ISOLATION OF TOBACCO NECROSIS VIRUS FROM STRAWBERRY LEAVES IN THE CZECH REPUBLIC

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Summary. – Leaves of symptomless *Fragaria ananassa* Duch cv. Čačanská raná were grafted onto *Fragaria vesca* indicator clones. Thirty-five of 72 grafted indicator plants developed leaf mottle symptoms. Isometric virus-like particles were observed in purified preparations from symptomatic leaves of *F. vesca*. The latter were mechanically inoculated to herbaceous host plants. A virus was successfully purified from *Nicotiana occidentalis* 37 B symptomatic plants by differential and sucrose density gradient centrifugations and a polyclonal antiserum to the virus was prepared. On the basis of serological reactions, symptomatology on herbaceous hosts and electron microscopy studies the virus was identified as tobacco necrosis virus (TNV) D-strain. This is the first isolation of TNV from strawberry leaves and its first finding on strawberry in the Czech Republic. The new experimental hosts *N. aucalis*, *N. bentamiana*, *N. occidentalis* 37 B (systemic hosts), and *Ammobium alatum*, *N. bigelovi*, *Petunia hybrida* (local hosts) for TNV are reported. These results may not exclude the presence of strawberry mottle virus as a causal agent of mottle symptoms in the tested plant samples. Further research is necessary to clarify the aetiology of the strawberry mottle.

Key words: tobacco necrosis virus; fragaria; virus isolation; electron microscopy; enzyme-linked immunosorbent assay; strawberry mottle

Introduction

TNV belongs to the *Necrovirus* genus according to its isometric particles of about 26 nm in diameter, single-stranded linear genomic RNA of positive polarity, and single 30 K coat protein (Brunt *et al.*, 1995).

TNV was first found in *Nicotiana tabacum* and *N. glutinosa* seedlings in Cambridge, England (Smith, 1937). It was later demonstrated to be soilborne and transmitted by the zoospores of the chytrid fungus *Olpidium brassicae* (Teakle, 1962). It was common in irrigated fields and in unsterilized soil in glasshouses (Kassanis, 1970). Although TNV is readily transmitted by mechanical inoculation to a wide range of plants, usually does not infect them systemically and causes only a few diseases, e.g. tulip necrosis, bean stipple streak, ABC disease of potato tubers, and a form of cucumber necrosis (Brunt *et al.*, 1995; Kassanis, 1964, 1970).

Twenty-nine viral diseases of strawberry plants have been so far detected and it is probable that still further diseases will be found (Converse, 1987; Spiegel *et al.*, 1993). Strawberry viruses occur naturally in complexes and low concentration in plant tissues. Their isolation and transmission to herbaceous host plants is very problematic. Therefore even antisera for the detection of fundamental viruses in strawberry plants (strawberry mottle, crinkle, vein banding and mild yellow edge viruses) are not commercially available and strawberry is currently indexed for viruses by time-consuming leaf-graft bioassay on indicator plants.

Abbreviations: BSA = bovine serum albumin; DAS-ELISA = double-antibody sandwich enzyme-linked immunosorbent assay; PBS = phosphate-buffered saline; PVP = polyvinyl pyrrolidone; SMV = strawberry mottle virus; TNV = tobacco necrosis virus

Strawberry mottle is one of the most important and widespread virus diseases occurring wherever strawberries are grown. Many virus strains and variants were described on the basis of symptoms on *F. vesca* indicator clones varying from barely discernible mottle to severe degeneration (Mellor and Krczal, 1987). This disease has been associated with a virus transmissible by grafting, aphids (semi-persistent way) (Frazier, 1968; Frazier and Sylvester, 1960) and mechanical inoculation (Adams and Barbara, 1986; Leone *et al.*, 1992, 1995; Polák and Bezpalcová, 1988; Hepp and Converse, 1990). Isometric virus particles of variable size (14 – 37 nm) have been described by different authors for strawberry mottle virus (SMV) isolates (Polák and Jokeš, 1992; Hepp and Converse, 1990; Kitajima *et al.*, 1971; Leone *et al.*, 1992, 1995; Yoshikawa and Converse, 1991). Because of difficulties so far encountered in many laboratories in thorough purifying of SMV, the virus is still virtually uncharacterised and unidentified (Converse, 1992; Leone *et al.*, 1995). According to Mellor and Krczal (1987), the term „mottle" is used to characterise the involvement of numerous strains of a single virus. However, this possibility was not yet confirmed.

