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Plant Viruses in Soil and Water of Forest Ecosystems in Croatia

By

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With 1 Figure

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Summary

PLEŠE N., JURETIĆ N., MAMULA D., POLÁK Z. & KRAJAČIĆ M. 1996. Plant viruses in soil and water of forest ecosystems in Croatia. – *Phyton* (Horn, Austria) 36 (1): 135-143, 1 figure. – English with German summary.

Soil and water in three forest districts in central Croatia (the vicinity of Zagreb) were examined for the presence of plant viruses. Using bait- and test plants 40 out of 70 soil/root samples were found to contain tobacco necrosis virus (TNV). In *Chenopodium amaranticolor* and *C. quinoa*, symptoms were caused by the virus isolates only in leaves that were inoculated. One of the isolates, which was examined serologically, appears to be a variant of TNV. Also isolated were the typical tobacco mosaic virus (TMV) from two forest ditches and one brook, and the typical tomato mosaic virus (ToMV) from two other brooks. The isolation was performed by inoculation of test plants with water concentrates obtained by high speed centrifugation (both TMV and ToMV), low speed centrifugation and by inoculation with native water silt (trials with TMV only).

Zusammenfassung

PLEŠE N., JURÉTIĆ N., MAMULA D., POLÁK Z. & KRAJAČIĆ M. 1996. Pflanzenviren in Boden und Wasser von Waldökosystemen in Kroatien. – *Phyton* (Horn, Austria) 36 (1): 135-143, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

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Boden und Gewässer von drei Waldgebieten in Kroatien unweit von Zagreb wurden auf das Vorkommen von Pflanzenviren untersucht. Mittels Fang- und Testpflanzen wurde festgestellt, daß 40 von 70 Boden/Wurzelproben das Tabaknekrose-Virus enthielten. In *Chenopodium amaranticolor* und *C. quinoa* wurden Symptome nur an den inokulierten Blättern festgestellt. Eines der Isolate, welches serologisch untersucht wurde, scheint eine Variante von TNV zu sein. Das typische Tabakmosaik-Virus (TMV) wurde aus zwei Drainagekanälen und einem Waldbach isoliert, das typische Tomatenmosaik-Virus (ToMV) aus zwei anderen Waldbächen. Die Isolierung von TMV und ToMV wurde nach Inokulierung der Testpflanzen mit Wasserkonzentraten, gewonnen durch hochtourige Zentrifugation, ausgeführt. TMV wurde auch aus Testpflanzen isoliert, die mit Wasserkonzentraten nach Niedriggeschwindigkeitszentrifugation oder mit frischem Schlamm inokuliert worden waren.

Introduction

Numerous plant viruses occur in soil and surface water, especially in agricultural areas (reviewed by NIENHAUS & CASTELLO 1989, KOENIG 1986, cf. LESEMANN & al. 1992). After reaching these media via decomposing infected plant material and also from the presumably undamaged roots of living plants (the latter phenomenon was discussed by SMITH & al. 1969), they more or less become adsorbed to colloid (soil) particles. The degree of the adsorption and the length of the period over which the infectivity is retained, which can last for weeks or sometimes for months, depends on virus properties and, considerably, on environmental factors. Among the latter are soil type and level of moisture, pH reaction, temperature, content of organic substances and especially, connected with these, the biological activity of the medium (KEGLER & al. 1991, NIENHAUS & CASTELLO 1989). Many of the viruses are stable and capable of infecting plants without being mediated by vectors, i.e. nematodes and soilborne fungi zoospores. This occurs through virus uptake from soil or water by the apparently uninjured plant roots (HOLLINGS & al. 1977, KEGLER & KEGLER 1981, cf. KEGLER & al. 1991, HEIN 1984). Viruses occurring in streams can be spread over large distances. Occasionally, new viruses, not found previously in a host plant, are isolated from water and soil (KOENIG 1986, BÜTTNER & NIENHAUS 1989a, LESEMANN & al. 1992).

