

Phyton (Austria)	Vol. 25	Fasc. 2	241–251	30. 11. 1985
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A Study of two Isometric Viruses Infecting Turnip in Austria¹⁾

By

Djordje MAMULA and Nikola JURETIĆ*)

With 4 Figures (3 Plates)

Received August 28, 1984

Key words: Turnip yellow mosaic virus, radish mosaic virus, *Brassica rapa* var. *rapa*, turnip; Austria

Summary

MAMULA Dj. & JURETIĆ N. 1985. A study of two isometric viruses infecting turnip in Austria. – *Phyton* (Austria) 25 (2): 241–251, with 4 figures (3 plates). – English with German summary.

The paper deals with turnip yellow mosaic virus (TYMV) and radish mosaic virus (RdMV) isolated from turnip plants (*Brassica rapa* var. *rapa*) in Austria. A total of 8 virus isolates were studied: 3 isolates of TYMV, 3 isolates of RdMV and 2 isolates which represented mixtures of both viruses.

On basis of test plant reactions and serological experiments it was established that Austrian TYMV isolates were mutually identical and also closely related to strain 1 of TYMV. By the same criteria Austrian RdMV isolates were found to be identical with one another as well as with the European strain of RdMV. In addition, properties in vitro, inclusion bodies and type of virus particles of Austrian TYMV and RdMV were investigated. In turnip plants grown in fields between Villach and Klagenfurt, TYMV, RdMV and mixed infection of the two viruses were present in about 3%, 6% and 1% of specimens, respectively.

Zusammenfassung

MAMULA Dj. & JURETIĆ N. 1985. Untersuchungen von zwei isometrischen Viren, die Wasserrübe in Österreich befallen. – *Phyton* (Austria) 25 (2): 241–251, mit 4 Abbildungen (3 Tafeln). – Englisch mit deutscher Zusammenfassung.

¹⁾ Dedicated to our esteemed mentor professor Davor MILIČIĆ on the occasion of his 70th birthday.

*) Dr. Djordje MAMULA, Dr. Nikola JURETIĆ, Botanical Institute, Marulićev trg 20/II, YU-41000 Zagreb, Yugoslavia.

Die Arbeit befaßt sich mit Wasserrübelgelbmosaik-Virus (turnip yellow mosaic virus, TYMV) und mit Rettichmosaik-Virus (radish mosaic virus, RdMV), die in Wasserrüben (*Brassica rapa* var. *rapa*) in Österreich gefunden wurden. Insgesamt wurden 8 Virusisolate untersucht: 3 Isolate von TYMV, 3 Isolate von RdMV und 2 Isolate, die eine Mischung von beiden Viren darstellten.

Auf Grund der Reaktionen der Testpflanzen und der serologischen Versuche wurde festgestellt, daß TYMV-Isolate aus Österreich identisch miteinander und auch mit dem Stamm 1 des Virus waren. Nach denselben Kriterien sind die österreichischen RdMV-Isolate identisch miteinander sowie mit dem europäischen Stamm des RdMV. Außerdem, wurden die Eigenschaften *in vitro*, Zelleinschlußkörper und der Typ der Viruspartikeln der österreichischen TYMV und RdMV untersucht. In Wasserrübenfeldern des untersuchten Gebietes zwischen Villach und Klagenfurt waren TYMV, RdMV und die Mischinfektion an annähernd 3%, 6% und bzw. 1% der Wasserrübenpflanzen anwesend.

