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BRIEF COMMUNICATION

Mycoplasmas in Apple Trees Infected with the Proliferation Disease

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Abstract. A mycoplasma-like microorganism was identified on electron micrographs of ultrathin sections of roots and abnormal underground sprouts growing from roots of young apple trees artificially infected with the proliferation disease. Specimens taken in November, contained mycoplasmas at various stages of reproduction in sieve tubes of the host. Mycoplasma-like bodies occurred especially in abnormal underground sprouts, and rarely also in lateral roots.

Only Giannotti *et al.* (1968) reported the presence of *Mycoplasma*-like bodies (vernacular terms mycoplasma and mycoplasmas are preferred nowadays also for phytopathogenic members of the class *Mollicutes*) within stems and petiols of abnormal sprouts formed on roots and being a typical symptom of apple trees invaded with the proliferation disease in France. Mycoplasmas (present in sieve tubes) occurred rarely in proliferation infected apple trees. Apparently, apple trees may be infected with other mycoplasmas: Beakbane *et al.* (1971) discovered them also within trees infected with the rubbery wood disease or with the chat fruit disease. However, the agent described with the chat fruit disease showed rather different properties as far as its internal structure and size were concerned.

The apple proliferation disease occurring in Czechoslovakia seems to be identical with that known in France. Transmission esperiments implied a high concentration of its causal agent in roots. Using root grafting or root chip budding, Seidl (1965a, 1967, 1968) obtained 96 per cent transmission, even in investigating infection sources from which normally the proliferation disease could not be transmitted by grafting or budding. Therefore, we focused our attention on underground abnormal sprouts growing from lateral roots of infected apple trees and being typical for apple proliferation disease (Seidl

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1965 b). Besides the paper by GIANNOTTI *et al.* (1968) a recent report by SEIDL and KOMÁRKOVÁ (1972) strongly supported the presumed mycoplasma origin of apple proliferation disease: tetracycline treatment suppressed its symptoms in apple trees. The present paper demonstrates mycoplasmas in the same plant material by means of electron microscopy.

Young apple trees infected by budding six years ago were investigated. Specimens of phloem tissues were taken (November 15, 1971) from lateral roots (of about 10 mm in diameter) and from abnormal sprouts (1–3 mm in diam.) growing from the roots (Fig. 1) underground. Thin strips of phloem tissues were prefixed with 6.25% glutaraldehyde in phosphate buffer (pH 7.5) for two hours at 0 °C under vacuum and postfixed in buffered 1% osmium tetroxide at room temperature for two hours as well. Specimens were stained in 70% acetone saturated with uranylacetate overnight and after ordinary dehydratation gradually transferred into Durcupan ACM which was thinned by propylenoxide for a better penetration. After polymerization ultrathin sections were made by means of a Reichert ultramicrotome using a diamond knife Ge-Fe-Ri; the sections were additionally stained with lead citrate (RAYNOLDS 1963). An electron microscope Tesla BS 413 was used for taking micrographs.

Within sieve tubes of abnormal underground sprouts mycoplasma-like bodies occurred being oval or filamentous in shape (Figs. 2, 3, 5 to 9). These bodies were less abundant in roots (Figs. 4, 10), usually adhering to the cell wall (Fig. 10, left) or sieve pores (Fig. 4). Some of elongated bodies showed constrictions (Figs. 6, 9). Constrictions within mycoplasmal filaments could rarely be seen, giving rise to a chain of coccoid bodies (Fig. 5). Besides smaller (elementary) bodies (Fig. 5) larger bodies occurred showing net-like structures inside (Figs. 2, 3), or vacuole-like areas (Fig. 7 — arrows). Some elongated bodies showed a presumed formation of elementary bodies within filaments (Figs. 7, 9). Fig. 10 (right) shows unidentified membraneous structures in a cell containing also mitochondria.

