

Isolation of Cherry Leaf Roll and Brome Mosaic Viruses from European Beech and Transmission to Beech Seedlings

JOACHIM HAMACHER, Associate Professor, and ANDREA QUADT, Research Associate, Institut für Pflanzenkrankheiten, Rheinische Friedrich-Wilhelms-Universität, Nussallee 9, 53115 Bonn, Germany

ABSTRACT

Hamacher, J., and Quadt, A. 1994. Isolation of cherry leaf roll and brome mosaic viruses from European beech and transmission to beech seedlings. *Plant Dis.* 78:849-853.

Cherry leaf roll virus (CLRV) and brome mosaic virus (BMV) were transmitted from foliage of European beech to herbaceous plants and then back-transmitted to beech seedlings. Beech sampling sites were located in a nature reserve in Northrhine-Westfalia for CLRV and near the village of Hilberath, between the borders of the states of Rhineland-Pfalz and Northrhine-Westfalia, for BMV. Viruses were identified by bioassay, enzyme-linked immunosorbent assay, and immune electron microscopy. Transmission succeeded when seedlings in the four-leaf stage were inoculated by stem slashing. Infection rates averaged 24% for BMV and 17% for CLRV. Injection of concentrated BMV suspension into stratified beechnuts resulted in 15% infected seedlings 1 yr after infection. Symptoms developed in some inoculated beech seedlings about 4 wk after inoculation. Electron microscopy of infected tissue showed ultrastructural alterations.

Viruses infect many tree species in forest ecosystems throughout the world (5,24). Most of these viruses have been detected in deciduous trees (5,18,23,24), although recently some have been detected in conifers (10,11,17). The most economically important deciduous tree species in Germany is European beech (*Fagus sylvatica* L.). Nepoviruses, bromoviruses, potexviruses, and potyviruses have been reported and charac-

terized in this species (29,30). Our observations of beech stands in the mountain ranges of the Rhineland and the Alps (southeastern France) suggest a more widespread occurrence of virus infection and viruslike symptoms in European beech than formerly assumed. European beech trees infected with cherry leaf roll virus (CLRV) or brome mosaic virus (BMV) often exhibit irregular, meandering growth of branches and sometimes develop clawlike twigs with reduced internodes. Single branches or twigs, particularly in the upper part of the canopy, may die, thus giving the tree a bristle appearance. The wood of virus-

infected branches and twigs is brittle and dry. Leaf symptoms appear on single twigs or branches beginning during June. Young trees usually show more pronounced leaf symptoms than older trees. CLRV-infected leaves exhibit chlorotic line patterns, mosaic, or yellow stippling. Common leaf symptoms are small size, curling, and reduced growth of veins accompanied by chlorosis that becomes bright yellow (Fig. 1A). These symptoms were observed on many young trees and some older ones in a nature reserve in Northrhine-Westfalia, Germany, distributed over several square kilometers. BMV-infected beech were observed at another sampling site in the Eifel mountain range. Foliage of infected trees generally showed similar symptoms but no line patterns (Fig. 1B). Leaf symptoms were most distinct on sprouting rootstocks.

Because various techniques, including enzyme-linked immunosorbent assay (ELISA), electron microscopy, and mechanical transmission to herbaceous plants, did not yield a diagnosis from symptomatic foliage, we initiated an investigation to prove the virus etiology of the symptoms observed in beech trees in Northrhine-Westfalia and Rhineland-

Accepted for publication 16 May 1994.

© 1994 The American Phytopathological Society

Pfalz and to complete Koch's postulates by back-transmission to European beech.

MATERIALS AND METHODS

Virus isolation. The beech trees sampled were located in the nature reserve *Siebengebirge* near Bonn, Northrhine-Westfalia, at an elevation between 200 and 450 m, and in a forested area 30 km southwest of Bonn, at the border between Northrhine-Westfalia and Rhineland-Pfalz near the village of Hilberath, at an elevation of 360 m. Twigs of young trees exhibiting leaves with typical chlorotic mottling and/or line patterns as well as curling and blade reduction were labeled with colored tape during late summer of 1991 and were collected

