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Virus-diseased *Ulmus laevis* in Eastern Germany

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Abstract

Virus-like leaf symptoms and dieback were observed on elm trees in a public park near Potsdam. Infection with *Cherry leaf roll virus* (CLRV), *Elm mottle virus* (EMV), *Arabis mosaic virus* (ArMV) and *Tobacco ringspot virus* (TRSV), well known viruses to infected elm trees was excluded by bioassays and serological tests. Poty- or carlavirus-like flexible particles of approximately 750 nm in length were isolated repeatedly from diseased elms. The particles were transmissible to diverse *Chenopodium* species, a herbaceous indicator. The virus was not a member of the Potyviridae family, based on an ELISA and an RT-PCR assay using a potyvirus genus-specific broad-spectrum polyclonal antibody and family-specific primers, respectively. Also no potyvirus-like pinwheel inclusions were found in leaf cells of infected indicator plants in electron microscopic studies. Further molecular characterization of these virus isolates is under way.

Key words: elm, plant virus, symptoms, transmission, *Chenopodiaceae.*

Resumen

Patologías víricas en *Ulmus laevis* en el este de Alemania

En olmos situados en un parque cercano a Postdam, se ha observado la presencia de síntomas foliares similares a los producidos por virus. Mediante bioensayos y pruebas serológicas se descartó la presencia de infecciones originados por el virus del enrollamiento de la hoja del cerezo (CLRV), el virus del moteado del olmo (EMV), el virus del mosaico de Arabis (ArMV) y el virus del anillamiento del tabaco (TRSV), todos ellos bien conocidos por afectar a los olmos. Repetidamente se aisló, en olmos enfermos, partículas flexibles de aproximadamente 750 mm de longitud similares a las de Potyvirus y Carlavirus. Las partículas fueron transmisibles a diversas especies de *Chenopodium*, un indicador herbáceo. Según una prueba ELISA y un ensayo RT-PCR en que se usaron, respectivamente, un anticuerpo policlonal específico de género de Potyvirus de amplio espectro, y cebadores específicos de la familia, el virus no es miembro de la familia Potyviridae. Tampoco se ha encontrado, en estudios mediante microscopía electrónica, inclusiones del tipo potyvirus en las células de las hojas de plantas indicadoras infectadas. En la actualidad se están realizando nuevas caracterizaciones moleculares de estos aislamientos víricos.

Palabras clave: olmo, virus en plantas, síntomas, transmisión, *Chenopodiaceae.*

Introduction

Plant viruses are widely spread in deciduous trees. They may cause symptoms and lead to a decline of affected trees. Virus infections also alter a plant's predisposition with trees becoming more susceptible to abiotic and biotic stress impact. Therefore tree seedlings with a long generation cycle planted in forests, public gardens or along streets should ideally be healthy and virus-free to resist longterm stress impact.

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Investigations in forest, nurseries and public gardens have shown that viruses are widely spread in deciduous trees including elm trees (*Ulmus* sp.). Elm trees have become rare in Europe during the last decades due to Dutch elm disease caused by the fungus *Ophiostoma novo ulmi*. However, elm trees are popular in urban areas and are often cultivated in public gardens. Virus-like leaf symptoms and dieback were observed on several elm trees in a public garden close to Berlin. The oldest elm trees were planted in 1830. No fungal or bacterial pathogens were found to be associated with the symptoms.

Following biological, serological and electron microscopic assays, infection with previously described

viruses of elm trees such as *Cherry leaf roll virus* (CLRV), *Elm mottle virus* (EMoV), *Arabis mosaic virus* (ArMV) and *Tobacco ringspot virus* (TRSV) was excluded.

This investigation focuses on the identification and characterization of the viral agent with the aim of developing a specific assay suitable for routine diagnosis. Such an assay should help to conserve and preserve endangered elm species.

