to be resistant to the pathogen.

Several authors studied mechanical transmission of CLRV to forest trees (COOPER and ATKINSON, 1975; NIENHAUS et al., 1990; HAMACHER and QUADT, 1994). NIENHAUS et al. (1990) described an artificial infection method using a polycation to aid the attachment of virus particles to leaf surfaces and the penetration of the virus into leaf cells. Back transmission trials using stem-slashing as an inoculation tool proved CLRV to be the causal agent for chlorotic leaf mottling and spotting of European beech (*Fagus sylvatica* L.) (HAMACHER and QUADT, 1994). COOPER and ATKINSON (1975) observed symptoms on birch seedlings not

before one year or more after mechanical inoculation of CLRV. Despite the possibly long latent period, graft-transmissibility of the virus may be used for diagnostic purposes. All viruses are experimentally transmissible via grafts, if the virus-infected budwood grows together with the healthy tissue. Hypersensitive reactions or tissue incompatibilities are of diagnostic value, as shown by MIRCETICH et al. (1980) when investigating CLRV-infected walnut.

According to JONES (1986) there are no reports on the transmission of the virus between plants by direct plant to plant contact. Up to now there are no further studies on this matter.

Transmission by seed and pollen

MINK (1993) supposed that the interaction of three factors is required to spread the virus in the field via pollen. The factors are virus-contaminated pollen grains, insects or wind to move virus contaminated pollen to flowers of healthy trees and pollen-feeding arthropods in the pollen receptor flowers to create wounds needed for the mechanical transmission to non-gametophytic tissue. CLRV is reported to be spread through seed and pollen (BENNETT, 1969; MINK, 1993; JOHANSEN et al., 1994), but the mechanisms of dispersion through pollen remain unclear. CLRV adheres to the surface of pollen, slightly to anemophilous pollen such as birch or walnut and strongly to the surface of cherry pollen, which is entomophilous (MASSALSKI and COOPER, 1984). Furthermore, CLRV can be introduced into the embryo, multiplied within the embryo and distributed through seeds from infected birch trees (COOPER et al., 1984).

COOPER (1976) showed that healthy birch mother trees were not infected when their flowers were pollinated with CLRV-contaminated pollen. Nevertheless, some of the seeds which were produced on these trees were infected. Perhaps symptom development induced by specific CLRV-strains is related to the reproductive phase of the virus-infected plant. As described by MIRCETICH et al. (1980), walnut blackline disease induced by CLRV did not appear in young plantings until the trees began to flower. The authors concluded that the disease incidence was related to precocity of the cultivars.

COOPER et al. (1984) observed increased seed abortion in birch infected with CLRV. The measured proportion of germinating seed was highest when both parents were CLRV-free and an infection of either or both parents led to a decreased germination of the seeds. Following controlled crosses, the lowest germination rate occurred when the female parent was CLRV-infected. The authors found about 17% seed transmission of the virus when only females were infected and about 30% when only males were infected. WALKEY et al. (1985) supposed that the lower rate of germination of seeds of virus-infected plants may be caused by disease effects on the mother plant rather than the presence of the virus in the seed.

Seed transmission depends on the virus-host combination as demonstrated by SCHIMANSKI (1987). With CLRV the seed transmission-rate seems to vary depending on the CLRV isolate, the herbaceous host plant and the seed storage temperature. QUACQUARELLI and SAVINO (1977) showed that the frequency of CLRV transmission through walnut seeds varied with the storage temperature. The infection rate increased from 4% at 2 - 5 °C up to 32 % at room temperature.

CLRV has been proven to be seed transmissible in black cherry (*Prunus serotina* Ehrh.) to a low extent (0.5% to 0.8%) (SCHIMANSKI et al., 1976). In *Sambucus* however, SCHIMANSKI and SCHMELZER (1972) showed that from 8% up to 44% of the seedlings from seeds of CLRV-infected *Sambucus racemosa* L. were infected. The highest seed transmission-rate (100%) was found in the herbaceous host *Glycine max* L. (LISTER and MURANT, 1967). Furthermore, CLRV was shown to be seed transmissible in *Rheum rhaponticum* L., *Chenopodium amaranticolor* Coste et Reyn (TOMLINSON and WALKEY, 1967), *Nicotiana clevelandii*, *N. megalosiphon* (HANSEN and STACE-SMITH, 1971) and *Chenopodium quinoa* (LARSEN et al., 1990).

Although the pollen was infective there was no evidence of seed transmission of the elm-

strain of CLRV in *Chenopodium quinoa* (FORD et al., 1972). The authors tested more than 5.000 *C. quinoa* seedlings grown from plants with systemic CLRV-infection.