Our results confirm the mycoplasma etiology of the apple proliferation disease. Mycoplasma-like microorganisms were found in underground abnormal sprouts as well as in roots of artificially infected young trees in the winter period. Some of the observed forms of this agent featured its possible reproduction in underground host organs during winter.

References

BEAKBANE, A. B., MISHRA, M. D., POSNETTE, A. F., SLATER, C. H. W.: Mycoplasma-like organisms associated with chat fruit and rubbery wood diseases of apple, *Malus domestica* BORKH., compared with those in strawberry with green petal disease. — J. gen. Microbiol. **66** : 55–62, 1971.

GIANNOTTI, J., MORVAN, G., VAGO, C.: Micro-organismes de type mycoplasma dans les cellules libériennes de *Malus sylvestris* L. atteint de la maladie des prolifération. — Compt. rend. Acad. Sci. Paris **267**-D : 76–77, 1968.

RAYNOLDS, E. S.: Use of lead citrate at high pH as an electron-opaque stain in electron-microscopy. — J. cell. Biol. **17** : 209, 1963.

SEIDL, V.: The possibility of using root grafting method of testing for apple proliferation virus disease. — Zaštita Bilja **85–88** : 323–327, 1965a.

SEIDL, V.: Some observations and experiments on proliferation virus of apple tree. — Zaštita Bilja **85–88** : 317–321, 1965b.

SEIDL, V.: O přenosu infekce, její lokalizaci a metodách testu u virové proliferace jabloně. [Concerning transmission, localization, and testing methods of apple proliferation disease.] — Vědecké práce ovocnářské (Holovousy) **4** : in press, 1967.

SEIDL, V.: Weitere Versuche mit der virösen Hexenbesenkrankheit des Apfels (proliferation disease). — VII. Europäisches Symposium über Viruskrankheiten der Obstbäume, Aschersleben DDR, 1967 : 77—86, 1968.

SEIDL, V., KOMÁRKOVÁ, V.: Příspěvek k etiologii, chemoterapii a přenosu proliferace jabloně. [Concerning etiology, chemotherapy, and transmission of apple proliferation disease.] — Ochrana Rostlin (Praha) **8** : 43—46, 1972.