at the end of February and during April 1992. The twigs were allowed to leaf out in the greenhouse. A composite leaf sample (approximately 250 g for each extraction) from each beech collection (five trees for CLRV, two trees for BMV) was harvested, crushed in liquid nitrogen, and mixed in a Waring blender with 500 ml of 100 mM phosphate buffer (pH 7.0) containing 10 mM sodium diethyldithiocarbamate, 10 mM sodium thioglycolate, and 150 ml of chloroform. After filtration through cheesecloth, the mixture was centrifuged at 3,500 g for 30 min at 4 C. The aqueous phase was then centrifuged at 125,000 g at 4 C for 3.2 hr. The pellets were suspended in 10 mM phosphate buffer (pH 7.0) and immedi-

ately rubbed on the foliage of *Nicotiana tabacum* L. cvs. Samsun and Xanthi-nc, *N. glutinosa* L., and *Chenopodium quinoa* Willd. Herbaceous hosts were in the six-leaf stage and maintained in the dark for 24 hr prior to inoculation.

Virus identification. As CLRV and BMV had already been extracted from other beech trees and identified (29), we inoculated test plants known to be susceptible to these viruses. CLRV causes ring spots on the older leaves of Xanthi-nc tobacco and chlorotic spotting on *C. quinoa*, followed by tip necrosis. BMV causes chlorotic spotting on *C. quinoa* and no symptoms on tobacco. After appearance of symptoms on the test plants, leaves were homogenized in 10