Up to now about nine viruses have been identified, and a few others isolated, from the soil and water of forest ecosystems. They mainly belong to potex-, tombus-, tobamo-, poty- and necrovirus groups (BÜTTNER & NIENHAUS 1989a, b, JACOBI & CASTELLO 1991). Besides, the viruses of these and of at least seven other groups, particularly the nepo- and ilarviruses, have been found in woody forest plants, the related infections being more or less harmful to the plants. Also, many viruses were detected in herbaceous forest plants. Within the complex of agents causing forest damage or decline, which is a serious ecological and economic problem, the viruses contained in the soil and water of forest ecosystems are considered to be a contributory component (NIENHAUS & CASTELLO 1989). The sparsity

of investigations into virus occurrence in these substrates prompted us to undertake this work on the identification and spread of infectious viruses in the forest soil and drainage water in a Croatian region.

Material and Methods

Soil/root (further: soil) and water samples were taken in central Croatia in three forest districts outside inhabited areas in 1990, 1991 and 1992 during May. Two of the districts, south of Zagreb, were flat (Turopolje = D1) or slightly hilly (Vukomeričke Gorice = D2) areas, 110–180 m above sea level. The third one (D3), north of Zagreb, was the Medvednica Mountain region, a locality called Ponikve, ca 500 m above sea level. The soil type in the districts belongs to eutric brown soil, washed-out brown soil (sols bruns lessivés) and calc brown soil (calc Braunerde), respectively. The forests – the communities of *Quercus robur**) (D1) and *Quercus petraea*/*Carpinus betulus* (D2 and D3) – showed a moderate degree of exploitation and of decline. *Pinus strobus* and *Picea abies* were artificially grown in small stands within natural forests. Most plants of *Acer campestre*, *Cornus* sp., *Corylus avellana*, *Crataegus* sp., *Pyrus pyraeaster* and *Sambucus nigra* were 20 to 40 years old, while others were up to 100 or (some deciduous trees) even older (Table 1).

Experiments with soil samples were performed, in general, according to BÜTTNER & NIENHAUS 1989a. The samples, each weighing 6–8 kg, were taken at the stem base of a single tree or shrub, together with cut roots of the plant, from 20–50 cm depth under the soil surface, and at least 20 m away from the investigated water courses. Healthy bait plants *Chenopodium quinoa* Willd., *Cucumis sativus* L. 'Delicatess', *Nicotiana megalosiphon* HEURCK. et MUELL. and *Plantago lanceolata* L. were planted in each soil sample in a greenhouse at the few or (*C. quinoa*) several true leaves stage. Five to six weeks later the roots of all the bait plants of a given sample were crushed together in liquid nitrogen, homogenized in 30 mM Sørensen's phosphate buffer pH 7.0 and inoculated into common test plants.

Water samples, collected simultaneously with soil samples, originated from two forest brooks in district D3 and one in D2, and from two ditches in D1. Besides, silt samples were taken at the same time from the latter three water courses in D1 and D2 districts. Water samples, of 250 ml, were centrifuged for 100 min at 90,000 × g. The pellets were resuspended in 0.25 ml of the mentioned buffer, centrifuged 15 min at 2,500 × g and the supernatants inoculated onto test plants. In addition, suspensions of pellets obtained after only low speed centrifugation of some samples of the quoted volume at 4,000 × g (PIAZZOLLA & al. 1986) and 2 ml samples of untreated water silt were used as inocula.

Tobacco necrosis virus (TNV) was serologically examined by the electron microscope serology 'decoration' test (WALKEY 1991). Tobamoviruses were purified by PEG precipitation from systemically infected tobacco leaves, and analysed by the double radial immunodiffusion method. The reference reactants were typical tobacco mosaic (TMV) and tomato mosaic (ToMV) viruses, and their rabbit polyclonal antisera with homologous titres of 1/256 (MAMULA & al. 1974). Investigations of tobamovirus cell inclusions were performed by light microscopy. In order to ascertain the presence and morphology of virus particles, electron microscope examination of negatively

*) Complete names of forest (woody) plants are given in Table 1.

stained 'quick-dip' preparations of infected leaf tissue and purified preparations was carried out (WALKEY 1991).