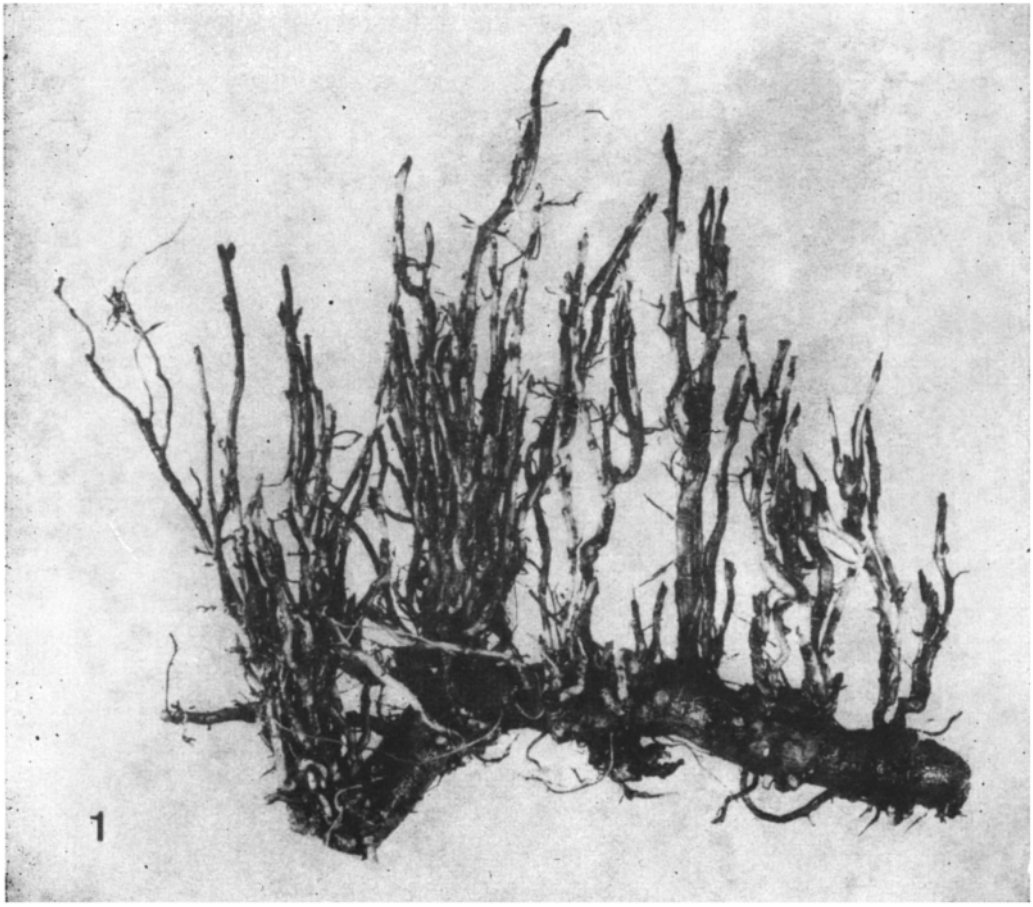
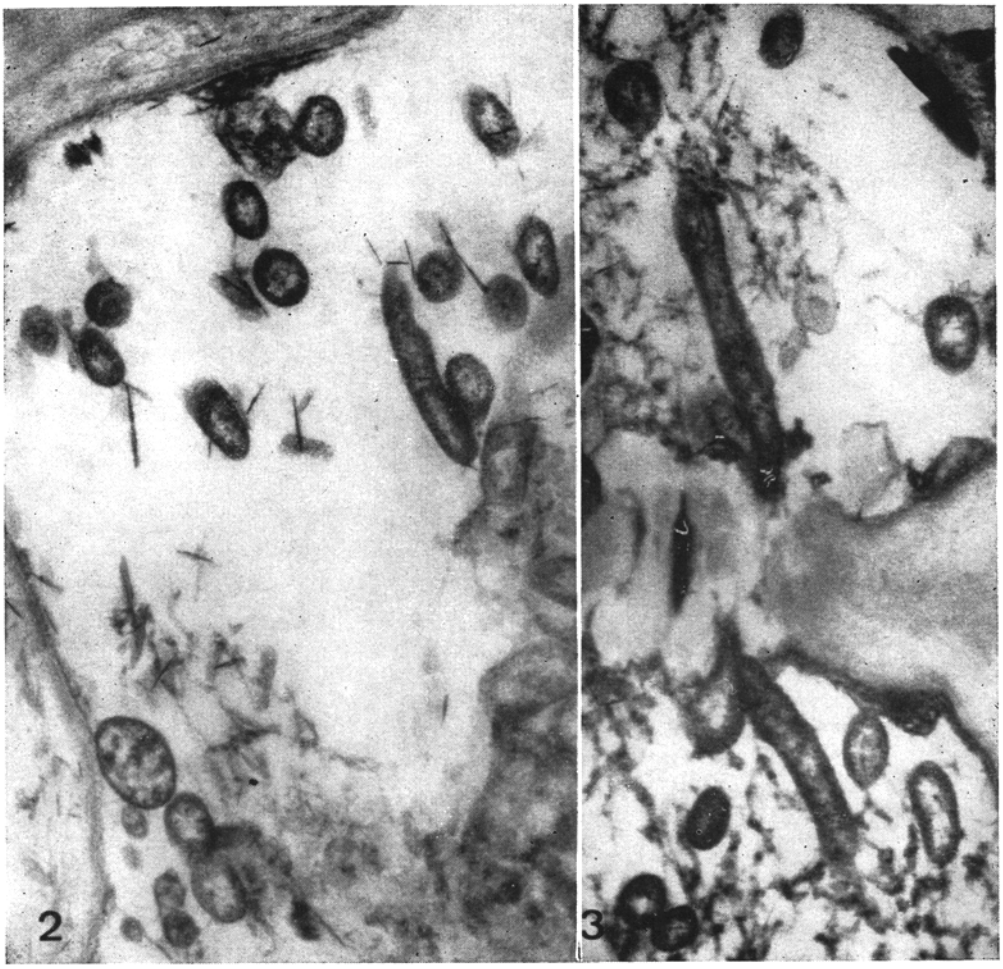


Fig. 1. Abnormal sprouts growing underground from a root of an apple tree artificially infected with apple proliferation disease.