A leaflet graft to *F. vesca* from a plant of *F. ananassa* Duch. cv. Čačanská raná imported to the Czech Republic from former Yugoslavia in 1989 revealed typical mottle symptoms. For the detection, identification, isolation and characterisation of viruses present in symptomatic indicator clones, electron microscopic examination, virus purification, mechanical transmission to herbaceous host plants and enzyme-linked immunosorbent assay (ELISA) were applied. A preliminary report of this work has been already published (Fránová-Honetšlegrová *et al.*, in press).

Materials and Methods

Origin of samples and grafting. Symptomless *F. ananassa* Duch. cv. Čačanská raná was imported to the Czech Republic from former Yugoslavia in 1989. The leaves were grafted onto indicator clones of *F. vesca* FV-72, EMC, EMK, UC-2 and UC-6 (12 plants per clone), UC-5 (8 plants) and UC-4 (4 plants) for virus indexing. The symptoms were evaluated at 2 – 5 weeks after grafting and observed for 1 year.

Virus purification. Leaves from graft-inoculated *F. vesca* FV-72 (50 g) and mechanically infected *N. occidentalis* Wheeler, accession 37 B (100 g) plants as well as from healthy controls were homogenised with phosphate-buffered saline (PBS) pH 7.4 containing 0.1 g/l polyvinyl pyrrolidone (PVP), 0.1 g/l bovine serum albumin (BSA), 0.25 ml/l Triton X-100, 1 mol/l urea and 0.001 g/l thioglycolic acid. The homogenate was filtered and the sap was clarified by stirring with 6% suspension of Mg-activated bentonite (Dunn and Hitchborn, 1965). The supernatant was subjected to 2 cycles of differential centrifugation (29,000 rpm for 2 hrs, 7,000 rpm for 10 mins, 50,000 rpm for 1hr, 12,000 rpm for 10 mins). Final pellets were resuspended in 0.02 mol/l phosphate buffer pH 7.2 and centrifuged at 6,000 rpm for 10 mins. Further purification was conducted by density gradient centrifugation through 10 – 40% sucrose in 0.02 mol/l phosphate buffer pH 7.2 at 24,000 rpm for 4 hrs in Beckman SW 28 rotor. Virus bands were collected and concentrated by centrifugation at 50,000 rpm for 1 hr in Beckman 50.2 Ti rotor.

Mechanical transmission. Young symptomatic leaves of *F. vesca* indicator plants were ground in 0.066 mol/l phosphate buffer pH 7.0, and the resulting saps were rubbed onto carborundum-dusted leaves of *Chenopodium quinoa* Willd. Moreover, partially purified preparations from strawberry leaves (supernatants collected before the sucrose gradient centrifugation) were diluted with 0.02 mol/l phosphate buffer pH 7.2 (1:4 v/v) and used for mechanical inoculation of *C. quinoa*, *N. aucalis*, *N. benthamiana* Domin. and *N. occidentalis* 37 B. Subsequent mechanical transfer from these plants to herbaceous hosts was done by sap extracts in 0.066 mol/l phosphate buffer pH 7.0.

Electron microscopy. Crude sap preparations from diseased and healthy plants as well as purified sap preparations negatively stained with 2% uranyl acetate were examined under Philips EM420 or Jeol 100 MB transmission electron microscopes.

Serological tests. An antiserum was prepared from a rabbit immunised with one subcutaneous and one intramuscular injection followed by 3 intravenous injections given at weekly intervals. For each injection, about 0.1 mg of purified virions was used. A double diffusion serological test in gel (Ouchterlony) was done in 0.07 g/l Difco Noble agar in 0.018 mol/l McIlvaine buffer pH 7.0 containing 0.002 g/l sodium azide.