Seed transmission is important in regard to virus disease epidemiology as it provides a mean for virus dispersal over time and distance. Regarding horizontal transmission, the infected seed may provide initial infection sites for vector dispersal of the virus or the infection of other species. COOPER (1988) discussed the transmission of CLRV-strains to different tree species by mechanical rubbing of leaves loaded with pollen and penetration through wounds or by sucking insects. This possible interspecific distribution may explain the close relationship between the CLRV-strains isolated from birch, beech and cherry. Investigations on vertical transmission of CLRV were carried out by COOPER et al. (1984), who showed that 3% of the seedlings in the field were CLRV-infected. The samples were taken up to 3 m outside the drop zone from infected birch mother trees.

MANDAHAR and GILL (1984) rated CLRV as an epidemiologically important pollen-transmitted virus as the pathogen is spread horizontally by pollen from infected plants to healthy mother plants in orchards. These plants get back-infected during pollination and fertilization. Therefore these internally pollen-borne viruses may spread very rapidly not only in orchards but also in forest stands.

Nematode and insect vectors

Field studies showed that nematode vectors often contaminate soils in commercial nurseries (SWEET, 1974; SWEET and COOPER, 1976). CLRV is discussed as being transmissible by soil-inhabiting nematodes (JONES, 1986). Using modern diagnosis techniques, the transmissibility found in former days (FRITZSCHE and KEGLER, 1964) could not be confirmed.

FRITZSCHE and KEGLER (1964) recorded *Xiphinema coxi, X. diversicaudatum* and *X. vuittenezi* as vectors to the cherry isolates of CLRV, whereas X. *americanum* was shown not to transmit the elm mosaic strain of CLRV (FULTON and FULTON, 1970).

In the 80ies the transmissibility of CLRV by nematodes could not be confirmed, as shown by experiments carried out by COOPER and EDWARDS (1980) and JONES et al. (1981). COOPER and EDWARDS (1980) treated the soil at two of five nurseries growing CLRV-infected walnut seedlings with a nematicide immediately before planting. As neither trichodorids nor longidorids were detected in these soils, the authors concluded that nematodes did not facilitate the spread of CLRV between walnut transplants. These results were confirmed by JONES et al. (1981), who tested the ability of 10 nematode species to transmit CLRV. The authors assessed the recovery of CLRV by direct root assays in a few bait plants exposed to *Longidorus elongatus, L.macrosoma, Xiphinema diversicaudatum, X. vuittenezi* or *Longidorus leptocephalus* due to contamination. CLRV could not be detected when the virus source plants were removed before the bait plants were cultivated in the same pot. Nematodes extracted from the soil after access to virus infected plants did not induce a viral infection of the indicator plants.

In laboratory tests different North American *Xiphinema* populations were screened for their ability to transmit selected nepoviruses such as cherry rasp leaf virus (CRLV), tobacco ringspot virus (TRSV) and tomato ringspot virus (ToRSV). Although there is a narrow specifity of the transmission between European nepoviruses and their vector species, three tested viruses could be transmitted by individuals of the *Xiphinema* species (BROWN et al., 1994). Nevertheless, there are several reports describing the dependence of transmission on the virus strain, vector population and plant species. ALLEN et al. (1984) measured the transmission frequency of *Longidorus diadecturus* and *Xiphinema americanum* when transmitting orchard-acquired peach rosette mosaic virus (PRMV) to cucumber plants over three years. *X. americanum* infected about 22% of the test plants and *L. diadecturus* about 53%. No data were obtained on the transmissibility to woody seedlings such as peach trees. In laboratory studies JONES et

al. (1994) showed that raspberry ringspot nepovirus (RRV-P) may be transmitted by *Paralongidorus maximus* at a very low level between grapevines, whereas no transmission could be observed to or within herbaceous hosts.

Further studies on the possible role of nematodes as vectors in the transmission of CLRV to other host plants and its distribution in nurseries, forest stands and other managed populations are certainly required. As shown by YUAN et al. (1990) a nematode may be virus-contaminated but fail in virus transmission. So *Criconemella xenoplax* picked from the root zone of PNRV-infected peach trees were a carrier of the virus, but further studies showed that the nematode failed to transmit the pathogen to cucumber or peach seedlings.

A transmissibility of CLRV by insects is not known. Elm mosaic virus, the elm-strain of CLRV, was proved non transmissible by the green peach aphid, *Myzus persicae* (FORD et al., 1972). The involvement of the bug *Cleidocerys resedae* in the transmission of CLRV is discussed (WERNER et al., 1997 b). The authors detected the virus in the bugs, but transmission trails to seedlings are still missing.