Figs. 2 and 3. Large oval and elongated mycoplasmas in abnormal sprouts of an apple tree artificially infected with apple proliferation disease. Note net-like structures inside mycoplasma-bodies (39 000 ×).

Fig. 4. Mycoplasma-like bodies (arrow) adhering to sieve pores in a root of a proliferation diseased apple tree (26 000 ×).

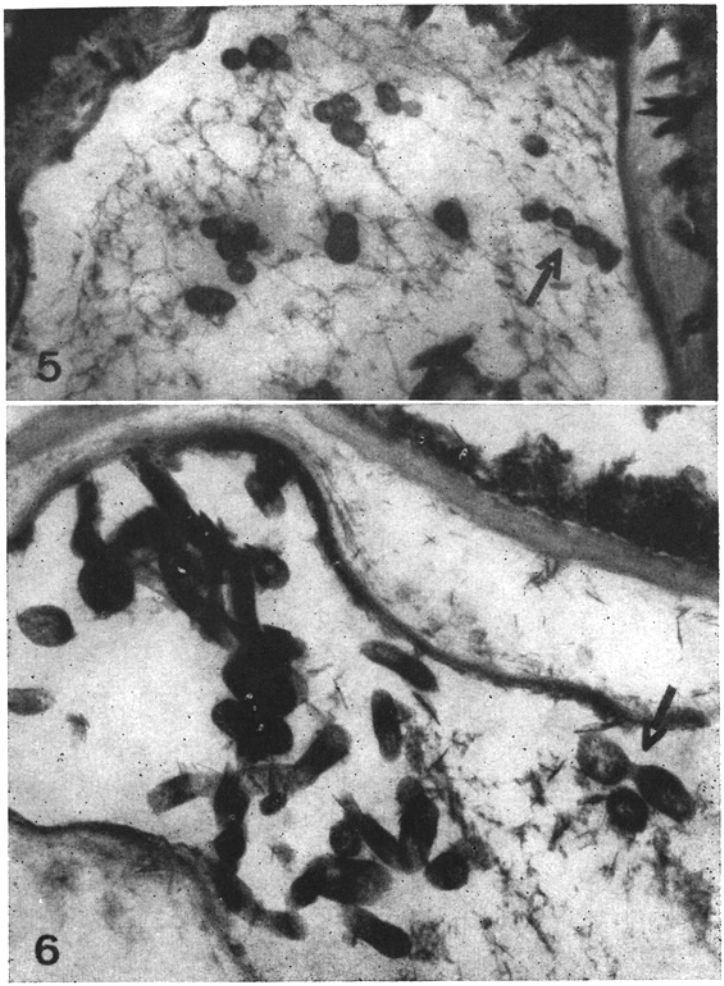


Fig. 5. A chain of dense coccoid bodies (arrow) in an abnormal sprout (13 000×).
Fig. 6. The same object as in Fig. 5. Note constriction of a body (arrow) (26 000×).