1. Introduction

During our two visits to Austria in October 1969 and 1982, a pronounced yellow variegation, mosaic and vein-clearing symptoms were observed on leaves of about 3% of turnip plants (*Brassica rapa* L. var. *rapa*) in fields in the area between Villach and Klagenfurt (Austria, Fig. 1 a). A larger number of turnip specimens (in average 6%, but sporadically up to 14%) revealed mosaic, necrosis of leaf veins and petiole, malformations of leaves and plant stunting (Fig. 1 b). Sometimes ochre yellow was present on leaves. Also, the root ("bulb") of some of the plants was dwarfed. Some plants (about 1%) displayed a third type of symptoms. They included moderate yellow mosaic and variegation, especially in older leaves, necrosis of leaves accompanied by various malformations (younger leaves), and mostly severe rosetting and stunting. The first type of symptoms was similar to that caused by turnip yellow mosaic virus (TYMV, MAMULA & MILIČIĆ 1971, MATTHEWS 1980). Mosaic, necrosis and dwarf symptoms, present on the majority of plants, resembled symptoms of radish mosaic virus (RdMV, ŠTEFANAC & MAMULA 1971, CAMPBELL 1973) and rarely symptoms of a few other crucifer viruses. On basis of the third type of symptoms it was more difficult to infer the causal virus.

About twenty different viruses have been found infecting under natural conditions plants from family *Cruciferae*, many of them having been recorded in Europe (ŠTEFANAC 1967, SHUKLA & SCHMELZER 1972, 1975). In Central Europe, cruciferous plants, either wild or cultivated ones, are often attacked by TYMV and RdMV (SHUKLA & SCHMELZER 1973). The distribution of these two viruses in turnip plants in this part of Europe was somewhat more studied in Yugoslavia and Hungary (MAMULA & MILIČIĆ 1971, MAMULA & al. 1972, JURETIĆ & al. 1973). Data on the occurrence of crucifer viruses in Austria are rather scarce (VUKOVITS 1956, GLAESER 1970, MAMULA & MILIČIĆ 1971). This fact was an additional reason for studying virus isolates which we found on turnips in Austria.

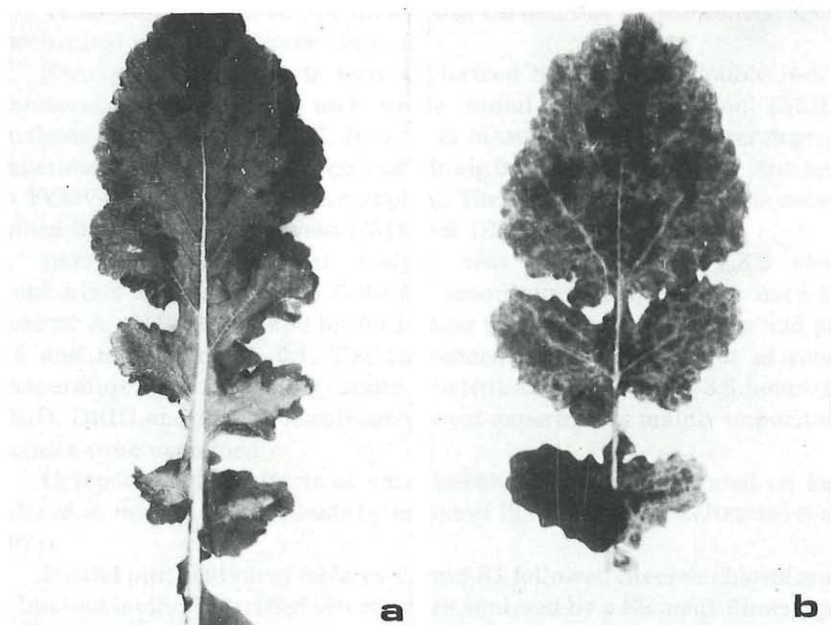


Fig. 1. Two types of leaf symptoms on naturally infected turnip plants in Austria:
a – symptoms caused by isolate T1, b – symptoms caused by isolate R1.



2. Material and Methods

Eight virus isolates from turnip plants collected in Austria (see Introduction) were included in the investigations. They were marked: T1, T2, T3 (isolates of group No. 1), R1, R2, R3 (isolates of group No. 2), M1 and M2 (isolates of group No. 3). Isolates T3 and R3 were collected in 1969, the others in 1982. The Austrian isolates were compared with the Yugoslav TYMV (MAMULA & al. 1966) and Yugoslav RdMV (ŠTEFANAC & MAMULA 1971). In the present paper the Yugoslav viruses are marked as TYMV-Y and RdMV-Y, respectively.