Virus distribution in the tree

To gain reliable results on detecting CLRV in woody plants, it is imperative to know about the distribution of the virus within the plant. Material has to be collected in such a way that, in the case of an infected tree, the sample definitely contains virus-infected tissue.

Studies in commercial walnut orchards showed that CLRV is erratically but uniformly distributed in trees (MIRCETICH et al., 1985). Concerning the distribution of viruses in woody host plants, FUCHS and GRÜNTZIG (1994) classified viruses in 3 groups: those with a systemic, a partly systemic or a sporadic distribution. CLRV is characterized by a sporadic distribution. By testing dormant buds and naturally-forced buds of bird cherry *Cerasus avium* (L. MOENCH) and birch trees it could be confirmed that many branches of diseased trees remain virus-free. The mechanism of the differences in the behaviour of distribution of the viruses remains unclear. The influence of the host plant is supposed to be subordinate (FUCHS and GRÜNTZIG, 1994). In contrast, DELBOS et al. (1984) reported on interspecific differences in the virus distribution following graft transmission of the pathogen. Grafting *Juglans regia* on CLRV-infected *J. nigra* led to a heterogenous virus distribution. But homogenous distribution was observed by grafting *J. regia* on diseased *J. regia*. These investigations were supplemented by BAUMGARTNEROVA and SLOVAKOVA (1995) working on the detection of CLRV in walnut flowers and leaves. The virus concentration varied due to the heterogenous virus distribution and was higher in flowers than in diseased leaf tissue.

QUADT-HALLMANN et al. (1996) increased our knowledge through studies on the localization of CLRV in leaves of herbaceous host plants and birch and cherry trees. In woody hosts the detection of CLRV was generally confined to chlorotic spots and line patterns. Virus distribution in herbaceous hosts varied depending on the host species. *Nicotiana tabacum* var. Xanthi-nc developed distribution patterns similar to those of woody hosts. The virus was also present in green areas of *Chenopodium quinoa* WILLD. Analysing the distribution pattern of CLRV in artificially infected tobacco plants, MAS and PALLAS (1996) detected viral RNA before the symptoms appeared. As the virus did not replicate in fully matured leaves, systemic infections could only be detected in young leaves inoculated with the pathogen.

Diagnosis

Transmission to test plants

Transmission experiments to woody and herbaceous test plants have been carried out to confirm suspected CLRV-infection and to fulfil Koch's postulates. SCHMELZER et al. (1966)

succeeded in transmitting the pathogen by grafting, whereas the transmisson failed by mechanical inoculation. MIRCETICH and ROWHANI (1984) transmitted CLRV to walnut indicator plants by bark patches from English walnut seedlings mechanically inoculated with a virus isolate. Within two years 23 out of 27 seedlings developed the characteristic disease symptoms. ROWHANI and MIRCETICH (1988) reported on strain specifity in regard to the host plant: only the walnut strain of CLRV caused the blackline symptoms of the English walnut (*Juglans regia* L.). Pathogenicity tests demonstrated that healthy walnut trees and trees inoculated with CLRV cherry- and golden elderberry-strains remained virus-free. GIERSIEPEN(1993) successfully inoculated only two of 47 formerly healthy ash seedlings by implantating bark patches of diseased ash trees. The low transmission rate was explained by the heterogenous virus distribution and the low virus titer which is often observed in woody plant tissue. Furthermore, a long latent period of several months to a few years from the time of grafting to the appearance of symptoms has to be bridged (NIENHAUS and CASTELLO, 1989).

Electron microscopy

Electron microscopy is applied for different purposes in virological work such as studies of size and structure of the pathogen, detection and identification of viruses in infected plants and investigations on cellular changes caused by virus infection (RUBIO HUERTOS et al., 1985; NILSSON and TOMENIUS, 1987). QUADT and HAMACHER (1991) demonstrated histological alterations in tissue of European ash (*Fraxinus excelsior*) caused by CLRV.

GOODMANN et al. (1986) summarized significant alterations in cell walls and intercellular membrane structures due to virus infection. Cell wall thickening with deposition of callose and lignin and other polymers are particularly pronounced when the host responds hypersensitively to limit the virus to the area surrounding the infection site. In contrast, the formation of paramural bodies and cell wall outgrowths are often observed in systemic infection. HAMACHER et al. (1994) showed capsid protein of viral origin restricted to chlorotic areas of CLRV-infected leaves. The capsid protein was observed in tubular inclusion bodies associated with plasmodesmatas and along the cell walls. Callose was already found in non-infected leaves close to plasmodesmata. Labeled particles were detected in diseased leaf material located along cell walls. These histological investigations are useful to understand alterations due to the CLRV-infection process and in virus replication, but cannot be applied for virus diagnosis.