Results

Detection of TNV in forest soil

In all virus isolates recovered from soil in forest districts D1, D2 and D3 an identical test plant reaction was found. The symptoms in inoculated

Table 1

Distribution of tobacco necrosis virus (TNV) in soil/root samples taken near woody forest plants in central Croatia*)

District	Plant species	No. of samples	No. of virus positive samples
D1	<i>Acer campestre</i> L.	2	2
	<i>Alnus glutinosa</i> (L.) Gaertn.	2	2
	<i>Carpinus betulus</i> L.	5	3
	<i>Cornus</i> sp.	2	—
	<i>Corylus avellana</i> L.	1	1
	<i>Crataegus</i> sp.	2	1
	<i>Pyrus pyraaster</i> Burgsd.	2	2
	<i>Quercus robur</i> L.	9	6
D1 Total		25	17
D2	<i>Betula pendula</i> Roth	5	3
	<i>C. betulus</i>	1	1
	<i>Castanea sativa</i> Mill.	5	2
	<i>C. avellana</i>	1	—
	<i>Fagus sylvatica</i> L.	6	4
	<i>Larix decidua</i> Mill.	2	1
	<i>Picea abies</i> (L.) Karsten	3	2
	<i>Pinus strobus</i> L.	2	1
	<i>Populus</i> sp. div.	3	2
	<i>Prunus avium</i> L.	1	1
	<i>Quercus petraea</i> (Matt.) Liebl.	2	2
<i>Robinia pseudacacia</i> L.	3	3	
<i>Sambucus nigra</i> L.	1	—	
D2 Total		35	22
D3	<i>A. glutinosa</i>	2	—
	<i>C. betulus</i>	2	—
	<i>Populus tremula</i> L.	1	1
	<i>Q. petraea</i>	4	—
	<i>R. pseudacacia</i>	1	—
D3 Total		10	1
D1, D2, D3 TOTAL		70	40

*) For explanations see the text.

leaves included very prominent and rapidly appearing and necrotizing lesions in 2–3 days on *Chenopodium amaranticolor* Coste et Reyn. and *C. quinoa* (Fig. 1a), and necrotic and chlorotic flecks and dots on *Nicotiana glutinosa* L., *N. megalosiphon* and *N. tabacum* L. 'Samsun'. Often, systemic necrotic flecks developed in *N. benthamiana* Domin. This indicated that in all cases, that is from more than half of the investigated soil samples (Table 1), only one virus was isolated. According to the symptomatology, it was very similar to TNV. Lesions from *C. quinoa* contained spherical particles of ca 30 nm in diameter (Fig. 1b). An isolate selected at random showed a weak decoration with antibodies to TNV strains A, NFT and B and none with those to D strain.

Detection of TMV and ToMV in forest brooks and ditches

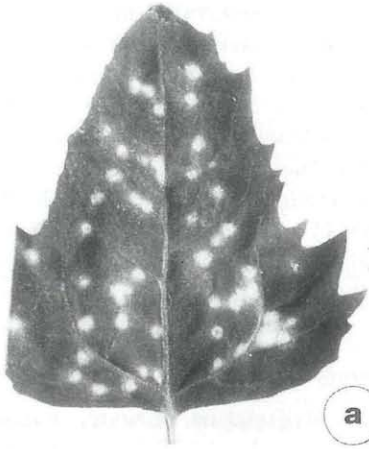
Sediments obtained by high speed centrifugation of water samples from the five water courses in D1, D2 and D3 districts yielded virus isolates which provoked local lesions in *C. amaranticolor* and *C. quinoa*, and especially characteristic necrotic ones in *N. glutinosa* and *Datura stramonium* L. (Fig. 1c). Systemic symptoms developed in *N. megalosiphon* and *N. tabacum* 'Samsun'. Additionally, two of the isolates (from D3) induced sporadic systemic chlorotic flecks and some malformation in leaves of both *Chenopodium* species.

Symptomatically, the latter isolates resembled ToMV and, somewhat, ribgrass mosaic virus, while the other three, from D1 and D2, resembled TMV. All isolates produced cell inclusions in the form of hexagonal prisms and also, sporadically, paracrystalline needles. The sap of infected plants and purified virus suspensions contained rod-shaped virus particles mostly 300 nm long (Fig. 1d). When tested serologically with TMV-antiserum the three TMV-like isolates did not differ from each other or from typical TMV (Fig. 1e). However, distinct spurs were formed by their precipitation lines over the lines of the two isolates from D3 and of typical ToMV. With ToMV-antiserum analogous reactions were obtained (Fig. 1f). In them, spurs were formed by the latter two isolates as well as by typical ToMV. Accordingly, typical TMV and ToMV were present in forest waters.