Virus transmission to test plants was carried out by the conventional mechanical inoculation procedure.

Serological experiments were performed by means of double radial immunodiffusion (DRID) and single radial immunodiffusion (SRID) methods (JURETIĆ & al. 1973, JURETIĆ & MAMULA 1980). In all serological experiments 0.9% bacto agar gel containing 0.05% NaN_3 was used. Antisera to TYMV-Y and RdMV-Y were applied. Their homologous titres, as determined by DRID method, were 1/512 and 1/256, respectively.

Immunoelectrophoretical analysis was performed on LKB electrophoresis apparatus (type 6800-A1) according to a procedure used by JURETIĆ & al. 1973. Veronal buffer in agar and in electrode vessels had pH 8.6 and ionic strength 0.1. The experiments were carried out at room temperature without cooling, under a potential of 8 V/cm for 3.5 hours. In SRID, DRID and immunoelectrophoretical experiments mainly unpurified isolates were examined.

Cytopathological effects of virus infections were investigated on leaf cells of *B. rapa* var. *rapa* plants by means of light microscope (JURETIĆ & al. 1973).

Partial purification of isolates T1 and R1 followed Steere's chloroform/n-butanol method. Purified viruses were analysed by a Siemens Elmiskop I electron microscope using negative staining with 4% phosphotungstate.

The number of particle types was established by centrifugation of purified virus suspensions in concentration 1 mg/ml on sucrose gradients of 7–25% at 25,000 rpm for 3 hours in a Spinco SW 25.1 rotor. The gradients were prepared in 0.03 M phosphate buffer at pH 7. Zones were collected by means of an ISCO density gradient fractionator with a recording UV (254 nm) densitometer.

3. Results

On basis of the symptoms observed in field and according to some preliminar experiments the eight investigated isolates were at the beginning of investigations classified in three groups (see Material and Methods). The first virus transmission tests showed that the three types of symptoms were caused by different causal viruses or virus combinations. The results ob-

tained from the investigations of the three groups of isolates will be here separately presented.

3.1. Investigations of isolates T1, T2 and T3

3.1.1. Test plant reaction and host range

Each of the three isolates was mechanically inoculated to 10 test plants. The reactions of the plants were the same in each isolate (Table 1).

Table 1

Reactions of test plants after inoculation with isolates T1, T2 and T3. L = symptoms in inoculated leaves, S = symptoms in non-inoculated leaves, 0 = insusceptible.

Test plant	Symptoms
<i>Brassica chinensis</i> L.	L: local lesions; S: vein-clearing and yellowing, mosaic, variegation
<i>B. rapa</i> var. <i>rapa</i>	Nearly the same as in <i>B. chinensis</i>
<i>Chenopodium amaranticolor</i> COSTE & REYN.	L: 0; S: 0
<i>Ch. murale</i> L.	L: 0; S: 0
<i>Ch. quinoa</i> WILLD.	L: 0; S: 0
<i>Nicotiana megalosiphon</i> HEURCK & MUELL.	L: 0; S: 0
<i>N. tabacum</i> L. cv. "Xanthi"-nc	L: 0; S: 0
<i>Raphanus sativus</i> L. var. <i>niger</i> KERNER	L: local lesions; S: vein-clearing and yellowing, yellow spotting, variegation
<i>Reseda odorata</i> L.	L: local lesions; S: vein-clearing and yellowing, yellow spotting
<i>Sinapis arvensis</i> L.	Similar as in <i>B. chinensis</i>

Local lesions (in cruciferous plants and in *R. odorata*) were most frequently chlorotic. Some leaf necrosis and deformation and plant stunting were expressed mainly at lower temperatures. In general, host range and test plant reactions of isolates T1, T2 and T3 resembled markedly those of TYMV, however to some extent also those of Erysimum latent virus.