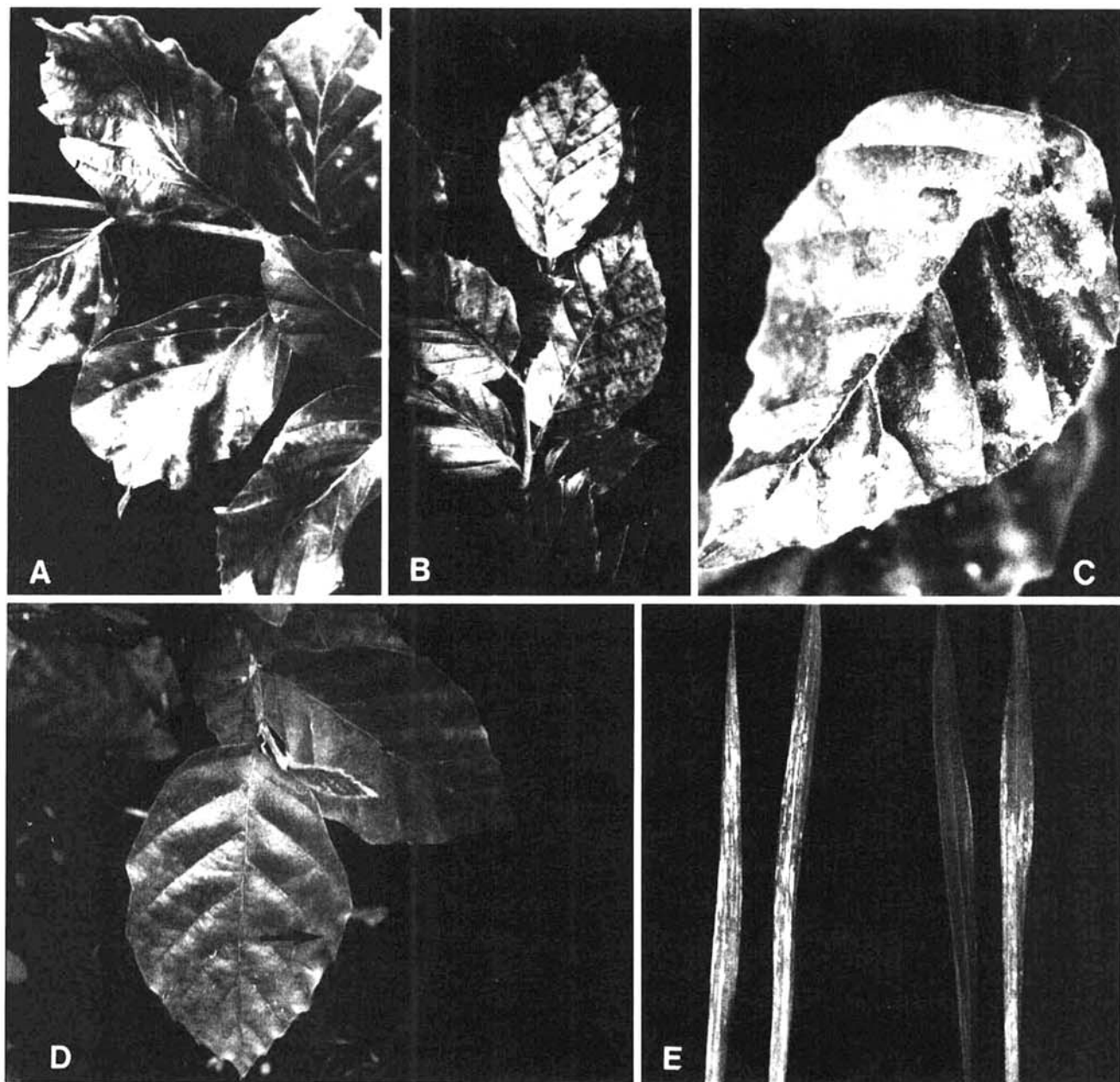


Fig. 1. (A) Chlorotic spots, line patterns, and blade deformation of leaves from a beech tree infected with cherry leaf roll virus (CLRV). (B) Chlorotic flecking and some blade deformation of leaves from a young beech tree infected with bromec mosaic virus (BMV). (C) Chlorotic line patterns and ring spots on a young leaf from a beech seedling after back-transmission of CLRV. (D) Chlorotic mottling (arrow) of young leaf on beech seedling infected with BMV. (E) Whitish streaks on blades of (left) winter wheat cv. Apollo and (right) winter barley cv. Jana 3 wk after inoculation with an isolate of BMV from beech.

mM phosphate buffer (pH 7.0). The homogenate was then inoculated to herbaceous plants to enhance symptom development and virus titer. The BMV isolate was also transmitted to two cultivars each of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) and one cultivar each of oat (*Avena sativa* L.) and triticale (\times *Triticosecale* Wittmack).

Antiserum used for serological assays for BMV was prepared by one of us (J.H.) according to the protocol of Winter (29), and antiserum to an ash strain of CLRV was obtained from R. Giersiepen (Institut für Pflanzenkrankheiten, University of Bonn, Germany; 16,25). For double antibody sandwich ELISA (DAS-ELISA) (3,4), coating antibody and alkaline phosphatase conjugated antibody were used at a concentration of 1 μ g/ml. Plant sap was diluted 1:40 with extraction buffer (phosphate buffered saline, pH 7.4; 2% polyvinylpyrrolidone, M_r 11,000; 0.2% ovalbumin; 0.05% Tween 20). After *p*-nitrophenylphosphate substrate was added, absorbance at 405 nm was measured with an SLT 400 AT ELISA reader after 1, 10, 30, and 60 min at room temperature and then for an additional 15 hr at 4 C. All buffers were maintained at the same temperature as the sample extracts. Immunosorbent electron microscopy (ISEM) was conducted as described elsewhere (1,9,20,21), using crude virus antisera diluted to 1:500 for trapping and to 1:50 or 1:100 for decoration.

Partial virus purification. Each of the two virus isolates was propagated in 50 *C. quinoa* plants for back-transmission trials. CLRV-infected tissue was extracted in a 500 mM (pH 6.8) borate buffer containing 10 mM sodium diethyldithiocarbamate and 10 mM thioglycolate (27,29). BMV-infected tissue was extracted in 10 mM phosphate buffer (pH 5) containing the same additives as described earlier but omitting chloroform. The extraction procedure was the same as described for virus isolation from beech. The sediments obtained after ultracentrifugation were resuspended in 10 mM phosphate buffer, pH 7 for CLRV and pH 5 for BMV.

Virus transmission to beech seedlings. Beech seedlings obtained from a national forest nursery in Nagold, Black Forest, Germany, were raised in the greenhouse with a 23/19 C day/night cycle and 16 hr of additional artificial illumination after 2 mo of stratification at 4 C in moist peat soil. Before inoculation, the foliage of approximately 40 seedlings was harvested, extracted, processed as described above, and tested by DAS-ELISA and bioassay for BMV and CLRV. Results of these assays were negative, so five different mechanical inoculation procedures were used to inoculate the remaining seedlings when they had devel-

oped the second leaf pair.

