Studies on tobacco mosaic virus synthesis in apical parts of tomato roots

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The problem of virus distribution within the meristematic zone both in the roots and shoots, has been since a long time the subject of extensive investigations. The results of experiments of many authors revealed that meristematic tissues were free of viruses [12, 13, 20, 25, 27]. As yet no virus has been detected in growing-points either in the roots or in shoots of infected plants. This statement made basis for the method of curing some plants from virus infections on the way of cultivating their excised meristems on nutrient solution.

Many authors obtained healthy plants from excised apical parts of stem meristems originating from infected plants [9-11, 14-16, 23, 24]. Till now quite a number of investigations were carried out aiming to liberate infected plants or tissues from the virus by means of cultivating their excised meristems, or by use of various chemicals inhibiting multiplication of the virus [2, 17, 19]. On the other hand, there were only few attempts to infect maristematic tissues with the view to elucidate the mechanism of their infection. The defense mechanism as well as the physiological state of these tissues which prevent infection are as yet unknown.

It results from the hitherto investigations that there are substances which could cause some changes in the meristematic tissues so as to make them susceptible to infection, namely ethylenediaminetetraacetic acid (EDTA) [6] and 5-fluorodeoxyuridine (FUDR) [7].

The purpose of the present work was to test the influence of the two above mentioned chemicals both on the multiplication of viruses in root meristems and on cytological and histological changes occuring in healthy tissues treated with the same compounds. It should be emphasized that roots are a particularly good material for cytological investigations concerning multiplication of virus because they do not contain chloroplast, and their cells are rich in cytoplasm. They are also suitable for tests on the influence of various chemicals because they can be tested in immediate contact with the examined compuonds.

Experiments were carried out according to the previously described method [26]. Tomato plants were germinated in moist sand in sterile conditions. When the first leaves appeared, plants were transferred to glass jars containing 200 ml of nutrient

solution prepared according to Hewitt [8]. In the stage of three leaves they were transferred to a similar nutrient solution containing either ethyleno-diamino-tetra-acetic acid (EDTA) in concentration 6.4×10^{-4} M or 5-fluorodeoxyuridine (FUDR) in concentration 7.6×10^{-4} M. On the next day leaves of these plants were infected with a purified suspension of tobacco mosaic virus. The concentration of the inoculum was 0.001%. Inoculations was always made on all three or four leaves. Plants infected with TMV simultaneously, but cultivated on nutrient solution without chemicals were used as a control.

To establish the presence or absence of tobacco mosaic virus in roots they were removed from plants on the 10th to 13th day following inoculation. The root tips ca 1 mm long were collected together and from each cathegory 12-15 roots were homogenized together in 0.7 ml of phosphate buffer pH = 7. Then each homogenate was used to infect five whole leaves of tests plants. The number of local lesions appearing on the 3rd day following inoculation on leaves of test plants was taken as a measure of the virus concentration in examined roots. Results of these experiments are presented in Table 1.

Table 1

Total number of local lesions per 5 leaves of *N. glutinosa* infected with roots of tomato plants cultivated on different nutrient solution

Nutrient solution		
without chemicals	with EDTA 6.4×10^{-4}M	with FUDR 7.6×10^{-6}M
0	43	52

In other experiments each root was divided into small sections about 1 mm long and homogenized individually on a glass slide in 0.3 ml of phosphate buffer at pH = 7 and used for inoculation on two halves of different leaves of *Nicotiana glutinosa*. The results of these experiments are presented in Fig. 1.