Material and Methods

Elm trees investigated in this study are cultivated in a public garden in Caputh which is located close to Potsdam, approximately 40 km southeast of Berlin. Samples were taken regulary twice a month from March to October. About 30 out of 60 elm trees (*Ulmus laevis*) were included in the study. The oldest trees were planted in approximately 1830. Dates for supplementary planting and natural regeneration are not available.

Mechanical inoculations were carried out to transmit the causal agent from leaves of symptomatic elm plant material to herbaceous indicator plants. The following indicator plants belonging to different families were treated by rub inoculation at the 4-8 leaves stage: *Allium cepa* L., *Brassica campestris* L., *B. oleracea* L., *B. rapa* L, *B. pekinensis* Rupr., *B. chinensis* L, *Beta vulgaris* L, *Chenopodium album* L. , *C. amaranticolor* Coste et Reyn., *C. bonus-heuricus* L., *C. capitatum Asch., C. foetidum Asch., C. quinoa* Willd., *Cucumis sativus* L, *C. melo* L, *Datura stramonium L., Lycopersicon esculentum* Mill., *Matthiola incana, N. benthamiana* L, *N. clevelandii* L, *N. glauca* Graham, *N. glutinosa* L, *N. tabacum* var. «Samsun», *N. tabacum* var. «Xanthi», *Ocimum basilicum* L, *Pisum sativum* L., *Physalis wrightii* Gray, *Urtica* sp. and *Vica faba* L.

Procedures to concentrate virus particles in homogenates of plant sap from diseased elm trees and treated indicator plants were modified after Dijkstra and DeJager (1998).

Electron microscopic analyses were done by negative staining of leave homogenates (Milne, 1993) and ultrathin sections embedded in Spurr's medium (Bozzola and Russell, 1999). Images were generated and evaluated with a EM 10 C electron microscope (Zeiss, Oberkochen, Germany).

Double-antibody sandwich enzyme-linked-immunosorbent assay (DAS-ELISA) was performed according to Clark *et al.* (1976). Specific antibodies to detect *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Tomato bushy stunt virus* (TBSV), *Tomato ringspot virus* (TRSV), and *Tobacco mosaic virus* (TMV) were obtained by the DSMZ (Braunschweig, Germany).

Figure 1. Location of samples. Samples were taken in Caputh (arrow), southwest of Potsdam in the northeast of Germany.

Reverse-transcriptase polymerase chain reaction was applied using the potyvirus family-specific primers Poty-M4 and Poty-S (Chen and Adams, 2001). PCR-fragments were visualized after agarose-gelelectrophoresis under UV-light.

Results and Discussion

Twenty seven out of the thirty elm trees examined in Caputh showed virus-like leaf symptoms. The trees exhibited chlorotic ringspots, chlorotic line patterns, and distinct chlorotic or necrotic spots (Fig. 2). Chlorotic ringspots on *Ulmus glabra* Huds and *U. minor* Mill. in-

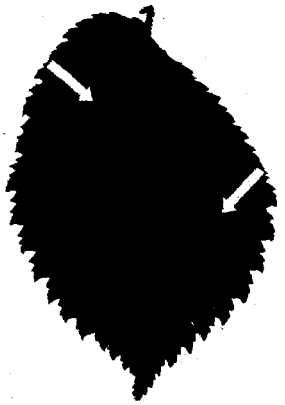

Figure 2. Elm leaf with chlorotic ringspots (arrow left) and chlorotic patterns along the veins (arrow right).

duced by *Elm mottle virus* were observed previously in Great Britain and Germany (Jones and Mayo, 1973; Schmelzer *et al.*, 1966). Ford *et al.* (1972) described *Cherry leaf roll virus*-infected *Ulmus americana* L. as exhibiting chlorotic ringspots, mosaic, leaf deformations and enations. However, all symptomatic *U. laevis* Pall trees from Caputh tested negative in ELISA or electron microscopy for *Cherry leaf roll virus* and *Elm mosaic virus*. There is a single report of virus-infection of *U. laevis* Pall. by *Tomato bushy stunt virus* (TBSV) (Novak and Lanzova, 1980). Symptomatic elm trees from Caputh were negative for TBSV in ELISA.