3.1.2. Properties in vitro

Thermal inactivation point of isolates T1, T2 and T3 was higher than 341 K but lower than 345 K, and longevity in vitro at 294 K was between 3

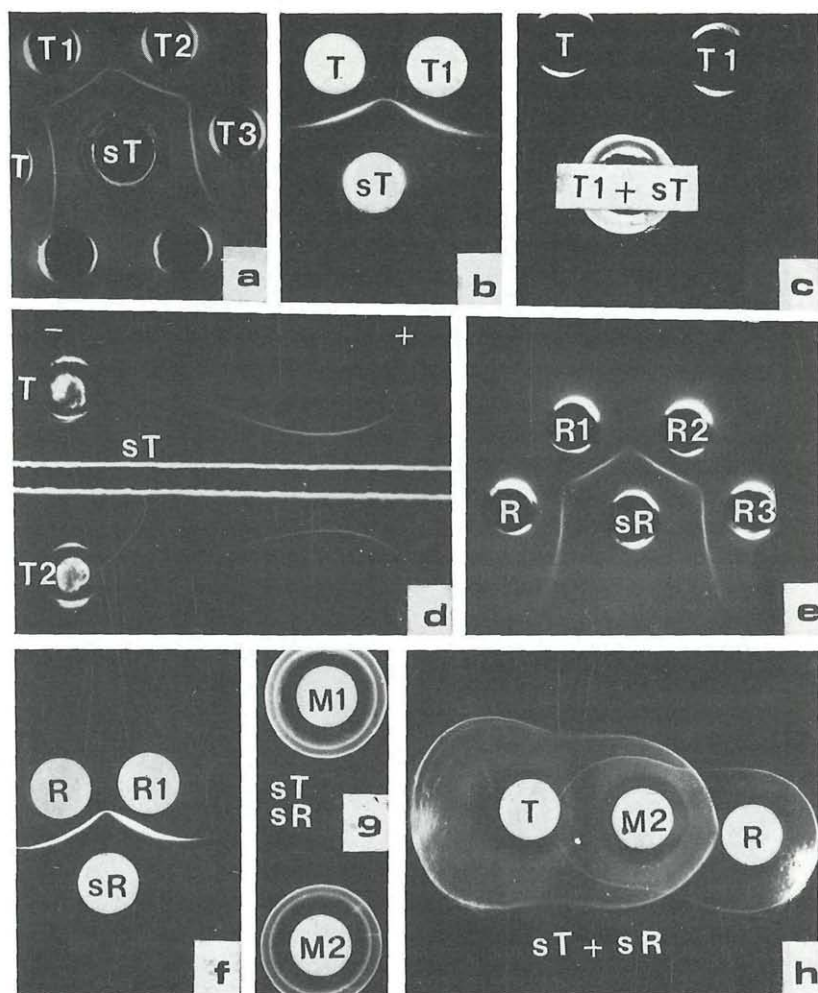


Fig. 2. Serological reactions in agar gel diffusion tests obtained with virus isolates found in turnips in Austria: a, b, c, e, f – double radial immunodiffusion tests; d – immunoelectrophoretic test; g, h – tests performed by single radial immunodiffusion (inner ring represents RdMV and outer one TYMV). T1, T2, T3 – virus isolates of turnip yellow mosaic virus (TYMV), R1, R2, R3 – virus isolates of radish mosaic virus (RdMV), M1 and M2 – virus isolates representing mixtures of TYMV and RdMV; T – Yugoslav TYMV, R – Yugoslav RdMV, sT – antiserum to T, sR – antiserum to R.

