Investigations on virus-diseased elm trees (*Ulmus laevis* L.) in eastern Germany

Martina Bandte, Marius Essing, Aksana Tarasevics and Carmen Büttner
Humboldt-Universität zu Berlin, Faculty of Agriculture and Horticulture, Section Phytomedicine
Lentzeallee 55/57, D-14195 Berlin
phytomedizin@agrar.hu-berlin.de

Introduction

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 ulmi*. However, elm trees are popular in urban areas and are often cultivated in public gardens.
Virus-like leaf symptoms (Fig. 1) and dieback were observed on elm trees in a public garden (Fig. 2) close to Berlin. The oldest elm trees were planted in 1830.

The investigation focuses on the identification and characterization of the casual pathogen to develop a specific assay which is suitable for routine diagnosis and necessary to conserve and to preserve endangered elm species.

Fig. 1
Virus suspected elm leaf symptoms
left: chlorotic pattern along the veins (arrows)
right: chlorotic ringspots (arrows)

Fig. 2
Palace Garden in Caputh , approx. 40 km South West of Berlin

Material and Methods

Elm samples were taken regularly twice a month from March to October. About 30 out of 60 elm trees were included in the study. Exact dates for supplementary planting and natural regenerations were not available. An overview of the applied methods is given in figure 3. Mechanical inoculations were carried out to transmit the disease causing agent from leaves of symptomatic elm plant material to herbaceous indicator plants. 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. Images were generated and evaluated with a EM 10 C electron microscope. 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). Furthermore a Potyvirus-group specific antibody (TuMV-314, RICHTER et al. (1995) was applied. Reverse-transcriptase polymerase chain reaction (RT-PCR) was applied using the potyvirus family-specific primers Poty-M4 and Poty-S (Chen and Adams, 2001).

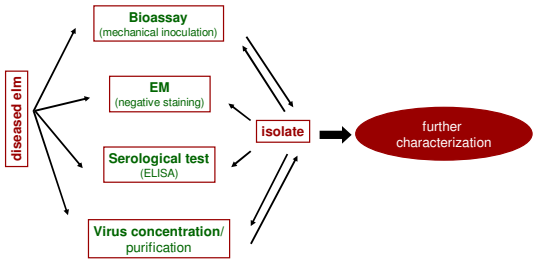

Abb. 3: The course of action investigations of leaf and bark samples of elm follow

Previous Results and Discussion

Twenty seven out of thirty elm trees showed virus-like leaf symptoms. The diseased elm trees developed chlorotic ringspots, chlorotic line patterns, and distinct chlorotic or necrotic spots. Because no bacterial or fungal organisms could be cultivated from plant material from diseased elm leaves on culture medium or in a humid chamber bacteria or fungi were excluded as the putative causal agents of the disease.

Bioassay – host range

Only *Chenopodium species* developed characteristic symptoms after mechanical inoculation with extracts from diseased elm leaves (Fig. 4). *C. quinoa* L. and *C. album L.* showed chlorotic local lesions whereas red ringspots developed on *C. amaranticolor* Coste & Reyn. leaves and necrotic spots on *C. foetidum* Lam.
Overall 23 plant species out of 12 families (*Apiaceae, Asteraceae, Brassicaceae, Cariophyllaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Liliaceae, Poaceae, Solanaceae* und *Ulmaceae)* were included in our investigations on the host range. Beside *Chenopodium* ssp. the agent was transmissible to tobacco plants - *Nicotiana clevelandii* Gray. and *Nicotiana benthamiana* Domin. Although virus particles could be visualized tobacco plants remain without visible symptoms. The other species have considered to be no host plant.

Electronenmicroscopy

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. 5). Particles belonging to the Carlavirus group are 600-710 nm in length, those belonging to the Potyvirus group 680-900 nm.

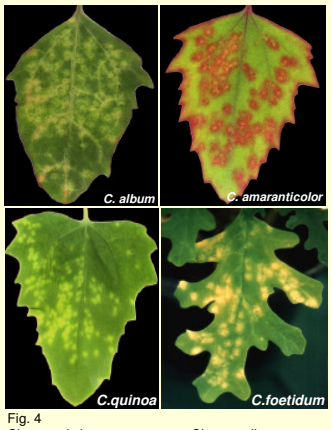

Fig. 4
Characteristic symptoms on *Chenopodium* ssp. after mechanical inoculation of an agent with leaf sap of diseased elm

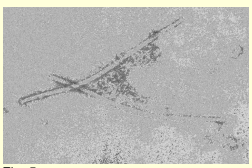

Fig. 5
Flexible virus particles (~ 800 nm in length) isolated from diseased elm leaves

ELISA

Confirmatory to the biological tests, *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, could be excluded to cause the disease by ELISA. Furthermore the causing agent disqualifies as a member of the Potyviridae family based on an ELISA using the specific antibody TuMV-314.

RT-PCR

The assays using Potyvirus- familiy specific primers (DNA-fragment 1700 bp) indicate that the obtained virus isolates do not belong to the potyvirus group.

Summary

The investigations focused on the identification of the agent causing a disease in elm trees. Bacterial or fungal organisms could be excluded as well as viruses (CLRV, EMV, ArMV and TRSV) well known to infect elm. Flexible particles of approx. 750 nm were isolated repeatedly and transmitted to *Chenopodium* ssp. The virus could not be identified yet but disqualifies as a member of the Potyviridae family.

References

Chen, J., Adams, M.J., 2001, Archives of Virology **146**, 757-766.
Clark, M.F., Adams, A.N., Barbara, D.J.; 1976, Acta horticulturae **67**, 43-49.
Dijkstra, J., DeJager, C.P., 1998: Springer Verlag Berlin, 459 p.
Richter, J., Rabenstein, F., Proll, E., Vetten, H.-J., 1995, J. Phytopathology **143**, 459-464.