Isolation of IgG, its conjugation with alkaline phosphatase and double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) were described by Clark and Adams (1977). The coating was done with 1.0 – 2.5 mg/l IgG, the leaf tissue was homogenised in 0.02 mol/l PBS pH 7.4 containing 0.5 ml/l Tween, 0.1 g/l PVP, and the conjugate was diluted to 1:500 or 1:1000 (v/v).

Identification of the purified virus was accomplished by testing of the prepared antiserum against a range of small fruit viruses including fragaria chiloensis (Spiegel *et al.*, 1993), raspberry bushy dwarf, strawberry mottle (Yoshikawa and Converse, 1991), strawberry crinkle, strawberry vein banding, strawberry mild yellow edge, tobacco necrosis (isolates A, B, C, D, and F), tobacco streak, tomato ringspot, alfalfa mosaic, blueberry shock, blueberry scorch and cucumber mosaic viruses from the collection held at USDA-ARS-HCRL, Corvallis, OR, USA, and strawberry latent ringspot, raspberry ringspot, tomato blackring and arabis mosaic viruses obtained from DSMZ, Braunschweig, Germany under an APHIS permit. The virus isolated from strawberry was tested with antisera to fragaria chiloensis, raspberry bushy dwarf, strawberry mild yellow edge, tobacco necrosis, tobacco streak, tomato ringspot, cucumber mosaic, alfalfa mosaic, blueberry shock and blueberry scorch viruses from the collection held at USDA-ARS HCRL. Commercial kits (Loewe Biochemica, Germany) for arabis mosaic, cucumber mosaic, strawberry latent ringspot, raspberry ringspot and tomato black ring viruses were used according to the manufacturer's instructions.

Results

Grafting onto indicator clones

Thirty-five of 72 *F. vesca* indicator plants grafted with leaves of *F. ananassa* Duch. cv. Čačanská raná revealed leaf mottle symptoms. The first symptoms usually appeared on the youngest developing leaf 8 – 20 days after grafting. Such a leaf showed mottle occasionally combined with irregular vein clearing, deformation and dwarfing. Sporadically, a few dead mature leaves were observed on *F. vesca* clones some months after grafting. Symptoms developed most frequently on FV-72, EMC, UC-5 and EMK clones.

Mechanical transmission

Repeated attempts were made to mechanically transmit the agent from crude sap of *F. vesca* leaves with mottle symptoms to *C. quinoa* but without success. Therefore, a partially purified virus preparation was used as inoculum for mechanical transmission. Five days after inoculation, a few chlorotic/necrotic lesions developed on the inoculated leaves of *C. quinoa*, *N. aucalis*, *N. benthamiana* Domin. and *N. occidentalis* 37 B plants. Subsequently, *N. aucalis* and *N. occidentalis* 37 B produced mild systemic vein clearing and mosaic. *N. benthamiana* produced systemic necrosis and collapse of young leaves. The virus was successfully subtransferred by mechanical inoculation to other herbaceous hosts. The symptoms on *C. quinoa*, *N. aucalis*, *N. benthamiana* and *N. occidentalis* 37 B were the same as those on the initially inoculated plants, only local necrotic lesions on inoculated leaves were very conspicuous. *Ammobium alatum* R. Brown, *C. album* L., *N. bigelovi*, *N. clevelandii* Gray, *N. rustica* L., *N. tabacum* L. cv. Samsun, *N. tabacum* L. cv. White Burley, *N. tabacum* L. cv. Xanthi, *Petunia hybrida* Hort ex Vilm., *Phaseolus vulgaris* L. cv. Blanka, and *Tetragonia expanza* Murr. revealed severe local necrotic lesions on inoculated leaves too. No symptoms were observed on *Cucumis sativus* L., *Lactuca sativa* L. and *Spinacea oleracea* L. No symptoms developed on *C. quinoa* plants inoculated with partially purified preparations from healthy strawberry controls.

Electron microscopy and virion purification

Occasionally, isometric virus-like particles of about 21 nm in diameter were observed on negatively stained leaf dip preparations from affected *F. vesca* plants.