In some initial experiments, four different mechanical inoculation procedures were tried to infect seedlings through the foliage, roots, or stem (2,14,23). Only a stem-slashing technique resulted in successful transmission (2,12,23,29). Seedlings were inoculated with a suspension containing 1 vol of concentrated virus suspension in 10 mM phosphate buffer (pH 7 for CLRV and pH 5 for BMV), 1 vol of 10 mM phosphate buffer (pH 7 or 5) containing 10% saccharose, 2% polyvinylpyrrolidone (M_r 11,000), 1 mM EDTA, and 100 μ g/ml of PLO. A razor blade was dipped into the inoculum, and up to 100 slashes per seedling were made into the vascular system of the stem, beginning at the stem base and ending below the petioles of the upper leaf pair. A total of 162 seedlings (six experiments) were stem-slash inoculated with CLRV and a total of 66 seedlings (three experiments) were stem-slash inoculated with BMV. In addition, 20 stratified beechnuts were injected with approximately 20 μ l of concentrated BMV each, with 1 mg/ml injected into the radicle between the base of the cotyledons.

ELISA was performed on the foliage of all seedlings 5 wk postinoculation and, for three experiments, on new foliage produced the following year. The seedlings were maintained under open-air conditions during the following summer and winter. Some of the inoculated seedlings died during winter and thus could not be reevaluated. The first BMV inoculations were performed with the isolate prepared according to the protocol of Winter (29,30), and the inoculated seedlings of these experiments were only evaluated 5 wk postinoculation.

Tissue preparation for electron microscopy. When symptoms had developed, chlorotic tissue was prepared for electron microscopy (16). Negatively stained grids and ultrathin sections were observed in a Zeiss 109 electron microscope.

RESULTS

Symptom development in young beech seedlings began about 4 wk after stem-slash inoculation. One of 11 CLRV-infected beech seedlings showed bright yellow ring spots (Fig. 1C). Five of the

BMV-infected seedlings exhibited chlorotic mottling (Fig. 1D), whereas most of the infected seedlings remained symptomless. Mechanically inoculated gramineous hosts developed whitish streaks beginning 1 wk postinoculation. The most distinct symptoms appeared on winter wheat cultivars Apollo (Fig. 1E) and Xanthos; symptoms were less distinct on barley cultivars Jana (Fig. 1E) and Kendo. Symptoms on oat and triticale were very weak. Virus transmission from gramineous plants to *C. quinoa* resulted in the same symptom expression as transmission from beech.

In the back-transmission experiments, ELISA values higher than the twofold absorption of the mean of the negative control (crude sap from beech leaves) were considered to be positive for virus. Injection of BMV suspension into beechnuts resulted in infection of three of 20 seedlings 1 yr after inoculation. Back-transmission succeeded only by the stem-slashing method, not by any leaf-inoculation procedure or dipping-rotation method. BMV was transmitted with a mean frequency of 21% for the three preliminary back-transmission experiments started in 1990, including injection of beechnuts. The 1992 experiments yielded a back-transmission rate of 24% for BMV. No additional infections could be detected 1 yr later (Table 1). CLRV was transmitted less frequently in 1991 (10%) and 1992 (12%) than BMV. The virus concentration, however, increased in 1 yr (Table 2). The 1993 back-transmission experiments with CLRV were performed in the spring at three different dates. The decreasing transmission rates of 45, 16, and 8% may indicate that as day length increased, successful transmission declined. Virus titer, however, did not decrease (mean values were 1.47, 1.98, and 1.96, respectively). Plants that were ELISA-positive the first year were still positive the second year. No additional virus antigen was detected in any of the seedlings that were ELISA-negative the first year (*data not shown*). Some seedlings did not survive the winter.

Inoculated herbaceous plants and beech seedlings were tested by ISEM (trapping and decoration) for CLRV and BMV. Very young leaves of infected seedlings had positive results (Fig. 2A

Table 1. Evaluation by ELISA of beech seedlings inoculated with bromo mosaic virus by stem slashing or beechnuts inoculated by injection

No. inoculated	Postinoculation evaluation	No. positive in ELISA	A_{405} -positive samples ^a	A_{405} -negative control
Seedlings				
19	5 wk	5	0.535 \pm 0.172	0.238
14	5 wk	3	0.486 \pm 0.193	0.056
33 ^b	5 wk	8	1.957 \pm 0.769	0.184
	1 yr	8	1.284 \pm 0.545	0.134
Beechnuts				
20	5 wk	3	1.770 \pm 0.113	0.184

^aAbsorbance was measured after 15 hr at 4 C. Values are averages \pm standard deviations.