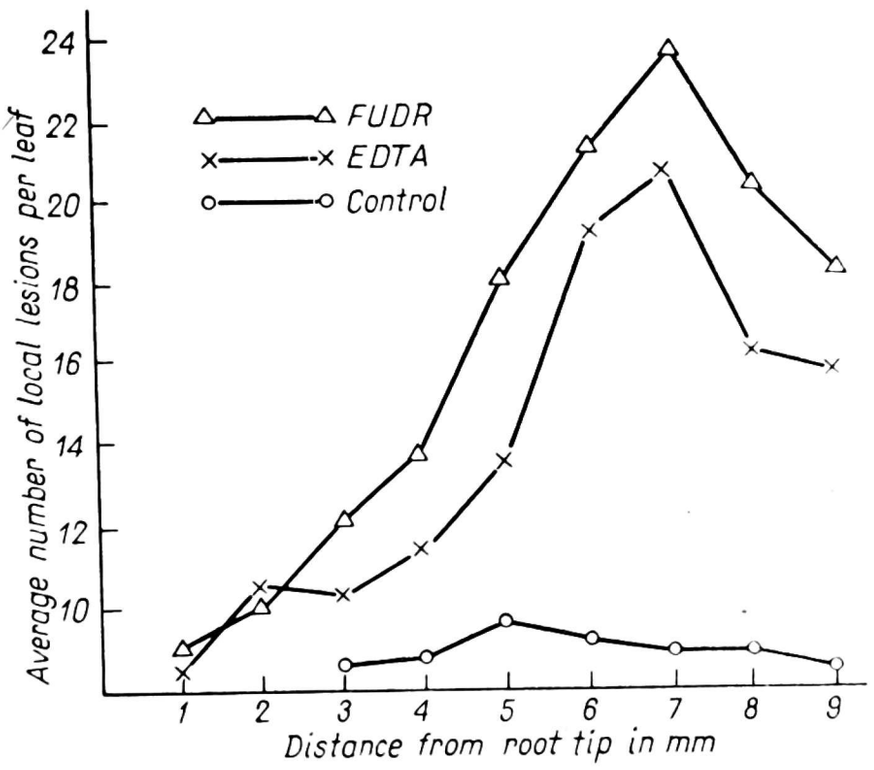

Fig. 1. Comparison of virus concentration in successive 1 mm root fragments on the 10th day after inoculation with TMV.