To develop a purification procedure for virus particles from symptom-bearing *F. vesca* leaves we tested several methods of sap clarification. Procedures using organic solvents like chloroform, n-butanol or their mixture completely destroyed the sap infectivity. Only partial purificates from strawberry leaves could be obtained. Numerous isometric virus-like particles of about 21 nm in diameter (Fig. 1) were observed in these purificates which were found to be a good inoculum for mechanical transmission of the virus to herbaceous host. Unfortunately, we could not find virus-specific peaks in UV absorption profiles of the purificates obtained after sucrose density gradient centrifugation. Only a sporadic occurrence of isometric virions was detected in the gradient fractions centrifugation and in final concentrates of the extracts.

Isometric particles of about 25 nm in diameter were detected in negatively stained preparations from crude sap of mechanically infected herbaceous hosts.

From 631 g of infected leaves of *N. occidentalis* 37 B 0.5938 mg of virus was obtained. The purified virus had a UV absorption spectrum characteristic for nucleoprotein ($A_{260/280}$ = 1.72) with a maximum between 259.5 and 262 nm and a minimum between 244 and 246 nm. Just one virus band was observed in sucrose density gradients. Electron micrographs of the virus purificates showed numerous isometric particles of about 25 nm in diameter and slightly hexagonal outline (Figs. 2 and 3). No virus-like particles were observed in negatively stained preparations from healthy controls.

Serological tests

In the double diffusion test, the polyclonal antiserum had a homologous titre of 1:512 against crude sap of mechanically infected host plants, virions purified from graft-inoculated *F. vesca* clones with mottle symptoms, and purified preparations from *N. occidentalis* 37 B. Only a weak reaction was detected with crude sap of symptom-bearing *F. vesca* leaves and antiserum diluted 1:1. No reaction was observed with corresponding healthy controls.

About 7.14 mg of IgG was purified per ml of the crude antiserum. A positive reaction was obtained in DAS-ELISA with partially purified virus preparation from leaves of *F. vesca* and *N. occidentalis* 37 B, crude sap from infected herbaceous host plants, and leaves of indicator clones with mottle symptoms. A negative reaction was obtained with partially purified preparations from healthy strawberry leaves, crude sap from healthy *N. occidentalis* 37 B, and healthy *F. vesca* clones. A_{405} values of DAS-ELISA for partially purified preparations from infected *F. vesca* and *N. occidentalis* 37 B tissues (diluted in distilled water 1: 4 (v/v)) and crude sap from mechanically inoculated herbaceous host plants were of about 1.7. A_{405} values of DAS-ELISA for crude sap of *F. vesca* clones with mottle symptoms varied from 0.15 to 0.50. Mean A_{405} values for partially purified preparations from healthy strawberry plants and crude sap from healthy strawberry and healthy herbaceous host ranged from 0.00 to 0.15.