^bSame sample was evaluated twice.

and B), whereas neither virus could be detected in older leaves of seedlings and trees. Decoration of virus particles with antiserum resulted in better resolution (Fig. 2C and D).

Electron microscopic investigations of ultrathin sections from CLRV-infected beech seedlings revealed ultrastructural alterations typical of nepoviruses. Plasmodesmata in mesophyll cells were wider and stained darker than normal

plasmodesmata and contained rows of viruslike particles (Fig. 3A). In BMV-infected leaves, cytoplasmic aggregates with proliferation of darkly staining endoplasmic reticulum, vesicles, and enhanced Golgi activity as well as filamentous structures were observed in some palisade and phloem parenchyma cells (Fig. 3B). Cell wall-like material was deposited infrequently next to slightly widened plasmodesmata, but no massive

accumulations of BMV particles in the cytoplasm, reported for BMV in grammeous hosts (19), was observed.

DISCUSSION

The back-transmission experiments showed that CLRV and BMV infected European beech. These viruses have been isolated previously and identified in European beech trees at other sites (29). We could not find evidence for mixed infections with BMV and CLRV, although both viruses were detected at the same site. Because of the very low virus titers in infected trees from forest stands, neither ELISA nor ISEM detected virus directly, even in foliage expressing severe viruslike symptoms. Diagnosis was possible only after a virus concentration procedure from material that had leafed out under greenhouse conditions. Thus, contamination of leaf material with infected pollen can be excluded. We encountered the same diagnostic difficulties with CLRV in European ash (16). Back-transmission experiments conducted earlier with 1- or 2-yr-old seedlings were inconclusive (23,29,30). Thus, very young beech seedlings seem to be more susceptible than older seedlings to mechanical transmission.

CLR V infects many species of trees and shrubs, including birch, ash, wild cherry, hazelnut, walnut, and possibly maple. Therefore, its detection in European beech is not surprising (5,6,15,24,25,29,30). The virus may be spread by pollen and seed, as has been shown for birch and wild cherry (7,8; A. Löw and J. Hamacher, unpublished). Changes of the ultrastructure of beech leaves, such as modified plasmodesmata with rows of viruslike particles, is often described for nepoviruses (6,7,15,16). It represents additional proof for CLR V infection in inoculated beech seedlings.

Experimental exchange of the respective 3a movement protein genes between BMV and cowpea chlorotic mottle virus (CCMV) indicates that this nonstructural movement gene is involved in host range (22). Perhaps the occurrence of such minor genetic changes may be an explanation for the unexpected host range of the beech isolate. The mode of BMV transmission in grasses is presumably mechanical. Some insects (chrysomelids and aphids) and stem and leaf rust fungi are reported as potential vectors (13,26). Seed transmission has also been reported (13,28) and may occur in European beech, since BMV-like particles have been observed by electron microscopy in cotyledons of a few beech seedlings derived from seeds collected from infected trees (15). Seed transmission in beech, however, needs to be substantiated. Our electron microscopic observations of BMV-inoculated seedlings include cytological changes other than those typically caused by nepoviruses. Filamentous inclusions are not

Table 2. Evaluation by ELISA of beech seedlings inoculated with cherry leaf roll virus by stem slashing

No. inoculated	Postinoculation evaluation	No. positive in ELISA	A_{405} -positive samples ^a	A_{405} -negative control
10	5 wk	1	1.001	0.02
39 ^b	5 wk	7	1.148 ± 0.647	0.02
	1 yr	5	1.338 ± 0.746	0.052
51 ^b	5 wk	3	1.488 ± 1.260	0.092
	1 yr	2	1.945 ± 1.299	0.046
11	5 wk	5	1.466 ± 0.872	0.134
25	5 wk	3	2.275 ± 1.078	0.126
26	5 wk	2	1.955 ± 1.439	0.120

^aAbsorbance was measured after 15 hr at 4 C. Values are averages ± standard deviations.

^bSame sample was evaluated twice, but number was reduced at 1 yr because some seedlings died during the winter.