Because no bacteria or fungi could be cultivated from plant material from portions of diseased elm le-

Table 1. Transmission of virus isolates recovered from diseased elm trees to herbaceous indicator plants and electron microscopic detection of virus particles

Indicator plant	Inoculation with			
	Crude extracts of diseased elms leaves		Partially purified suspensions from diseased elm leaves	
	Symptoms	Electron microscopy	Symptoms	Electron microscopy
Chenopodium album L.	–[a]	–	CLL	+
C. amaranticolor Coste et Reyn.	–	–	RR	+
C. quinoa Willd.	CLL	+	CLL	+
Cucumis sativus L.	nt	nt	–	–
Datura stramonium L.	–	–	–	–
Lycopersicon esculentum Mill.	nt	nt	–	–
Nicotiana benthamiana L.	–	–	–	–
N. clevelandii L.	nt	nt	–	+
N. glutinosa L.	nt	nt	–	–
N. tabacum L. var «Samsun»	–	–	–	–
N. tabacum L. var. «Xanthi»	–	–	–	–

[a] No symptoms (–). Chlorotic local lesions (CCL). Red ringspots (RR). Not tested (nt); virus particles (+).

Figure 3. Leaves of *Chenopodium* sp. showing characteristic symptoms after mechanical inoculation with crude extracts of diseased elm leaves. *C. quinoa* showing chlorotic local lesions (left); *C. album* showing chlorotic lesions (middle); *C. amaranticolor* showing red ringspots (right).

Figure 4. Flexuous virus particles detected in leaf extracts. Flexuous virus particles in partially purified leaf extracts of diseased elm trees (length ~ 800 nm) (left); flexuous virus particles detected in leaf extracts of herbaceous indicator plants (length ~ 800 nm) (right).

aves plated on culture medium or incubated in a humid chamber bacteria or fungi were excluded as the putative causal agents of the disease.

After mechanical inoculation of indicator plants with extracts from diseased elm leaves some species developed characteristic chlorotic local lesions (Table 1). The pathogen was only transmissible to *Chenopodium* species (Fig. 3). *C. quinoa* and *C. album* showed chlorotic local lesions whereas red ringspots developed on *C. amaranticolor* leaves. These symptoms induced on *Chenopodium* sp. also verify that the pathogen involved in the disease is not *Elm mottle virus* or *Cherry leaf roll virus* because these viruses cause other characteristic symptoms, as reported by Jones and Mayo (1973) and Ford *et al.* (1972).

Poty- or Carlavirus like particles with a length of approximately 800 nm could be found repeatedly in partially purified leaf extracts of diseased elm trees and herbaceous indicator plants by electron microscopy (Fig. 4, left). Furthermore virus particles with a particle length of approximately 800 nm were found in leaves of diseased plants (Fig. 4, right). The particles were similar to those observed in partially purified extracts of elm leaves. Particles belonging to the Carlavirus group are typically 600-710 nm in length (Wetter and Milne, 1981), those belonging to the Potyvirus-group 680-900 nm in length (Hollings and Brunt, 1981).

ELISA using potyvirus-specific antibodies and RT-PCR assays using potyvirus-specific primers indicate that the virus isolates obtained do not belong to the potyvirus group. Also, no potyvirus-like inclusion bodies were found in ultrathin sections. Potyviruses often induce unique cytoplasmatic cylindrical inclusions (Edwardson *et al.*, 1993). These inclusions are recognized as a main characteristic of the group (Fenner, 1976) and a diagnostic indicator for infections by potyviruses (Ewardson, 1966).

Further steps to characterize the virus isolates obtained from these diseased elm trees, to fullfill Koch´s postulates and to produce a specific antiserum for application in routine diagnosis are under way.

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