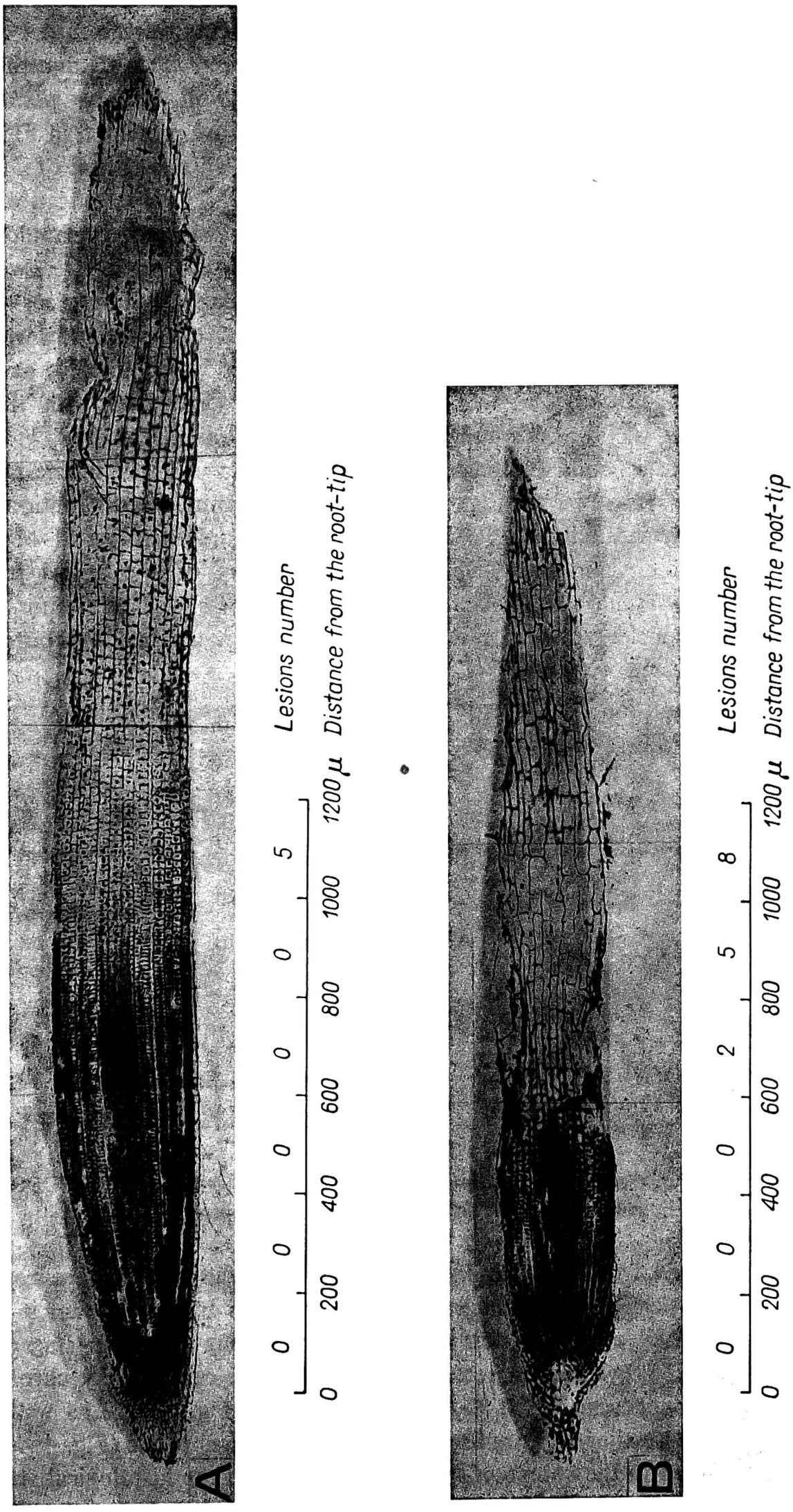

Fig. 2. A longitudinal section of a root tip of tomato plant with the length of sections and number of local lesions appearing on the leaves infected them. *A* — control, *B* — treated with EDTA.

In order to precise the localization of TMV in the first fragments about 1 mm long, the apical parts of tomato roots were cut into 5 fragments each 0.2 mm thick. These fragments were inoculated individually onto the leaves of *Nicotiana glutinosa* using the previously described method. The obtained results are presented in Fig. 2.

The obtained results show that EDTA like FUDR enabled replication of TMV in tomato root tips treated with these compounds (Fig. 1). The concentration of virus in treated roots was significantly higher than in control ones. It should be emphasized that results vary in dependence on the inoculation procedure. When test plants were inoculated with several root tips the tobacco mosaic virus was detectable in the first milimeter of roots. On the other hand in experiments in which test plants were inoculated with single root fragments TMV was detectable at the distance of 0.4-0.6 mm from the root tip. These results correspond with those obtained by Brants *et al.* [4] Smith and Schlegel [21] and Wajda [26].

In connection with obvious differences in the localization of TMV in the roots of untreated plants and of those treated with these compounds, examination of the mitotic index seemed advisable. Parallel cytological and histological observations were carried out on successive microtome sections of the apical parts of these roots. The obtained results show that the EDTA as well as 5-fluorodeoxyuridine reduces considerably the mitotic activity both in the healthy roots as well as in those infected with TMV (Fig. 3).

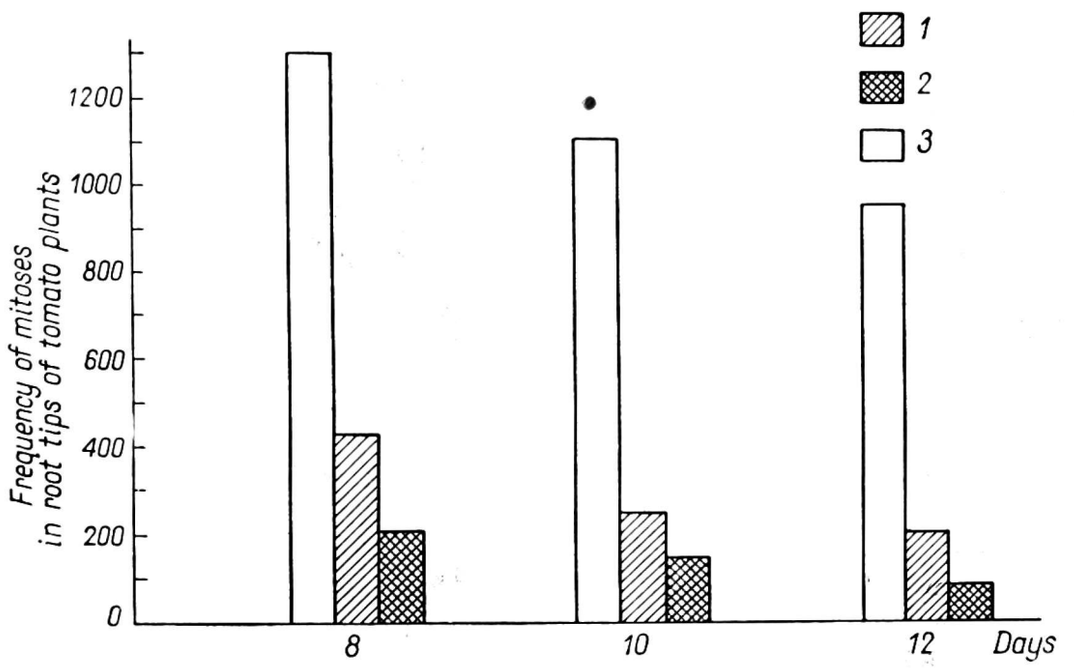

Fig. 3. Frequency of mitoses in the root tips of tomato plants treated with EDTA, FUDR and in control. *1* — FUDR, *2* — EDTA, *3* — control.