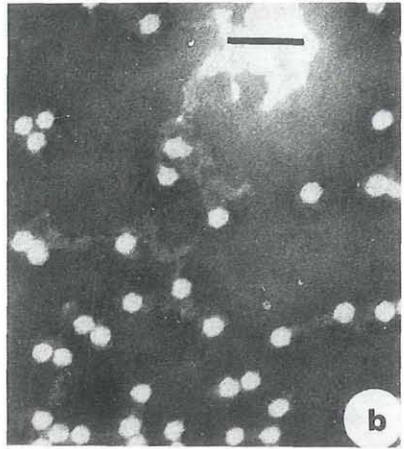
In addition, TMV was recovered from sediments of low speed centrifuged water samples. Among the sediments prepared from three water courses of districts D1 and D2 TMV was drawn from two, i.e. from one ditch and one brook. Native silt samples from the latter two water courses but not from the third one also caused symptoms of TMV.

Discussion

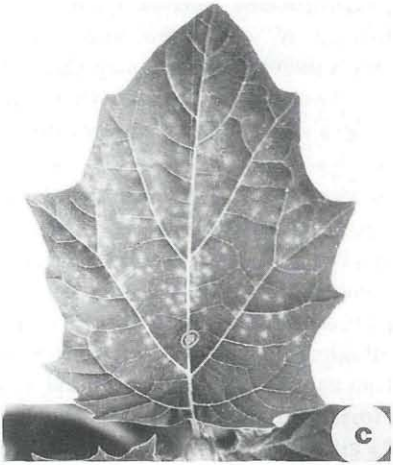
TNV isolates recovered from soil in the course of these investigations were not associated with a particular plant species (Table 1). Because of the virus stability it is likely that the most of the isolates recovered originated



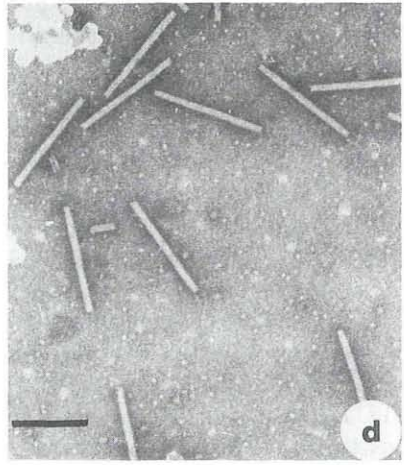
a



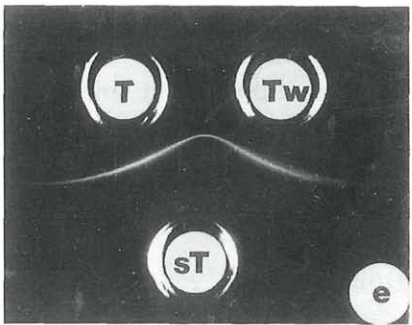
b



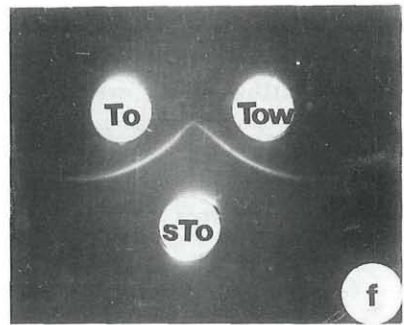
c



d



e



f

from the virus contained in the soil itself at the moment of sample taking. Compared to ca 2% of soil samples containing TNV in German forest ecosystems (BÜTTNER & NIENHAUS 1989a) our results revealed a conspicuously high rate, i.e. ca 57% of the samples harboured TNV. This could be due to differences in the properties of the viruses and in environmental conditions in Germany and Croatia or, partly, to the experimental conditions.