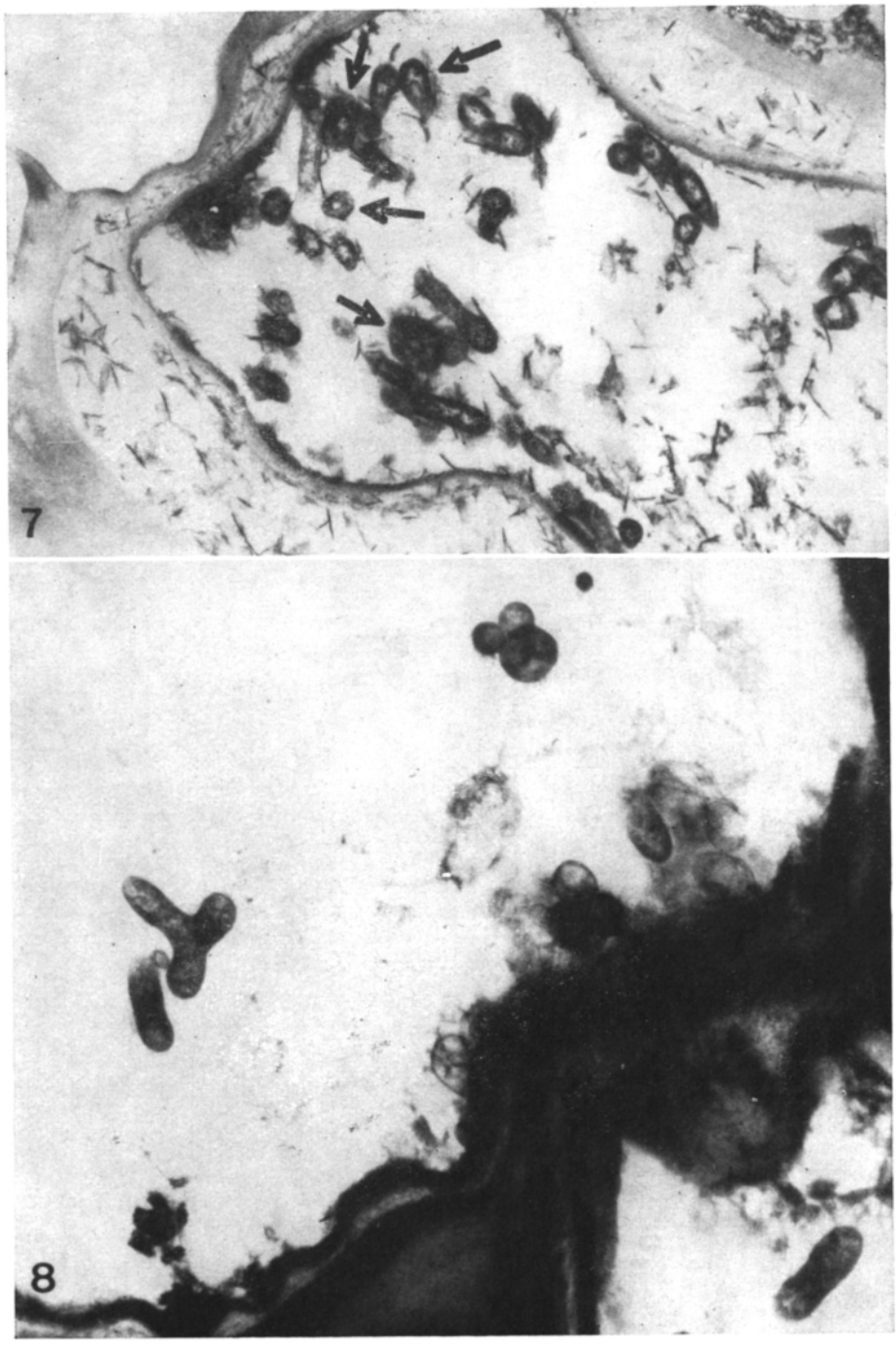


Fig. 7. Mycoplasmas in an abnormal underground sprout. Arrows indicate some bodies showing vacuole-like areas (13 800 ×).
Fig. 8. Various forms of mycoplasmas in an abnormal sprout (29 000 ×).