The specificity of serological tests and the visualization of the type of viral antigen is combined when using electron microscope serology. A number of different techniques are established to detect and identify virus particles in suspensions or in thin sections (DIJKSTRA and DE JAGER, 1998). LESEMANN (1982) reported that the detection by immunosorbent electron microscopy (ISEM) is in many cases as sensitive as the enzyme-linked-immunosorbent assay (ELISA), which is limited to 0,1-10 ng/ml. Regarding the detection of CLRV in leaf tissue of woody plants, it is very difficult to identify the particles, as there might be a low virus titer, and the small sphaeric particles of approximately 28 nm in diameter may be mixed up with cell components.

Immunoassays

Immunoassays use the binding specificitiy of an antibody for its specific antigen to measure the presence of antibody or antigen. The reaction may be quantified by sensitive measurements through enzymes with high turnover numbers like horseradish peroxidase or alkaline phosphatase. The investigations can be performed with either polyclonal or monoclonal antibodies or even a mixture of these compounds. As polyclonal antibodies are applied in routine diagnosis of virus infection, monoclonal antibodies are very useful for developing highly specific immunoassays for special purposes. In regard to sensitivity, the monospecificity of the monoclonal antibodies may be a disadvantage. The sensitivity of an immunoassay depends

mainly on the spectrum of antigen binding sites that the antiserum recognizes. Concerning the detection of CLRV by ELISA, many procedures and antisera have been developed.

Focusing on the detection of CLRV in ash trees, it is not possible to detect the pathogen in crude plant extracts by direct ELISA (D-ELISA) (GIERSIEPEN, 1993). Because of the low virus titer a concentration by ultracentrifugation has to be carried out prior to the serological investigation. In contrast, *Betula* plant material can be tested by D-ELISA (GRÜNTZIG et al., 1996). Testing more than 1000 birch trees, the authors presented test calenders for routine testing to detect CLRV in buds, leaves, young shoots, inflorescences and infructescences. The virus could be identified in birch trees with a reliability of 100% all year round because of a high virus concentration in the tested plant organs. In other tree species, e. g. cherry, the period of reliable serological testing in routine surveys is extendable by using emergent flower and leaf tissue (TORRANCE, 1981). Therefore, dormant budsticks can be cut in midwinter, stored at 4 °C and forced to sprout just before starting the testing period in the laboratory.

ROWHANI et al. (1985) showed that the indirect ELISA (I-ELISA) is efficient as well as reliable for detecting CLRV in walnut seedlings in nurseries and naturally infected orchard trees. Due to an uneven and erratic distribution of the pathogen in infected trees, the analysed sample of 0,1 g has to be taken from at least 80 g pollen of each tree to obtain a reliable result (ROWHANI et al., 1985). The authors showed that the I-ELISA was 8 or 16 times more sensitive than the D-ELISA, depending on whether inner bark tissue or pollen was analysed using walnut tissue.

The test sensitivity is relative and depends on the virus strain and the chosen method. To compare different methods, in-vitro tests using purified virus solutions mixed with plant sap are necessary. EDWARDS and COOPER (1985) compared the detection limit of the birch and golden elderberry isolates of CLRV applying a protein-A sandwich ELISA (PAS-ELISA). Whereas 6 ng/ml of the birch-strain could be detected, 24 ng/ml of the golden elderberry-strain were necessary to get an antigen-antibody reaction. The dilution end point of CLRV (walnut strain)-infected *Chenopodium quinoa* sap for ELISA was 1/5000 with PAS-ELISA and 1/1000 with D-ELISA.

Agar-gel-diffusion and immunoelectrophoresis are of particular value as they are simple to perform and provide information on immunological cross-reactivity and on the presence or absence of multiple antigen-antibody systems. The serotyping of CLRV isolates is very important in regard to the classification of viruses, epidemiological investigations and resistance breeding.

A study of serological and vector relationships indicated elm mosaic virus as the elm strain of CLRV (FULTON and FULTON, 1970; SCHMELZER 1972 b). By then, elm mosaic virus was known to be closley related to tomato ringspot virus (ToRSV), as these two viruses are strikingly similar in physical properties, host range and symptom development (VARNEY and MOORE, 1952 a, b; FULTON and FULTON, 1970). TOBIAS (1993) compared CLRV-isolates occurring in walnut trees.