Indeed, TNV isolates from Croatia differed from the German TNV isolates which, like *Chenopodium* necrosis virus (TNV-CN) from England (TOMLINSON & al. 1983), additionally provoked systemic symptoms in *C. quinoa* (BÜTTNER & NIENHAUS 1989a). The low frequency of virus occurrence in the soil of district D3 could also be influenced by environmental factors, such as a lower level of moisture in that soil (cf. KEGLER & al. 1991). It is likely that the Croatian TNV isolates, at least the one examined serologically, represent a variant of TNV. The present isolation from soil samples of no virus other than TNV could be due to the fact that in the joint bait plant inoculum, we used, some of the less infectious viruses might have been too diluted to infect test plants.

Tobamoviruses found in surface water (KOENIG 1986, HORVÁTH & al. 1986) include ToMV and some additional viruses other than TMV occurring in the water of forest ecosystems (BÜTTNER & NIENHAUS 1989b, JACOBI & CASTELLO 1991). Based on symptomatological, serological and cytopathological identification criteria (cf. MAMULA & al. 1974) our results give evidence of the presence of TMV in the latter medium as well.

The present recovery of TMV from pellets obtained by low speed centrifugation of water samples is congruent with the results of PIAZZOLLA & al. 1986 and their hypothesis that TMV is at least partially adsorbed to soil particles. This is supported by a finding of JURETIĆ & HORVÁTH 1991 of the high extent of TMV adsorption to sandy and humic soil. Moreover, we were able to extract TMV from water silt without any concentration.

We do not know definitely how TMV and ToMV appeared in the water, while they were not detected in the surrounding soil, or why TNV was not

Fig. 1. Symptoms, virus particles and serological reactions of viruses found in forest soil and water in Croatia. – a: necrotic lesions in a *Chenopodium quinoa* leaf inoculated with a TNV isolate from soil. – b: TNV particles from living border tissue of the lesions as in 1a; bar = 100 nm. – c: *Datura stramonium* leaf inoculated with a TMV isolate from ditch water showing pin point necrotic lesions with a chlorotic halo which soon becomes necrotized. – d: particles of the 1c TMV isolate from leaf sap of systemically infected „Samsun“ tobacco; bar = 200 nm. – e: Serological reactions of 1c TMV isolate (Tw) and typical TMV (T) with antiserum to T (sT). – f: serological reactions of a ToMV isolate from brook water (Tow) and ToMV typical (To) with antiserum to To (sTo). Figs. e and f show reaction of serological identity. For other explanations see the text.

found in water, although it was widespread in the soil. The possible source for the occurrence of tobamoviruses in the water courses could be infected woody and herbaceous plants growing in some kind of ecological niche near the courses, with virus release from their living roots into water occurring (HOLLINGS & al. 1977, TOMLINSON & al. 1983, cf. KEGLER & KEGLER 1981, KOENIG 1986). As stated before, TMV can be abundantly adsorbed at least to some soil types (JURETIĆ & HORVÁTH 1991, TRIOLO & MATERAZZI 1992); this is possibly the case with some other tobamoviruses as well. Thus, the surface washing out of locally scattered decaying infected plant residues and of the related forest soil surface layer also could bring the viruses into the water courses. By analogy with the related TNV-CN (TOMLINSON & al. 1983), TNV seems to be easily washed out from the soil (by drainage water), and probably passes through deeper soil layers (cf. KEGLER & al. 1991). Thus, a good deal of the virus could be lost before it reaches the water courses. This could result in an infective virus content too low to be recovered from the sample volumes we used.

We estimate the influence of virus infections from agricultural areas on the occurrence of TNV and tobamoviruses in the investigated forest soil and water, respectively, to be negligible. This can be supported by the instance of district D3 where such an influence can be ruled out because of the greater remoteness of agricultural areas and the district's height and configuration. The high frequency, in general, of TNV occurrence in the soil and the comparatively high TMV and ToMV concentration in the water of the three districts can also favour such a conclusion.

The role of clay minerals in virus adsorption to soil particles and retention of infectivity was emphasized (KEGLER & al. 1991, PIAZZOLLA & al. 1993). The absence of the isolation of some viruses from soil and water by the procedures used, together with the importance of considering the seasonal variation of virus content in both media, has already been discussed (NIENHAUS & CASTELLO 1989, JACOBI & CASTELLO 1991).

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