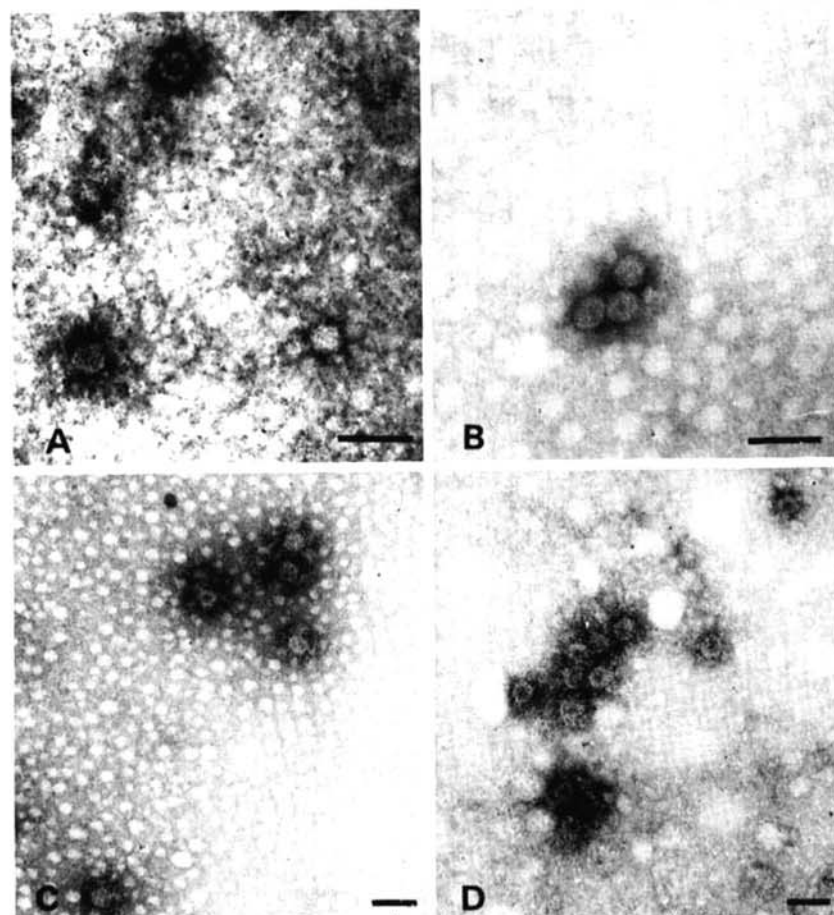


Fig. 2. Immune electron micrographs of viruses from beech: (A) Particles of cherry leaf roll virus (CLR V) after trapping with antiserum (dilution 1:500) in crude sap from beech. (B) Particles of bromo mosaic virus (BMV) after trapping with antiserum (dilution 1:500) in crude sap from beech. (C) Particles of CLR V after trapping and decoration with antiserum (1:50) in crude sap from *Nicotiana tabacum*. (D) Particles of BMV after trapping and decoration with antiserum (1:50) in crude sap from *Chenopodium quinoa*. Scale bars = 50 nm.