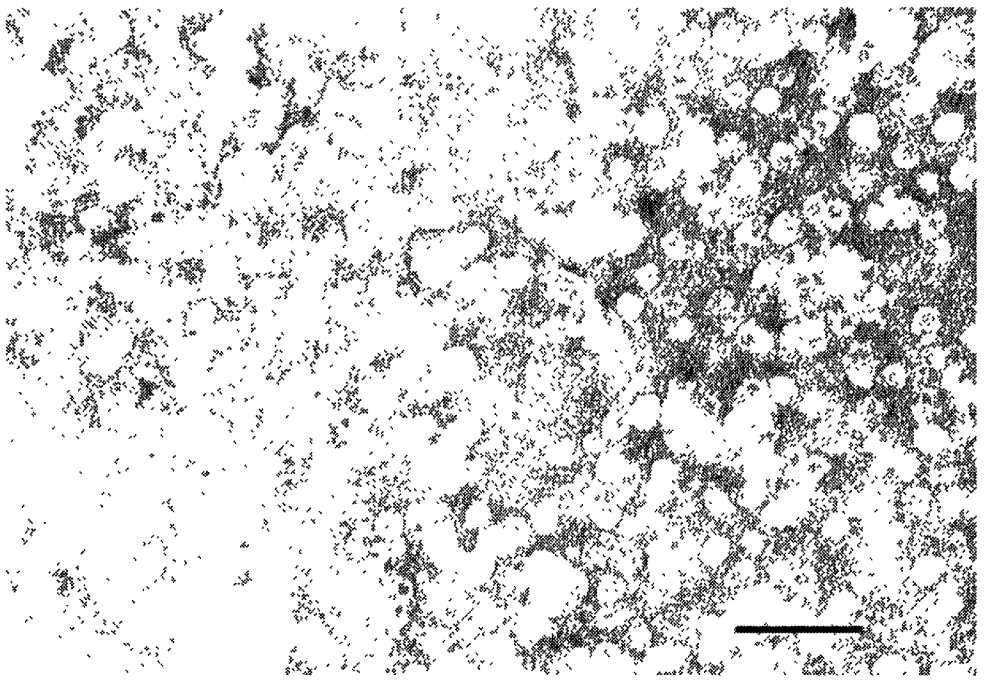

Fig. 1
Electron micrograph of partially purified virus-like particles of 21 ± 2 nm in diameter from *F. vesca* FV-72 clone
Bar = 100 nm

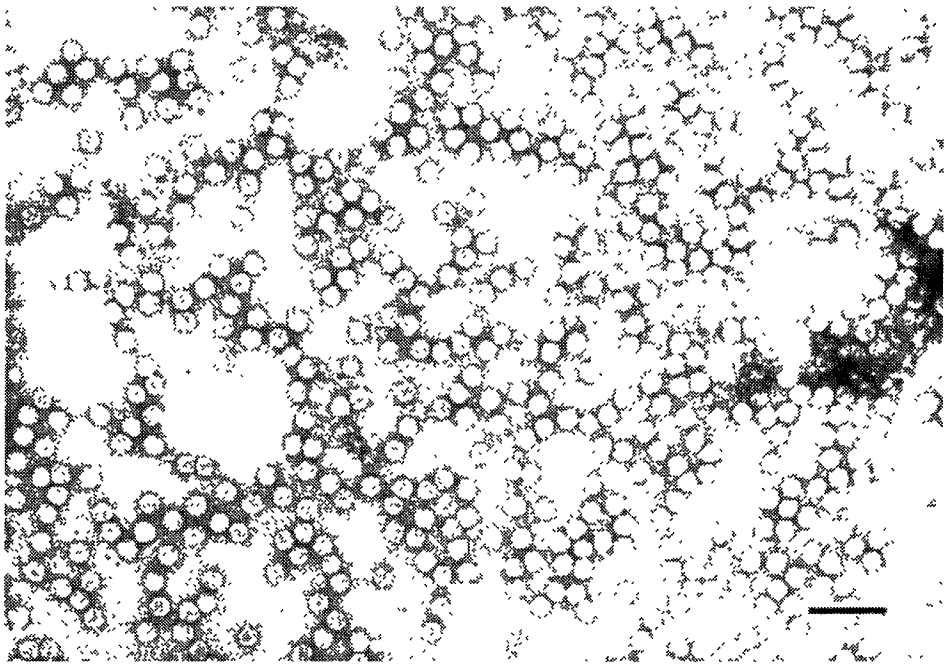

Fig. 2
Electron micrograph of purified virus-like particles of about 25 nm in diameter from *Nicotiana occidentalis* Wheeler accession 37 B leaf tissue systemically infected with TNV
Bar = 100 nm.