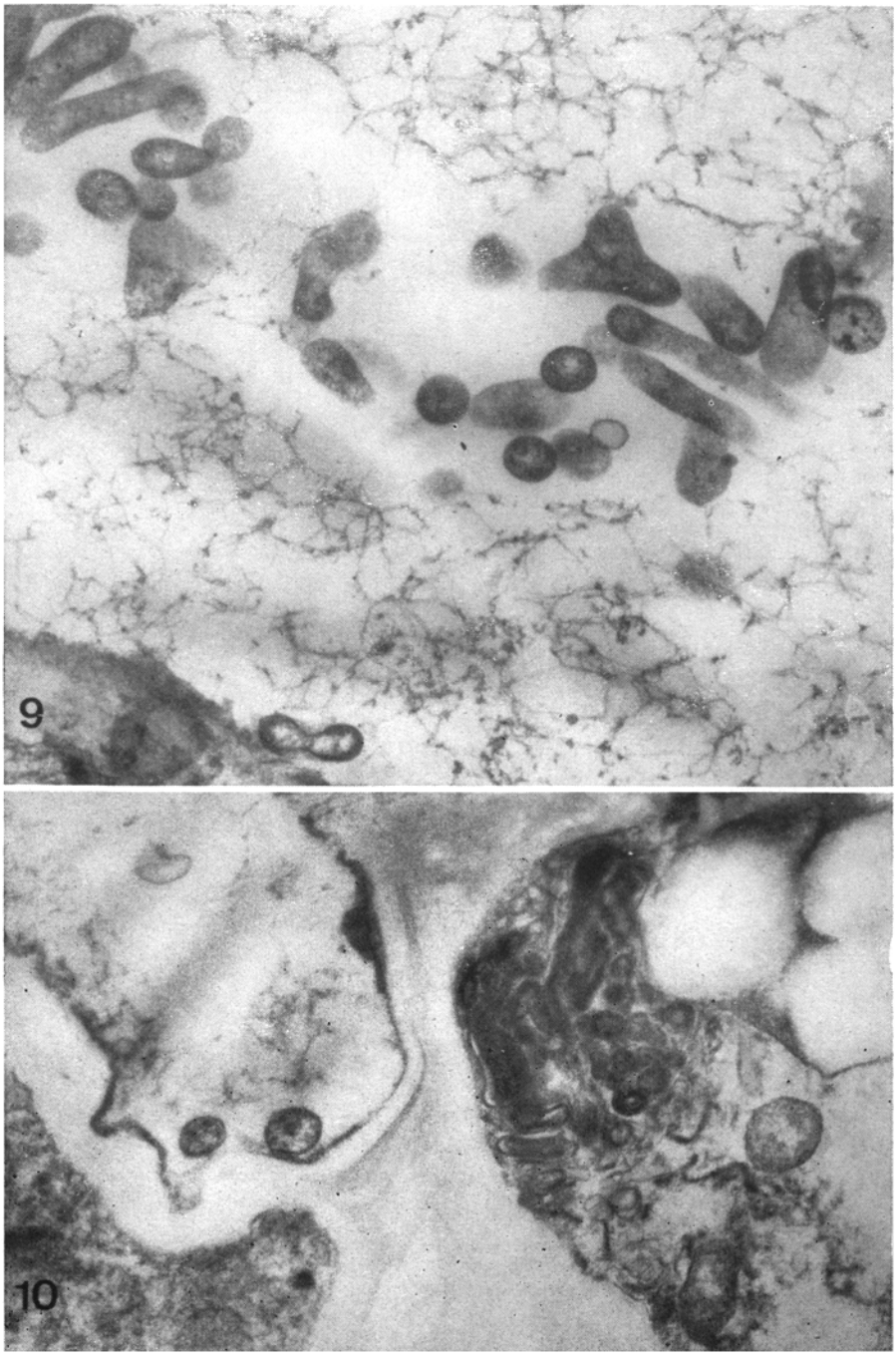


Fig. 9. Mycoplasmas in an abnormal sprout. Note internal structures, constrictions, and denser ends of some filaments (39 000×).

Fig. 10. An apple-proliferation infected root. The left cell contains two mycoplasma-like bodies, a part of the right cell is filled with membraneous structures (26 000×).