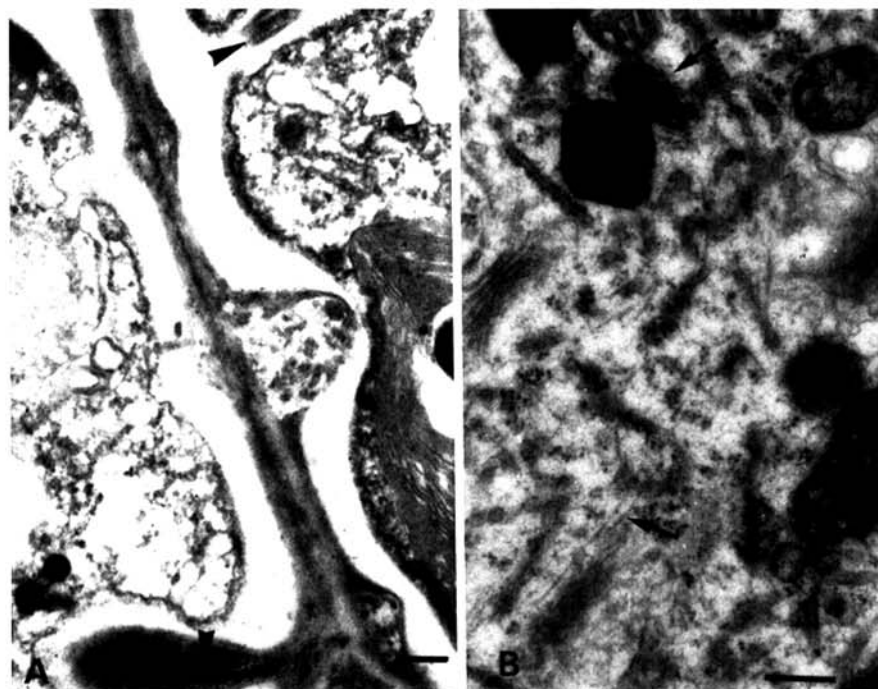


Fig. 3. Ultrathin sections of leaves from infected beech: (A) Mesophyll cells in a leaf from a seedling inoculated with cherry leaf roll virus showing rows of viruslike particles in tubules adjacent to plasmodesmata protruding into the cytoplasm (arrows). (B) Cytoplasmic aggregate with endoplasmic reticulum and Golgi activity and fibrillar inclusions (arrows) in a leaf from beech infected with brome mosaic virus. Scale bars = 200 nm.

common with BMV infection in monocots. They sometimes have been observed in CCMV infections in dicotyledonous hosts. In beech cells, however, filamentous inclusions should be regarded as stress reactions to virus infections, often observed in connection with cell wall modifications (A. Quadt, unpublished). Proliferation of endoplasmic reticulum in accumulated cytoplasm was not observed in noninfected or otherwise stressed beech leaves (15).

Unsuccessful efforts at virus isolation and identification in beech leaves exhibiting symptoms in late summer may be due to very low virus titers in adult leaves or to inhibition of infection in sap from beech leaves. Early sampling of twigs from symptomatic trees and sprouting these under favorable greenhouse conditions may be essential for successful diagnosis of viral infections in European beech. Virus replication in European beech may take place in very young tissue before symptoms become visible. The importance of virus infections in European beech is difficult to estimate because of the slow development of the full disease syndrome and the possible interaction with abiotic factors.

ACKNOWLEDGMENTS

The investigations were supported by the German Ministry of Research and Technology (BMFT, grant

no. 0339337 B). We thank R. Giersiepen for providing us with CLRV (ash strain) antiserum.