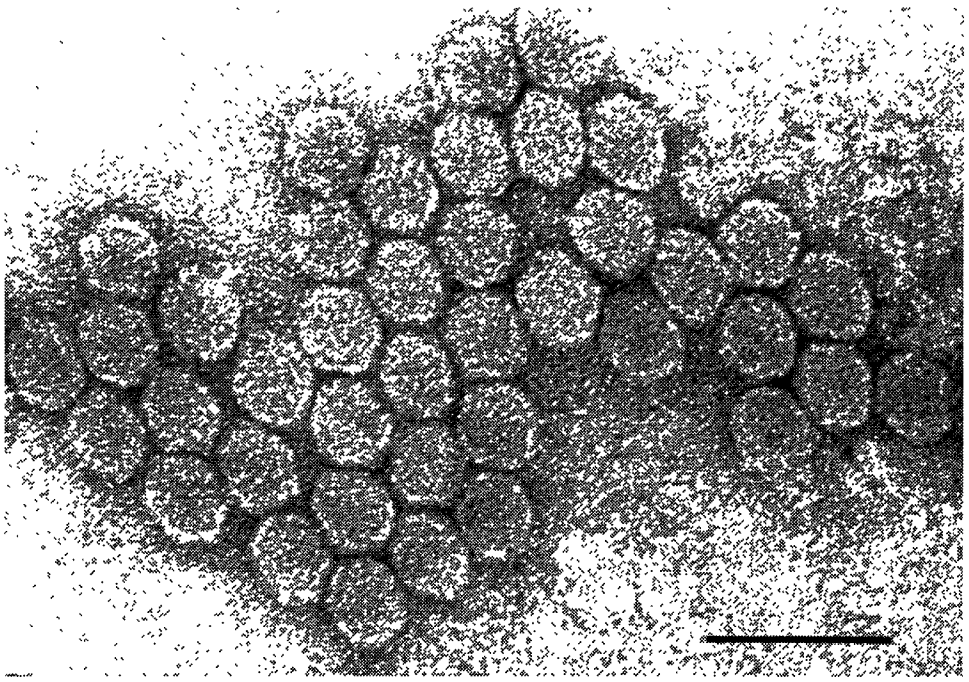

Fig. 3
Detail of isometric particles of TNV of slightly hexagonal outline
Bar = 50 nm.

With regard to the properties of the isolated virus, above all its symptomatology and host range-indicated similarities with TNV, 5 different isolates of this virus were tested against the prepared antiserum. The antiserum reacted strongly with the 14F and 14D isolates. No positive reaction was obtained in DAS-ELISA with other isolates tested.

Discussion

Although the graft-inoculated *F. vesca* clones revealed typical mottle symptoms, we did not succeed in isolation of a virus conformable to strawberry mottle virus (SMV) described by Mellor and Krczal (1987). Local lesions were the only symptoms on the mechanically inoculated *C. quinoa,* while Leone *et al.* (1995), Adams and Barbara (1986), Frazier (1968), Polák and Bezpalcová (1988), and Yoshikawa and Converse (1991) considered *C. quinoa* a systemic host. However, more than one virus may be involved in the term „mottle", and SMV is often accompanied by other viruses (Converse, 1987).

TNV has been first reported in *Fragaria* in Arkansas (Fulton, 1952) and California (Frazier, 1955), USA, primarily as a greenhouse disease of *Fragaria vesca* indicator root system, and later in cultivated strawberries in the field in Italy (Faccioli, 1969), Bulgaria (Yankulowa and Schmelzer, 1974), and Japan (Kaname and Kishi, 1973; Komuro *et al.*, 1973). TNV occurs commonly in roots of strawberry (Converse *et al.*, 1987; Faccioli, 1969, 1970, 1974), but its location in leaves of *Fragaria* is questionable. We believe, according to our results from DAS-ELISA, electron microscopy, mechanical transmission and symptoms in host plants that we have detected and isolated TNV from strawberry leaves.

In several standard *F. vesca* indicator clones, this virus is associated with premature death of older leaves (Converse *et al.*, 1987). In our experiments, graft-inoculated indicators did not develop or did only sporadically necrotic older leaves typical for TNV infection. The dominant symptoms were leaf mottle occasionally combined with irregular vein clearing, leaf deformation and dwarfing. In view of the fact that the presence of SMV could not be ruled out, it is probable that the mottle symptoms observed on *F. vesca* clones in our experiments could have been caused by SMV without any interaction with TNV. In addition, we were not able to reproduce the mottle disease symptoms using back transmission of TNV from herbaceous hosts to strawberry indicators by sap inoculation or aphids *Chaetosiphon fragaefoli* Kaltb. (data not shown).