Furthermore, an antigenic diversity based on the virus strain and the geographic origin exists. JONES et al. (1990) compared serologically isolates of CLRV from diseased beech and birch trees located in a forest decline area. As assessed by spur formation of precipitin lines in an agarose-gel-double-diffusion test, they were closely related but not identical. ROWHANI and MIRCETICH (1988) supported these results by analysing the serological properties of three walnut CLRV isolates originating from the United States, Italy and Spain. The Italian isolate was shown to be distinguishable from the other two closely related but not identical isolates. The birch and beech isolates observed by JONES et al. (1990) differed from ten other distinct CLRV isolates obtained from different natural hosts as well as from different countries. ROWHANI and MIRCETICH (1988) described the golden elderberry strain of CLRV as closely related to the walnut strain – in contrast to the cherry-, dogwood- and rhubarbstrains.

Molecular biological methods

The use of molecular biological methods in plant virus diagnosis requires knowledge of the virus genome, at least of the nucleic acid sequence of conserved parts of the virion. The high sensitivity of these methods – in comparison with serological techniques – facilitates the detection of virus fragments in soil, water, vectors, mixed samples, samples taken during early stages of infection and other applications with a small amount of sample material and/or a low virus titer. BORJA and PONZ (1992) described a dot-blot-hybridization with a 32P-labeled probe to detect CLRV. Samples representing 2,5 ng of infected tobacco leaf could be detected on nylon, compared with 0.3 mg on nitrocellulose. In our opinion the application of digoxigenin-labeled probes with its advantages in handling are as sensitive as radioactive ones if the procedure is, first of all, well optimized and, secondly detected by chemoluminescence. Whereas BORJA and PONZ (1992) tested purified virus and tobacco plant sap, MAS et al. (1993) applied crude extracts or partially purified plant sap. Using a digoxigenin-labeled RNA-probe for CLRV detection, MAS et al. (1993) compared the effect of visualization of the hybridization signals by colorigenic and chemoluminescent detection on the sensitivity. The chemoluminescent detection turned out to be five times more sensitive than the colorigenic one.

CLRV consists of a bipartite RNA genome with a genome-linked viral protein and a polyA tail (JONES, 1986). As shown from a CLRV-birch isolate, the 3'ends of RNA-1 and RNA-2 are identical, highly conserved and show tRNA-like structures (SCOTT et al., 1992). Sequencing data reveal 98.5% homology between RNA-1 and RNA-2 within the 1564 nt 3'-terminal stretch of the walnut strain of CLRV and about 80% sequence homology to other strains of CLRV (BORJA et al., 1995). These data confirm earlier studies by MASSALSKI and COOPER (1986), figuring out sequence homologies between the RNA genomes of CLRV strains naturally infecting woody hosts using cDNA hybridization analysis. Whereas the European and North American birch and cherry isolates of CLRV indicated a homology of 85-95%, lower levels (46-48%) were obtained when these isolates were compared with the herbaceous host rhubarb or the North American dogwood isolate of CLRV. This low homology of less than 50% leads to the question, whether there is a high variability of analysed sequence between woody and herbaceous host plants or the presence of two different but related viruses. These results have to be completed by comparative bio- and serological assays. Bioassays have to be carried out by virus transmission to herbaceous host plants and host determination by precise description of symptoms.

BORJA and PONZ (1992) reported on a RT(reverse transcription)-PCR amplifying a specific fragment of 448 bp from the 3' untranslated region of both viral RNAs. The technique can be applied to detect CLRV in walnut using minute amounts such as 50 ng of bud or 5 µg of twig tissue. But PCR and RT-PCR may often be inhibited by phenolic, cross-linking or oxidizing agents in crude plant extracts (DEMEKE and ADAMS, 1992). WERNER et al. (1997 b) avoided these problems when they established a modified immunocapture (IC) RT-PCR to detect CLRV in different hosts. The authors used polyclonal antibodies with a high specificity to CLRV to catch the virions and two short primers (conserved sequences within the 3'-terminal region) resulting in a 416 bp PCR product. Different isolates of CLRV isolated from birch, beech and petunia could be identified throughout the year in leaf buds, leaves, inflorescences, cortical tissue of young twigs and single seeds. Virions were detectable in plant sap dilutions of 10^{-7} to 10^{-8} corresponding to an initial amount of 0,5 to 5 ng plant material (WERNER et al., 1997 b). We successfully applied these primers to detect CLRV also *in Rhamnus alnus, Sambucus nigra, Fraxinus excelsior* and *Cornus* sp. This test procedure is especially suitable for routine diagnosis.

A method combining immunocapture and PCR amplification in a microtiter plate was presented by NOLASCO et al. (1993) for the detection of different plant viruses and subviral pathogens. This method allowed the identification of CLRV by reverse transcription of viral RNA performed directly on the retained material. The authors remarked that the procedure has the typical sensitivity of assays based on PCR. The use of a microtiter plate allows an

equivalent degree of automation as in the ELISA. This method may be applied for routine diagnosis for testing seed lots, mother trees and material stored in gene banks. Probably, other procedures combining not only molecular biological and serological methods but several molecular biological steps would obtain similar results.