LITERATURE CITED

- Ball, E. M., and Brakke, M. K. 1968. Leaf-dip serology for electron microscopic identification of plant viruses. *Virology* 36:152-155.
- Bitterlin, M. W., Gonsalves, D., and Scorza, R. 1987. Improved mechanical transmission of tomato ringspot virus to *Prunus* seedlings. *Phytopathology* 77:560-563.
- Casper, R., and Meyer, S. 1981. Die Anwendung des ELISA-Verfahrens zum Nachweis pflanzenpathogener Viren. *Nachrichtenbl. Dtsch. Pflanzenschutzdienst* 33:49-54.
- Clark, M. F., and Adams, A. M. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34:475-483.
- Cooper, J. I. 1993. *Virus Diseases of Trees and Shrubs*. 2nd ed. Chapman & Hall, New York.
- Cooper, J. I., and Atkinson, M. A. 1975. Cherry leaf roll virus, a cause of chlorotic symptoms in *Betula* spp. in the United Kingdom. *Forestry* 48:193-203.
- Cooper, J. I., and Massalski, P. R. 1984. Viruses and virus-like diseases affecting *Betula* spp. *Proc. R. Soc. Edinburgh Sect. B* 85:183-195.
- Cooper, J. I., Massalski, P. R., and Edwards, M. L. 1984. Cherry leaf roll virus in the female gametophyte and seed of birch and its relevance to vertical virus transmission. *Ann. Appl. Biol.* 105:55-64.
- Derrick, K. S. 1972. Immunospecific grids for electron microscopy of plant viruses. (Abstr.) *Phytopathology* 62:753-754.
- Flachmann, M. 1992. Untersuchungen zum Nachweis von Viren in Nadelbäumen aus Waldschadensgebieten Südwestdeutschlands. Ph.D. dissertation. University of Hohenheim, Germany.
- Flachmann, M., Lesemann, D. E. Frenzel, B., and Koenig, R. 1990. Isometric virus-like particles in *Abies alba* Mill. and other *Abies* species: Partial purification and improved detection by means of immunoelectron microscopy. *J. Phytopathol.* 129:193-202.
- Garnsey, S. M., and Whidden, R. 1973. Efficiency of mechanical inoculation procedures for citrus exocortis viroid. *Plant Dis. Rep.* 57:886-889.
- Garrett, R. G., Cooper, J. A., and Smith, P. R. 1985. Virus epidemiology and control. Pages 269-299 in: *The Plant Viruses. Vol. 1, Polyhedral Virions with Tripartite Genomes*. R. I. B Francki, ed. Plenum Press, New York.
- Giersiepen, R., Quadt, A., Hamacher, J., and Nienhaus, F. 1992. Untersuchungen zur Virusrückübertragung auf Forstgehölze. (Abstr.) *Mitt. BBA* 283:414.
- Hamacher, J. 1994. Licht- und elektronenmikroskopische Untersuchungen an Laubbäumen unter verschiedenen Stresseinwirkungen. Postdoctoral thesis. University of Bonn, Germany.
- Hamacher, J., and Quadt, A. 1991. Light and electron microscopic studies of cherry leaf roll virus (CLRV) on European ash (*Fraxinus excelsior* L.). *J. Phytopathol.* 131:215-226.
- Jacobi, V., Castello, J. D., and Flachmann, M. 1992. Isolation of tomato mosaic virus from red spruce. *Plant Dis.* 76:518-522.
- Jones, A. T., Koenig, R., Lesemann, D. E., Hamacher, J., Nienhaus, F., and Winter, S. 1990. Serological comparison of isolates of cherry leaf roll virus from diseased beech and birch trees in a forest decline area in Germany with other isolates of the virus. *J. Phytopathol.* 129:339-344.
- Martelli, G. P., and Russo, M. 1985. Virus-host relationships: Symptomatology and ultrastructural aspects. Pages 163-205 in: *The Plant Viruses. Vol. 1, Polyhedral Virions with Tripartite Genomes*. R. I. B Francki, ed. Plenum Press, New York.
- Milne, R. G. 1984. Electron microscopy for the identification of plant viruses in *in vitro* preparations. *Methods Virol.* 7:87-120.
- Milne, R. G., and Lesemann, D. E. 1984. Immunoabsorbent electron microscopy in plant virus studies. *Methods Virol.* 8:85-101.
- Mise, K., Allison, R. F., Janda, M., and Ahlquist, P. 1993. Bromovirus movement protein genes play a crucial role in host specificity. *J. Virol.* 67:2815-2823.
- Nienhaus, F., Büttner, C., and Hamacher, J. 1990. Virus infection of forest trees by mechanical transmission. *J. Phytopathol.* 129:141-150.
- Nienhaus, F., and Castello, J. D. 1989. Viruses in forest trees. *Annu. Rev. Phytopathol.* 27:165-186.
- Nienhaus, F., and Hamacher, J. 1989. Virose in Eschen. *Allg. Forstztg.* 16:385-386.
- Proeseler, G. 1978. Transmission of brome mosaic virus by cereal beetles (Coleoptera, Chrysomelidae). *Arch. Phytopathol. Pflanzenschutz* 14:267-268.
- Stace-Smith, R., Reichmann, M. E., and Wright, N. S. 1965. Purification and properties of tobacco ringspot virus and two RNA-deficient components. *Virology* 25:487-494.
- von Wechmar, M. B., Kaufmann, A., Desmarais, F. A., and Rybicki, E. P. 1984. Detection of seed-transmitted brome mosaic virus by ELISA (enzyme-linked immunosorbent assay), radial immunodiffusion and immunoelectroblotting tests. *Phytopathol. Z.* 109:341-352.
- Winter, S. 1987. Untersuchungen zum Nachweis von Viren in Rotbuchen *Fagus sylvatica* L. Ph.D. dissertation. University of Bonn, Germany.
- Winter, S., and Nienhaus, F. 1989. Identification of viruses from European beech (*Fagus sylvatica* L.) of declining forests in Northrhine-Westfalia (F.R.G.). *Eur. J. For. Pathol.* 19:111-118.