Concerning the difference in size of isometric particles observed in preparations from strawberry leaves and *N. occidentalis* 37 B plants, the variation in size of TNV virions has been already described. The diameter of TNV-D strain virions purified directly from strawberry in Italy varied from 30 to 38.5 nm in shadow-casted preparations (Faccioli, 1969, 1970). However, the examination of the same TNV isolate after transmission to *C. amaranticolor* Coste et Reyn. revealed a diameter of 25 – 26 nm in preparations negatively stained with 2% phosphotungstate buffer pH 5 (Faccioli, 1971, 1974). It seems, in agreement with our experience, that the condition of the virus or the way the specimens are prepared for electron microscopy may influence the apparent size of TNV particles (Babos and Kassanis, 1963).

The symptomatology of herbaceous hosts was very similar to those described for TNV (Brunt *et al.*, 1995). Moreover, Babos and Kassanis (1963) differentiated 7 strains of TNV on the basis of symptoms caused in French bean and young cucumber plants. The virus isolated from strawberry revealed small, round, dark reddish-brown necrotic local lesions on *Phaseolus vulgaris*. These lesions remained discrete and did not spread along the veins which is a characteristic of the D strain of TNV.

TNV may occur in infected plants as free RNA as well as intact virions (Faccioli, 1971, 1974). Therefore, ELISA results for TNV in infected sap from strawberry tissue are often faint or even negative (Converse *et al.*, 1987). Moreover, in ELISA, the viruses are in contact with inhibitory and denaturing compounds present in strawberry leaf tissue for a relatively long period of time, while during purification, the viruses are separated from the majority of these compounds rather quickly and therefore may remain serologically more active in the resulting sap (Martin and Converse, 1985). This could explain the low DAS-ELISA values of some infected strawberry tissues and the high DAS-ELISA values of infected herbaceous host plant and purified virus preparations in our experiments.

In conclusion, on the basis of the presented results we believe that this is the first isolation of TNV from strawberry leaves and the first detection of TNV in strawberry in the Czech Republic. Also new experimental hosts, namely *N. aucalis*, *N. bentamiana*, *N. occidentalis* 37 B (systemic hosts), and *Ammobium alatum*, *N. bigelovi*, *Petunia hybrida* (local hosts) for TNV are reported. Nevertheless, our investigation does not exclude SMV as a causal agent of mottle symptoms in the tested plants. Further research is necessary to clarify the aetiology of this disease.

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References

Adams AN, Barbara DJ (1986): Transmission of a virus from Fragaria vesca infected with strawberry mottle virus to *Chenopodium quinoa*. *Acta Hortic.* **186**, 71–76.

Babos P, Kassanis B (1963): Serological relationships and some properties of tobacco necrosis virus strains. *J. Gen. Microbiol.* **32**, 135–144.

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L (Eds) (1995): Tobacco necrosis necrovirus. In *Viruses of Plants*. University Press Cambridge, pp. 1256–1259.

Clark MF, Adams AN (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* **34**, 475–483.

Converse RH (1992): Modern approaches to strawberry virus research. *Acta Hortic.* **308**, 19–30.

Converse RH (Ed.) (1987): *Virus Diseases of Small Fruits. Agricultural Handbook.* No. **631**. Government Printing Office, Washington, DC.

Converse RH, Martin RR, Tanne E, Spiegel S (1987): Tobacco necrosis virus in strawberry. In Converse RH (Ed) *Virus Diseases of Small Fruits. Agricultural Handbook.* No. **631.** Government Printing Office, Washington, DC, pp. 64–66.

Dunn DB, Hitchborn J (1965): The use of bentonite in the purification of plant viruses. *Virology* **25**, 171–192.

Faccioli G (1969): Caratteristiche sintomatologiche e principali proprieta fisiche di un virus isolato da radici di fragola (*Fragaria vesca* L.) e della sua variante instabile. *Phytopathol. Mediterr.* **8**, 162–170.

Faccioli G (1970): Necrosi della rosetta: Nuova infezione de virus sulla fragola. *IV. National Strawberry Conference*, Cesena, Italy, pp. 351–355.

Faccioli G (1971): A new strawberry necrotic virus (SRNV): biophysical characterization of a free infectious ribonucleic acid. In *Atti 1° Congr. naz. Virologia*, Parma-Salsomaggiore, pp. 17–32.