It may be inferred from the detailed cytological examination of these roots, that EDTA inhibits the interphase nuclei entering prophase. It appears that it has not only a preprophasic effect [26] but acts on chromosomes in further stages of mitosis (Fig. 4 *A-F*). Ethylenediaminotetraacetic acid could cause sticky chromosomes, bridges in anaphases (Fig. 4 *C-D*) and telophases (Fig. 4 *E-F*) as well as disturbs a correct congression of chromosomes in the metaphases plates (Fig. 4 *A-B*). Disturbances observed in the course of mitosis manifested in the histological struc-

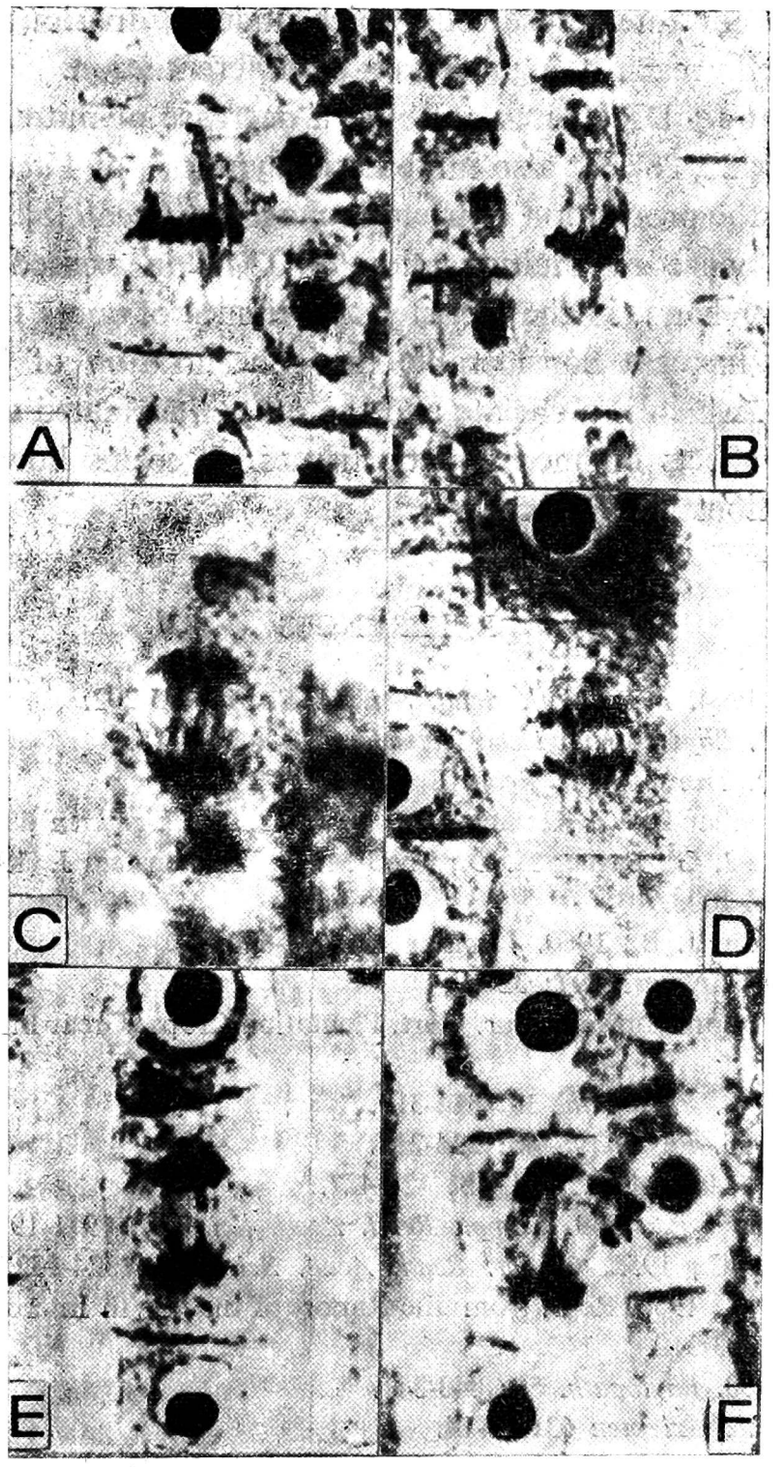

Fig. 4. Disturbances in mitoses after EDTA treatment. *A—B* — disturbances in chromosome congresion, *C—D* — bridges in anaphases caused by sticky chromosomes, *E—F* — bridges in telophases. Lagging chromosomes.