BORJA and PONZ (1992) appraised the use of different methods for the detection of the walnut strain of CLRV. The authors compared the ELISA, dot-blot hybridization and RT-PCR. The investigations were carried out with crude tobacco extracts. The RNA dot blot hybridization was 10 times more sensitive than the ELISA. In contrast, the RT-PCR was 10-8 times more sensitive than the hybridization, which required the virus extraction of 0,3 mg tobacco tissue. WERNER et al. (1997 b) confirmed these results comparing ELISA and an IC-RT-PCR for detecting the birch-strain of CLRV in different woody hosts. The authors demonstrated that the RT-PCR assay is 10^3 to 10^4 times more sensitive than the ELISA.

CLRV infection effects on gene banks, nurseries, public gardens and forest stands

For ecological and economical reasons it is very important to ensure the production and commercialization of healthy CLRV-free plant material, as infected seedlings and trees suffer from a loss of vigor which often results in a degeneration of the trees. Furthermore virus-infected plants are considered to be very sensitive towards changes in environmental conditions (NIENHAUS et al., 1985). Whilst investigating diseased declining beech trees in selected forest stands, the authors demonstrated that in some geographic regions viruses are involved in the decline. Abiotic and biotic factors, which may be connected with the decline, often lead to the death of trees. They are summarized and figured in the decline, disease spiral (NIENHAUS, 1985; MANION and LACHANCE, 1992). Authors are divided amongst themselves in regard to the grouping of viruses. While NIENHAUS classified viruses as predisposing and inciting factors of the decline, MANION and LACHANCE described these pathogens as contributing factors. In any case, viruses have to be taken into consideration when studying tree decline and establishing healthy stands.

In birch amenity trees the CLRV infection rate was six times higher than in unmanaged populations (COPPER and MASSALSKI, 1984). The authors concluded that the seedlings and trees cultivated in public areas were already infected with the virus before planting. Investigations on *Prunus avium* from a German gene bank confirmed this assumption: 31% of the tested seeds were CLRV-infected and an additional 5% were infected by CLRV together with prune dwarf virus (PDV) (LÖW, 1995). The germination rate of these virus-infected seeds was strongly reduced (23-54%) as compared to seeds of healthy control plants.

LÖW (1995) screened the CLRV-infection rate of two *Prunus avium* stands used for seed production. Testing more than 600 trees, the author showed that 1-4% of the mother trees were infected and about 12% were dead. The extent of losses is difficult to evaluate in forest tree production as there is a long time period between sowing in nurseries, planting in forest stands and wood-harvesting. An investigation of more than 1000 *Juglans regia* seedlings aged 5 years or less in Great Britain showed that about 0.4% of these plants were CLRV-infected (COOPER and EDWARDS, 1980). The diseased seedlings were described as shorter and thinner than the healthy ones. These observations were confirmed and complemented by COPPER and MASSALSKI (1984). According to the authors, seedlings as well as cuttings from naturally infected trees grew less rapidly than their healthy counterparts. Whereas 35% of the hardwood cuttings from healthy birch trees became established under mist propagation, only 20% of the cuttings from CLRV-infected birch trees did so. The annual increment of CLRV-infected seedlings cultivated under glasshouse conditions was half as large as those of healthy birch seedlings. KONTZOG et al. (1990) reported on the predisposing influence of a CLRV infection on the growth of birch plants exposed to chemical immission. The CLRV-infected birch seedlings showed a significant growth reduction, measured by length and dry weight of young

shoots at the end of the growing season, compared with exposed healthy seedlings. Thus production of dry matter was 65% lower in the case of CLRV-infected and fumigated trees compared with virus-infected trees not treated with sulphur dioxide and ozone.

These three reports concern the influence of a CLRV infection on the growth rate of young forest tree seedlings, and no data have been published referring to the yield loss of infected old forest trees. Because healthy and vital trees are selected in forest management, weak trees tend to be neglected. Regarding fruit trees, all planted trees get attention. In fruit orchards economic losses caused by viruses were measured by growth and yield parameters. Investigations in commercial orchards indicated infection levels from 5 up to 55%, depending on the geographic region, the species and the virus causing the disease (MYRTA et al., 1996; ZERAMDINI et al., 1996; JAWHAR et al., 1996; HAIDAR et al., 1996). The consequence is completely different management and research aims. But to achieve less yield losses in forests, the basic of management has to be changed.