Faccioli G (1974): Identificazione e caratteristiche fisico-cimiche di un ceppo di virus della necrosi della tobacco isolato di fragola. *Phytopathol. Mediterr.* **13**, 71–76.

Fránová-Honetšlegrová J, Erbenová M, Nebesářová J, Špak J, Martin RR (1998): Detection and identification of tobacco necrosis virus in strawberry leaves in the Czech Republic. *Acta Hortic.* **471**, 39–43.

Frazier NW (1955): Tobacco necrosis virus on strawberries. *Plant Dis. Reporter* **39**, 143–147.

Frazier NW (1968): Transmission of strawberry mottle virus by juice and aphids to herbaceous host. *Plant Dis. Reporter* **52**, 64–67.

Frazier NW, Sylvester ES (1960): Half-lives of transmissibility of two aphid-borne viruses. *Virology* **12**, 233–244.

Fulton JP (1952): A tobacco necrosis virus associated with strawberry plants. *Plant Dis. Reporter* **36**, 313–314.

Hepp RF, Converse RH (1990): Aphid transmission of strawberry mottle virus from *Chenopodium quinoa* to *Fragaria vesca*. *Plant Dis.* **74**, 320–321.

Kassanis B (1964): Properties of tobacco necrosis virus and its association with satelite virus. *Ann. Inst. Phytopathol. Benaki* **6**, 7–26.

Kassanis B (1970): Tobacco necrosis virus. *Description of Plant Viruses*. No. **14**. C.M.I./A.A.B., Kew, Surrey, England.

Kaname T, Kishi K (1973): On tobacco necrosis virus and tobacco mosaic virus isolated from strawberries. *Ann Phytopathol Soc. Jpn.* **39**, 134 (in Japanese).

Kitajima EW, Betti JA, Costa AS (1971): Isometric, viruslike particles in leaf tissues of *Fragaria vesca* L. infected with strawberry mottle virus. *Ciencia e Cult.* **23**, 649–655.

Komuro Y, Iwaki M, Makino T (1973): Occurrence of tobacco necrosis virus in roots of strawberry collected in the Watari District of *Miyagi* Prefecture. *Ann Phytopathol. Soc. Jpn.* **39**, 134 (in Japanese).

Leone G, Lindner JL, Schoen CD (1992): Attempts to purify strawberry viruses by non-convential separation methods. *Acta Hortic.* **308**, 121–129.

Leone G, Lindner JL, Schoen CD (1995): Unstable infectivity and abundant viral RNAs associated with strawberry mottle virus. *Acta Hortic.* **385**, 76–85.

Martin RR, Converse RH (1985): Purification, properties and serology of strawberry mild yellow-edge virus. *Phytopathol. Z.* **114**, 21–30.

Mellor FC, Krczal H (1987): Strawberry mottle. In Converse RH (Ed) *Virus Diseases of Small Fruits. Agricultural Handbook.* No. **631.** Government Printing Office, Washington, DC, pp. 10–16.

Polák J, Bezpalcová H (1988): Strawberry mottle virus in Czechoslovakia. *Acta Hortic.* **236**, 129–131.

Polák J, Jokeš M (1992): Cytoplasmic inclusions in leaves of *Chenopodium quinoa* Willd. infected with the strawberry mottle agent. *Acta Hortic.* **308**, 141–144.

Smith KM (1937): Studies on a virus found in the roots of certain normal-looking plants. *Parasitology* **29**, 70–85.

Spiegel S, Martin RR, Leggett F, ter Borg M, Postman J (1993): Characterization and geographical distribution of a new ilarvirus from *Fragaria chiloensis*. *Phytopathology* **83**, 901–905.

Teakle DS (1962): Transmission of tobacco necrosis virus by a fungus, *Olpidium brassicae*. *Virology* **18**, 224–231.

Yankulova M, Schmelzer K (1974): Determination of a mechanically transmitted strawberry virus in Bulgaria. In *Virusni Bolesti po Rasteniyata*. Bulgarian Academy of Sciences, pp. 33–42 (in Bulgarian).

Yoshikawa N, Converse RH (1991): Purification and some properties of strawberry mottle virus. *Ann. Appl. Biol.* **181**, 565–576.