ture of these roots; namely those treated with EDTA, have a considerable shortened division zone (Fig. 2 *B*). In control roots it is ca, 1 mm long from the root tip but in those treated with EDTA it is only 0.4-0.6 mm long (Figs 2*A* and 2*B*). Immediately behind this zone where innumerous divisions took place there is the zone of elongated cells. It results from the presented data that the length of the division zone, both in treated as well as in untreated roots, corresponds with high accuracy with the length of the virus free zone of these roots. It seems that there exists a correlation between the length of the division zone and the distance to which the virus could penetrate the meristematic tissue of the root tip. It semms

also that virus can-not enter those cells which undergo divisions, but can multiply there where the cells reach a high degree of differentiation.

It is known that FUDR causes not only a decrease of mitotic activity in roots of treated plants [1, 22, 28] but also inhibits the synthesis of *Vaccinia virus* in HeLa cells [18] as well as the production of bacteriophages in *E. coli* [5]. It exerts, however, only a limited influence on multiplication of TMV in excised disks of tobacco leaves. On the other hand, in the meristematic tissues of roots this compound like EDTA is able to increase considerable the concentration of TMV. Both these compounds, one of which is a chelate and the other fluorinated analoque of pirimidine exert similar effects on meristematic tissues. It seems however, that mechanisms of these actions are different.

REFERENCES

1. Bell S., Wolf S., 1964. *Proc. Natl. Acad. Sci. U. S.* **51:** 195-202.
2. Bergman L., 1958. *Phytopathologische Zeitschrift*, **34:** 209-215.
3. Bergman L., 1959. *Trans. N. Y. Acad. Sci.* **21:** 227-232.
4. Brants D. H., Graafland, W., Kerling L. C. P., 1962. *T. Pl.-ziekten* **68:** 198-207.
5. Cohen S. S., Flaks J. G., Barner H. D., Loeb M. R., Lichtenstein J., 1958. *Proc. Natl. Acad. Sci. U.S.* **44:** 1004-1008.
6. Crowley N., Hanson J. B., 1960. *Virology* **12:** 603-606.
7. Djordjevic B., 1965. *Nature* **207:** 327.
8. Hewitt E. J., 1952. Commonwelth Bur. Hort. Plantation Crops (Great Britain) Tech. Commun. **22.**
9. Holmes F. O., 1948. *Phytopath.* **38:** 314-319.
10. Holmes F. O., 1955. *Phytopath.* **45:** 224-228.
11. Kassanis B., 1957. *Ann. Appl. Biol.* **45:** 422-427.
12. Limasset P., Cornuet P., 1949. *Compt. Rend. Acad. Sci.* **228:** 1971-1972.
13. Morel G., Martin C., 1952. *Compt. Rend. Acad. Sci.* **235:** 1324-1325.
14. Morel G., Martin C., 1955. Repr. from the Raport of the XIVth Intern. Horticult. Congress, The Netherlands 303-310.
15. Nielsen J. W., 1960. *Phytopath.* **50:** 840-841.
16. Quak F., 1957. *T. Pl.-ziekten* **63:** 13-14.
17. Quak F., 1961. Advances in Horticular Science and their Applic., Pergamonn Press, Oxford——London—N. York—Paris.
18. Salzman N, P., 1960. *Virology* **10:** 150-162.
19. Schmelzer K., 1960. *Acad. Sci. Hung.* **7:** 176-181.
20. Sheffield F. M. L., 1942. *Ann. Appl. Biol.* **29:** 16-17.
21. Smith S. H., Schlegel D. E., 1964. *Phytopath.* **54:** 1273-1274.
22. Taylor J. H., Haut W. F., Tung J., 1962. *Proc. Natl. Acad. Sci. U.S.* **48:** 190-198.
23. Thomas A. D., 1956. *Nature* **177:** 709-711.
24. Thomas A. D., 1958. Proc. Third Conf. Potato Virus Dis. Lisse-Wageningen, 156-159.
25. Thung T. H., 1938. *Tijdschrift over Plantenziekten* **44:** 225-245.
26. Wajda L., 1966. *Acta Biol. Cracov.* **9:** 13-30.
27. White P. R., 1934. *Phytopath.* **24:** 1003-1011.
28. Kihlman B. A., 1962. *Exptl. Cell Research* **27:** 604-607.