Possible economic losses of long-standing forest tree species infected with seed-transmissible CLRV can be reduced only by the use of virus-free seeds or tolerant/resistant cultivars and the control of virus transmitting vectors (BÜTTNER and BANDTE, 2000). It has to be considered that selecting hypersensitive cultivars may counteract a distribution of CLRV, especially in regard to grafted trees produced for public gardens, embankments and roadsides.

NEMETH et al. (1993) investigated the influence of the rootstocks on spreading time of CLRV and decline in an old walnut plantation. The authors showed that the extent of total tree decline on the hypersensitive rootstock *J. nigra* developing CLRV-induced blackline was four times higher than on *J. regia*. The condition of the infected *J. regia* trees was described to be very bad, so that, in contrast to the dead *J. nigra*, this species provided a source of infection for a long time in the plantation. Another virus source in amenity areas exists through the open pollination of infected trees. COOPER and MASSALSKI (1984) described the pathway in regard to CLRV-infected birch trees. NIENHAUS and HAMACHER (1989) observed a high distribution of CLRV-infected trees in natural regeneration areas and assumed that seed is an important pathway in the transmission of the pathogen.

Concluding remarks

By the worldwide shift from extensive to intensive forest management, systems of high quality forest stands have been established. To preserve their value, more resources must be committed to the protection of forest stands. Forest tree pathology plays a major part in conserving the economic value of a forest stand (HUBBES, 1993). Therefore potential and existing disease threats must be identified and prophylactic methods for disease control have to be developed. Regarding the range of pathogens such as fungi, nematodes, insects, bacteria and viruses, the plant pathogenic viruses are of particular importance. In contrast to other pathogens viruses can not be controlled by curative treatments. Therefore prophylactic measures and the planting of virus-free seedlings are the basic requirement to ensure a long-term economic forest stand. For instance, CLRV is widely distributed, and early detection is an important part of the strategy for prevention of the spread of the disease. In regard to CLRV, new stands should be established with CLRV-free seedlings. As this pathogen is seed-transmissible to a high degree, seed lots as well as growing seedlings have to be tested for CLRV infection before planting into their final stand. A new infection in a stand may be introduced by contaminated soil or water, but these transmission modes are epidemiologically of minor importance. Molecular biological techniques may offer the basis for the establishment of a test suitable for routine diagnosis (BÜTTNER et al., 1996). The required method demands high reliability and sensitivity and has to be suitable for seeds as well as leaves and buds. A strain-specific test is of scientific importance for determining the relationship of different strains and their different properties, but it is not necessary for routine diagnosis, where the main question, CLRV-infected

or CLRV-free, has to be answered. In this case a high specifity may even hinder the reliability in regard to that question.

Investigations have to be carried out to provide more information on the transmission paths of CLRV in the field. Virus-resistent or -tolerant individual trees or varieties have to be selected for breeding programms. Infecting plants in nurseries with mild strains of CLRV may lead to more tolerant plants. But it has to be taken into consideration that infection with another virus may be dangerous and cause unacceptable losses. For instance, IEKI et al. (1997) described the effect of a preinoculation of navel orange with mild strains of citrus tristeza virus on trees affected with the severe strain. The treatment facilitated larger fruits, and the yield was 50% higher compared to trees infected only by the severe citrus tristeza virus-strain. There is no experience on such treatments with forest trees.

There are other virus species which also play an interesting and significant role in tree diseases. CLRV spreads to a great extend in forested areas. It is therefore necessary to have forest rangers and nurseryman well-informed about the potential risk of virus infections.

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Fig. 1: Leaf symptoms on deciduous trees infected with cherry leafroll nepovirus

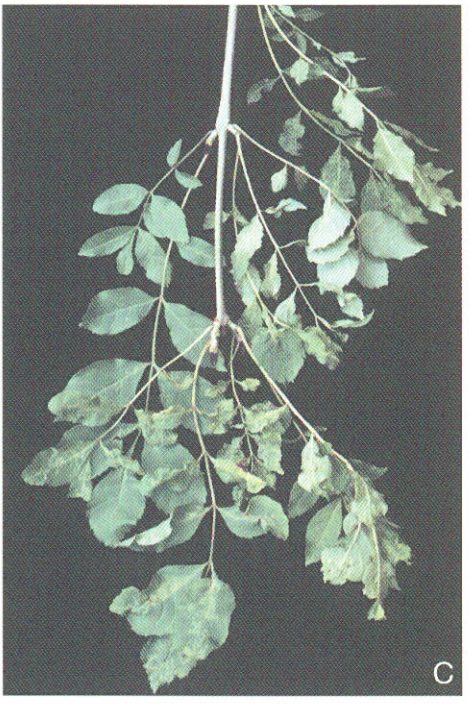
a: chlorotic ringspots in leaves of birch (*Betula pendula* Roth.)
b: chlorotic line pattern in leaf of beech (*Fagus sylvatica* L.)
c: deformation of the leaf (strap leaf) and chlorotic ringspots on ash (*Fraxinus excelsior* L.)
d: chlorotic mottling and line patterns on elder (*Sambucus nigra* L.)
e: yellow ring and line patterns (sometimes oak leaf patterns) on buckthorn (*Rhamnus frangula* L.)
f: chlorotic mottling on dogwood (*Cornus* sp.)