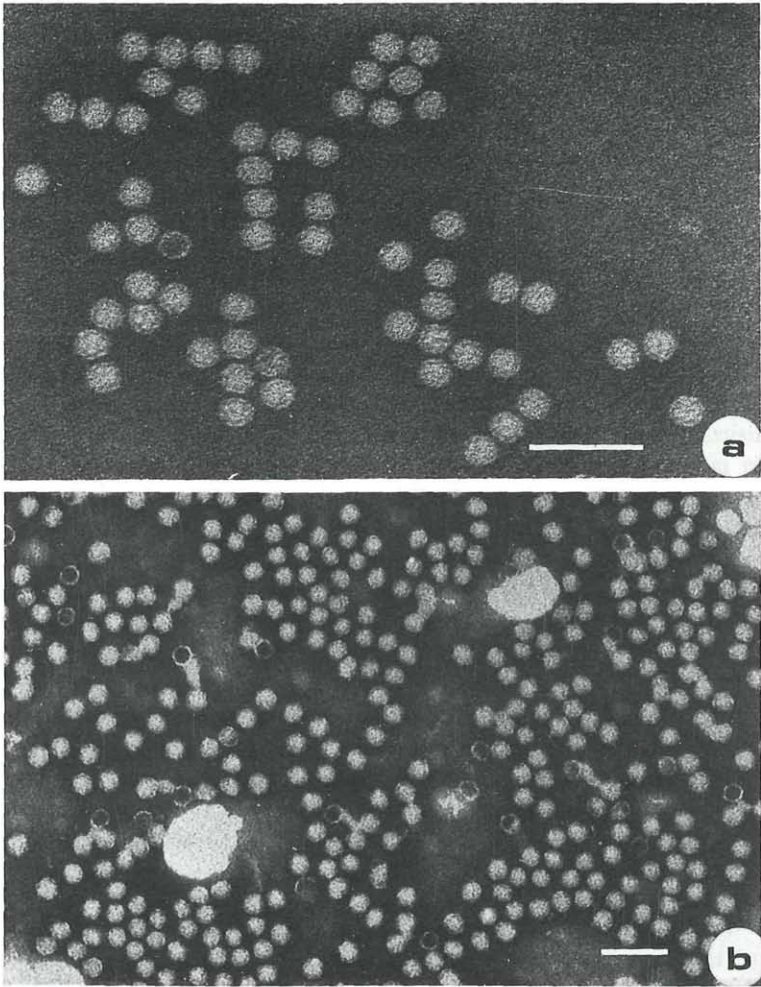


Fig. 3. Particles of TYMV-A (a) and RdMV-A (b) in partially purified preparations.
Bar equals 100 nm.

and 4 weeks. In desiccated infected leaves of *B. rapa* var. *rapa* plants stored at 268 K isolate T3 remained highly infective after a period of 14 years. The results showed that no considerable, if any, variations in the properties existed between the isolates.

3.1.3. Cytopathology

In leaf lower epidermis cells and in those of the adjacent mesophylla layer characteristic vacuolating, clumping and disintegration of chloroplasts occurred under the influence of each of the isolates T1, T2 and T3. This has been observed within several weeks following the inoculation of the plants. The same type of cell alterations is characteristic of TYMV and other tymoviruses.

3.1.4. Serology

The majority of serological experiments were performed by the DRID method and some of them by the SRID method. The three isolates (T1–T3) reacted with antiserum-TYMV-Y but not with antiserum-RdMV-Y (Fig. 2 a). This excluded the possibility that the isolates belong to Erysimum latent virus, a tymovirus being somewhat similar to TYMV (host range, symptoms, etc.) but serologically unrelated to it (SHUKLA & GOUGH 1980). By means of antiserum-TYMV-Y the isolates could neither be distinguished mutually nor from isolate TYMV-Y (Fig. 2 b, c). Consequently, isolates T1, T2 and T3 belong to turnip yellow mosaic virus (TYMV), at the same time being closely related to TYMV-Y.

Also, isolates T1–T3 did not differ electrophoretically from each other and from TYMV-Y (Fig. 2 d).

Since isolates T1, T2 and T3 were recognized as identical, not only on basis of test plant reactions and properties in vitro but also serologically and electrophoretically, they were considered as one virus which we marked as TYMV-A.

3.1.5. Purification, UV absorption and electron microscopy

Leaves of turnip plants systemically infected with TYMV-A (isolate T1) were used as source of virus for purification. Infectious sap was clarified by emulsification with 1/5 volume of chloroform/n-butanol mixture (1 : 1, v/v) and afterwards subjected to two cycles of differential centrifugation (5,000 g 15 min and 90,000 g 100 min). Purified virus suspension showed a typical absorption curve with maximum in ultraviolet at 262 nm. Ratio A 260/280 of the preparation was 1.37, indicating a satisfactory partial purification.

