BIOLOGIA PLANTARUM 34 (5-6): 471-474, 1992.

BRIEF COMMUNICATON

The occurrence of walnut ringspot on *Juglans regia* L. in Slovakia

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Abstract

The properties of a virus causing walnut ringspot of *Juglans regia* L. which had been identified by the visual examination of symptoms on leaves and fruits of walnut trees in Slovakia were studied. The virus was transmitted mechanically to *Chenopodium quinoa* Willd., *Chenopodium amaranticolor* Coste et Reyn., *Nicotiana clevelandii* Gray, *Phaseolus vulgaris* L. cv. Bountiful. Purified virus was used for antiserum production. The thermal inactivation point of the virus lies between 48 and 50 °C and the dilution end-point between 10^{-1} and 10^{-2}. The obtained antiserum had a titer 1:256. Virus isolates gave a positive immunological reaction with the Mircetich's antiserum against the cherry leaf roll virus obtained from walnut tree.

The infection of *Juglans regia* L. by the cherry leaf roll virus (CLRV) was described by Savino *et al.* (1976) in Italy. In Great Britain, Cooper (1979, 1980) isolated from *Juglans regia* L. as well as from other representatives of the *Juglans* family a virus identified as CLRV. Delbos *et al.* (1983) described the CLRV virus of attacked *Juglans nigra* trees in France. In the USA, Mircetich *et al.* (1980) isolated CLRV from *Juglans hindsii*. Németh (1986) throughly described the walnut ringspot in Hungary. The visual examination of *Juglans regia* L. in Slovakia revealed some localities where walnut ringspot occurred. The aim of our work was to identify the virus causing that disease.

The isolate of the virus obtained from *Juglans regia* L. (locality Bošáca) was used. The inoculum was prepared from buds and leaves of the attacked tree showing symptoms of infection, mixed with phosphate buffer 0.02 M pH 8.0,1 % of nicotine, 3.5 % of polyvinylpyrolidon, 0.1 % of sodium thioglycolate and 0.02 M Na-dietyl-dithiocarbamate. Test plants of *Chenopodium quinoa* Willd., *Chenopodium*

Received 20 March 1991, *accepted* 22 August 1991.

amaranticolor Coste et Reyn., *Nicotiana clevelandii* Gray, *N. glutinosa* L., *Nicotiana tabacum* L. cv. White Burley, *Phaseolus vulgaris* L. cv. Bountiful, *Zinnia elegans* Jackq., *Cucumis sativus* L. cv. *Delicates* were inoculated. The results were evaluated within 8 to 9 d. In symptomless plants a regressive transmission on *C. quinoa* was performed.

To determine the thermal inactivation point, the dilution end-point and longevity *in vitro*, the methods described by Baumgartnerova (1987) were applied.

Fig. 1. Chlorotic ringspots on the leaves of *Junglans regia* L.

The virus was purified from infected leaves of *C. quinoa* according to the modified method described by Cropley and Tomlinson (1971). The purified virus in concentration 1 mg cm^{-3} was used as antigen for antisera preparation. The antigen was mixed 1:1 with uncomplete Freund adjuvans and injected to rabbits. The immunization was performed 3 times intramuscularly in two-week intervals. Six weeks after the last injection the titer of antisera was determined by the agar gel test. The same method was used to test the attacked walnut leaves with antisera against the cherry leaf roll virus obtained from Dr. Jones (Invergowrie, G. Britain), Dr. Quacquarelli (Bari, Italy) and Dr. Mircetich (USA). The antiserum against the apple chlorotic leaf spot virus gained from Dr. Clark (G. Britain) was used as well.

On leaves of the diseased tree yellow chlorotic ringspots (Fig. 1), deformed leaves and a gradual drying up of the top small branches (Fig. 2) were observed. The fruits

of the attacked tree were much smaller than those of the healthy one. The kernels were deformed, shrunken and black.

Fig. 2. Drying up of small branches of *Juglans regia* L. attacked by the virus.

A positive transmission was made on *C. quinoa* - yellow ringspots gradually necrotizing with a subsequent systemic infection; *Nicotiana clevelandii* - yellow spots; *C. amaranticolor* - yellow spots; *P. vulgaris* - black lesions. The thermal inactivatioin point of the virus isolate in the juice obtained from infected leaves of *C. quinoa* was approximately 48 - 50 °C. The dillution end-point was 10^{-1} to 10^{-2}. In the juice held at room temperature the virus was infectious after two days. The virus showed a typical absorption spectrum in the UV sphere with a maximum at 260 nm and minimum at 240 nm. From immunized rabbits the antiserum with an antibody titer 1:256 was obtained. Virus isolates gave a positive immunological reaction with the Mircetich's antiserum against the cherry leaf roll virus obtained from walnut tree.

On the basis of results described by Savino *et al.* (1976), Quacquarelli and Savino (1977), Mircetich *et al.* (1984), Cooper (1980), Németh *et al.* (1982), Delbos (1983), Németh (1986) some types of that disease were identified as the walnut yellow mosaic, walnut ringspot (type Italian), walnut ringspot (English and Hungarian) and walnut blackline. The thermal inactivation point was 55-60 °C, longevity *in vitro* 8-16 days and dilution end-point 10^{-4} to 10^{-6}. The antiserum titer was obtained 1:512 to 1:2048 (Németh 1986).

On the basis of results obtained in our experiments we assume that the walnut ringspot in *Juglans regia* L. was caused by the CLRV type English and Hungarian. The lower values obtained by evaluating some properties of our virus isolate when compared with results of other authors, were obviously associated with the virulency of the used isolate. The study of the properties of the walnut ringspot virus will proceed in our further works.

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