Abb. 1: Blattsymptome induziert durch das Kirschenblattrollvirus (cherry leafroll nepovirus) an Laubgehölzen

a: chlorotische Ringflecken an Sandbirke (*Betula pendula* Roth.)
b: chlorotische Linienmuster entlang der Blattadern an Rotbuche (*Fagus sylvatica* L.)
c: Deformation der Blattspreite (Schmalblättrigkeit) und chlorotische Ringflecke an Esche (*Fraxinus excelsior* L.)
d: Chlorosen und chlorotische Linienmuster an Schwarzem Holunder (*Sambucus nigra* L.)
e: chlorotische Linienmuster z. T. Eichenblattmuster an Faulbaum (*Rhamnus frangula* L.)
f: Chlorosen an Hartriegel (*Cornus* sp.)


A


B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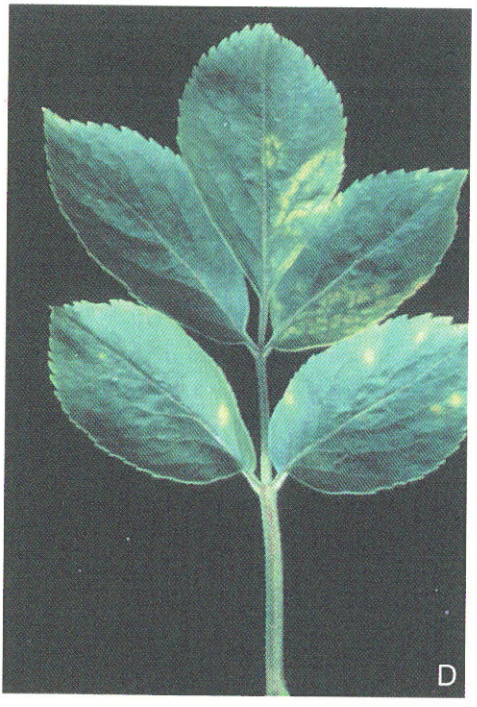
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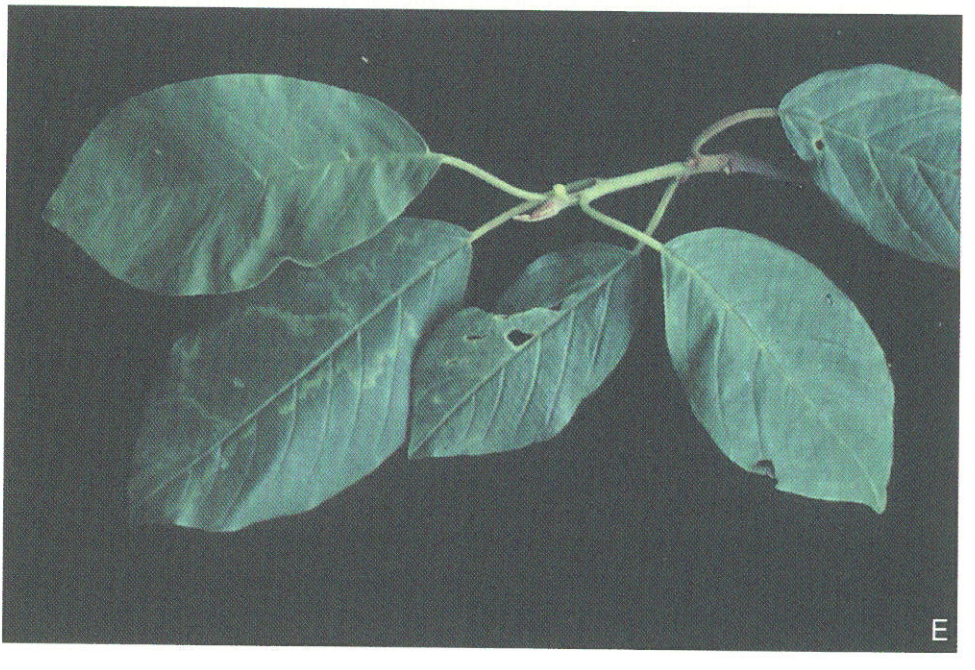
E

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