Electron microscope analysis of partially purified TYMV-A revealed isometric virus particles of about 30 nm diameter (Fig. 3 a).

3.1.6. Density gradient centrifugation

After density gradient centrifugation partially purified TYMV-A was separated into two opalescent zones, the lower one being wider and more dense. Scanning of the gradient columns gave two peaks (Fig. 4, left). By means of fractionator two components corresponding to the peaks were isolated. The top component had an A 260/280 ratio approx. 0.9, while the ratio of the bottom component was 1.56. Evidently the top component apparently did not contain any remarkable amount of nucleic acid. When used as inoculum the bottom component infected 9 out of 10 inoculated *B. chinensis* plants while the top component infected only 1 in 10 plants. Both components reacted identically with antiserum to TYMV-Y.

3.2. Investigations of isolates R1, R2 and R3

3.2.1. Test plant reaction and host range

Isolates R1, R2 and R3 were inoculated separately onto 12 test plants. The isolates did not differ in reactions of the plants (Table 2).

Table 2

Reactions of test plants after inoculation with isolates R1, R2 and R3
For plant names and for abbreviations see Table 1.

Test plant	Symptoms
<i>B. chinensis</i>	L: necrotic lesions; S: faint mosaic and vein-banding
<i>B. rapa</i> var. <i>rapa</i>	L: necrotic lesions; S: mosaic, vein-banding, nervature necrosis, stunting
<i>Ch. amaranticolor</i>	L: faint chlorotic lesions; S: 0
<i>Ch. murale</i>	L: necrotic lesions; S: 0
<i>Ch. quinoa</i>	L: faint chlorotic lesions; S: 0
<i>C. sativus</i> "Delicates"	L: faint chlorotic lesions; S: 0
<i>D. stramonium</i>	L: 0; S: 0
<i>Lycopersicum esculentum</i> L.	L: 0; S: 0
<i>N. glutinosa</i>	L: 0; S: 0
<i>N. megalosiphon</i>	L: necrotic lesions; S: chlorotic and necrotic spotting, necrosis along veins, malformations, stunting
<i>N. tabacum</i> "Xanthi"-nc	L: faint chlorotic lesions; S: 0
<i>R. sativus</i> var. <i>niger</i>	L: 0; S: 0

On basis of symptoms quoted in Table 2 it was obvious that isolates R1-R3 represented an other virus than TYMV. Symptoms in all test plants, but especially those in *B. rapa* var. *rapa* and in *N. megalosiphon*, were similar to those of radish mosaic virus (RdMV).

3.2.2. Properties *in vitro*

Some properties *in vitro* were investigated for isolates R1 and R3. The isolates were shown to have thermal inactivation point between 335 K and 338 K and longevity *in vitro* at 294 K 8–12 days. It is evident that in these properties isolates R1 and R3, and very likely the similar isolate R2, differed from TYMV. Isolate R3 remained infective after 14 years storage in desiccated infected leaves being kept at 268 K.

3.2.3. Cytopathology

In contrast to isolates T1–T3 (TYMV-A) the isolates R1, R2 and R3 did not cause vacuolating of chloroplasts in infected cells but they provoked appearance of amorphous cytoplasmic inclusion bodies. These bodies could be seen in leaf epidermal cells of systemically infected turnip plants 20–30 days after inoculation. Sometimes fairly transparent or sometimes opaque, oval in shape and often somewhat larger than nuclei, they contained several minute granula-like bodies or one to several vacuole-like structures.

3.2.4. Serology

In serological experiments performed in agar gel isolates R1, R2 and R3 did not react with antiserum to TYMV-Y. However, they reacted with antiserum-RdMV-Y. When the isolates were compared with each other using antiserum-RdMV-Y they were serologically identical (Fig. 2 e). Additional serological experiments showed that the three isolates did not differ from RdMV-Y when antiserum-RdMV-Y was used (Fig. 2 f). On basis of these data it was concluded that apart from TYMV, RdMV also occurs in turnip plants in Austria. RdMV found in Austria is marked here as RdMV-A.

3.2.5. Purification, UV absorption and electron microscopy

RdMV-A was easily purified by the earlier mentioned method using phosphate buffer 0.03 M, pH 7. Electron microscope analysis of purified virus suspension revealed isometric virus particles of about 30 nm diameter (Fig. 3 b). A typical nucleoprotein absorption curve of purified RdMV-A was obtained, having ratio A 260/280 1.48.

3.2.6. Density gradient centrifugation

When RdMV-A was centrifuged in sucrose density gradient three zones (components) could be revealed by density gradient fractionator (Fig. 4, right). Under the same conditions identical results were obtained by JURETIĆ & FULTON 1974 who have studied RdMV-Y with respect to the components.

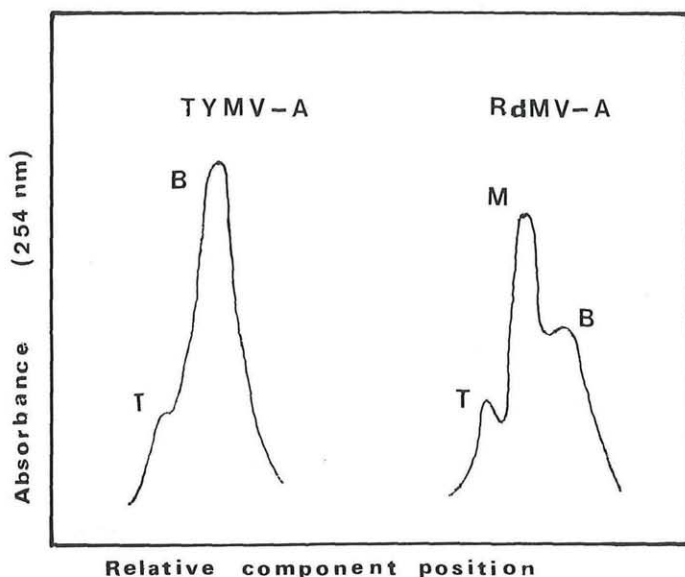


Fig. 4. Scans of density gradient tubes in which there were centrifuged TYMV-A (left) and RdMV-A (T, M and B – top, middle and bottom components, respectively).

3.3. Investigations of isolates M1 and M2

Transmission of isolates M1 and M2 to several differential test plants showed that they did not represent single TYMV or RdMV either. Notably, symptoms in *Reseda odorata* and *Raphanus sativus* var. *niger* were quite similar to the ones of TYMV (see Table 1), while those in *Nicotiana megalosiphon* and *Chenopodium* spp. resembled RdMV (see Table 2). On the basis of symptoms and host range these isolates could represent mixtures of both TYMV and RdMV.

Serological tests carried out by means of the SRID method (antisera to TYMV and RdMV were mixed with agar) revealed that isolates M1 and M2 formed two precipitation halos around the well in which they were separately placed. The control test showed that the inner halo corresponded to RdMV and the outer one to TYMV (Fig. 2 g).

In another experiment the two isolates were examined in the way that can be seen in Figure 2 h. Three wells were made in agar containing antisera to TYMV-Y and RdMV-Y. The left well was filled with TYMV-Y (T), the right one with RdMV-Y (R) and the central well with isolate M2. As can be seen in Figure 2 h, isolate M2 contained both TYMV and RdMV. The same result was obtained with isolate M1, i. e. isolates M1 and M2 represent an identical mixture of TYMV and RdMV. TYMV and RdMV found in mixed

infections did not differ with regard to test plant reactions and serology from the afore described TYMV-A and RdMV-A, respectively. No indication was obtained on the presence of any virus other than TYMV and RdMV in isolates M1 and M2.

4. Discussion

It has been shown in the present work that the investigated Austrian turnip yellow mosaic virus (TYMV-A) is serologically closely related to the Yugoslav isolate of that virus (TYMV-Y), which belongs to TYMV strain 1 (MAMULA 1984, unpublished). It should be pointed out that only antiserum to TYMV-Y was used. It can be expected that the use of the other antiserum (antiserum-TYMV-A) should not reveal a significant difference between TYMV-Y and TYMV-A, as it was found with three closely related, formerly investigated, isolates of the virus. As with TYMV-A, the majority of TYMV isolates investigated so far belong to strain 1 of the virus (MAMULA 1984, unpublished). Serological reaction of identity of Austrian radish mosaic virus (RdMV-A) and Yugoslav radish mosaic virus (RdMV-Y) was obtained when antiserum to the latter virus isolate was used. An antiserum-RdMV-A was not applied. However, on basis of earlier experiments with more RdMV isolates from Europe (PLAKOLLI & ŠTEFANAC 1976), similar as afore stated for TYMV-A, probably no significant difference could be found between RdMV-Y and RdMV-A in case of applying antiserum to RdMV-A. Since RdMV-Y represents the European type isolate, i. e. European type strain of RdMV (ŠTEFANAC & MAMULA 1971), it could be stated that RdMV-A belongs to European type strain of the virus. This is in concordance with earlier evidence that the majority of RdMV isolates from Europe belong to the European strain of RdMV (PLAKOLLI & ŠTEFANAC 1976, MAMULA 1984).

The discovery of TYMV and RdMV in Austria speaks in favour of the thesis that these two viruses are distributed continuously over wide area in Europe. This is supported by the fact that some wild (and also some ornamental) plants can harbour the viruses mostly lacking symptoms (SHUKLA & SCHMELZER 1973). As known, natural host ranges of TYMV and RdMV comprise only plants (wild and cultivated species) from family *Cruciferae* and they are broader (including genera, species and varieties) than with majority of other viruses attacking crucifers in nature (ŠTEFANAC 1967, SHUKLA & SCHMELZER 1973, MATTHEWS 1980, MAMULA 1984). TYMV has so far been recorded in 10 European countries including Austria (cf. BENETTI & al. 1978). Perhaps the virus is also spread in Poland (JASIŃSKA 1969). However, RdMV spreads over a larger area than TYMV as outside Europe, where it has been found altogether in 5 countries (cf. PLAKOLLI & ŠTEFANAC 1976), it has also been found in North America (USA) and Asia (Japan) (CAMPBELL 1973). From the available literature it is not clear whether a virus found and described under the name of radish mosaic virus

in China (Anonymous 1977) belongs to RdMV the present paper deals with. By its properties radish mosaic virus from India is probably a different virus (UPADHYAYA 1977).

BURCKHARDT 1958 described infection of three cultivated *Brassica*-species in Germany with Kräuselmosaik which was found to be caused by turnip mosaic virus or a virus belonging to turnip virus 1 group. The disease has been investigated and described by the same author in several papers up to 1963. Symptoms of Kräuselmosaik on some naturally infected turnip and two additional *Brassica* spp. specimens resemble very much those of RdMV (e. g. BURCKHARDT 1960: fig. 6, BURCKHARDT 1958: fig. 2). Moreover, some of the photographs presented (e. g. BURCKHARDT 1960: fig. 6) reveal damages on leaves of turnip plants which are very similar to the injuries produced by beetles, the vectors of RdMV. In addition, thermal inactivation point of the causal virus of Kräuselmosaik was similar to that of RdMV and at the same time lower than in some other beetle-transmitted crucifer viruses (see SHUKLA & GOUGH 1980). Thus, it is possible that some of the plants contained RdMV alone or in mixed infections with some other viruses. Therefore, it is not excluded that in the BURCKHARDT's papers there are some of the first indications on presence of RdMV in Europe.

Mixed infections of TYMV and RdMV have been reported from other countries; in addition, mixed infections of these two viruses with some other isometric or anisometric viruses have been recorded (MAMULA & MILIČIĆ 1971, SHUKLA & SCHMELZER 1975).

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Zeitschrift/Journal: [Phyton, Annales Rei Botanicae, Horn](#)

Jahr/Year: 1985

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Artikel/Article: [A Study of two Isometric Viruses Infecting Turnip in Austria. 241-251](#)