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LYCOPERSICON ESCULENTUM MILL. WITH
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PRESENCE OF MYCOPLASMA IN *LYCOPERSICON ESCULENTUM* MILL. WITH «MAL AZUL» *

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SINCE 1962 a disease called by the farmers «Mal Azul» (Blue disease) has been observed in tomato fields, chiefly in the Ribatejo region. A survey of the insect species occurring in those fields show that *Empoasca fabae* (Harris) is the most common (G. MAGALHÃES SILVA, personal communication). For this reason this Cicadellidae is supposed to be the vector.

OLIVEIRA (1965) gave a detailed description of flower abnormalities of tomatoes and other Solanaceae due to «Mal Azul». The same author (personal communication) obtained by grafting the transmission to several Solanaceae but all attempts to sap transmission and purification of a virus did not succeed.

Similar symptoms were observed by one of the authors (BORGES) in tomatoes received from Cabo Verde Islands in 1963; all attempts to sap transmission or purification of a virus from such material have been also unsuccessful.

Recently the presence of Mycoplasma in plants affected by diseases transmitted only by grafting and by *Cicadellidae*, and previously considered to be due to viruses, has been recognised (see Table I).

This note describes some abnormal aspects of the phloem in tomatoes with «Mal Azul» and reports the observation of Mycoplasma-like organisms in the phloem cells.

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MATERIAL AND METHODS

From tomatoes (*Lycopersicon esculentum* Mill.) with typical and severe symptoms of «Mal Azul», graft transmissions have been made to tomato and tobacco plants under insect proof glasshouse. In all these plants no viruses have been detected by sap inoculation in the conventional differential hosts.

Longitudinal sections of adventitious roots, petioles and sepals, including conducting tissues, were fixed in 3 % glutaraldehyde followed by 2 % osmium tetroxide (phosphate buffered). The tissues were embedded in Epon and the thin sections, cut in an LKB Ultratome, were collected on carbon coated grids. After double staining with uranyl acetate and lead citrate they were examined in a Siemens Elmiskop 1A at 80 Kv. Longitudinal and transversal sections of adventitious roots and stems obtained in a freezing microtome were observed in the light microscope after staining with HERTZ'aceto-carmine.

Naturally and experimentally infected tomatoes as well as healthy ones have been studied.

RESULTS AND DISCUSSION

Symptoms of «Mal Azul» have been observed in tomato plants a month after grafting. The symptoms begin by a yellowing and rolling of the leaf margins, followed by epinasty, which gives the gothic aspect, typic of Stolbur disease in tomato. The plant becomes thick and by development of lateral conical shoots, the stem looks successively bifurcated. Later on the leaves, due to changes in anthocyanins, become blue-purplish thus giving the name to the disease. The sepals frequently coalesce, the calices usually enlarge and the whole flower structure is disturbed.

In naturally infected plants the fruits are in limited number and usually show woodiness. The seeds are few and sterile. The presence of a great amount of adventitious roots is also a common symptom (fig. 2)

Severity of symptoms is rather dependent on environmental conditions of light and temperature. The symptoms

shown in fig. 1 are developed under low light intensity in a glasshouse.

By the symptoms in tomato and way of transmission «Mal Azul» resembles attenuated aspects of Big Bud disease (SAMUEL *et al.*, 1933), Stolbur disease (KLINKOWSKI, 1958; COUSIN *et al.*, 1966) and Potato Witches' Broom (WRIGHT, 1958).

Under the light microscope an accumulation of chromophilous substances is observed in the phloem cells of roots and stems (fig. 3-7).

An intense hyperplasia of the internal and external phloem is specially obvious in transversal sections of stems (fig. 4). In longitudinal sections, the chromophilous substances are seen in long chains of phloem cells. Under immersion (1250 \times) small granules were observed which may correspond to mycoplasma cells (fig. 7). In sections from healthy plants no similar aspects have been observed.

The alterations we described have been signaled by others in similar diseases. SAMUEL *et al.* (1933) and CICCARONE (1951) refer to the thickness of internal phloem in sections of petioles of tomatoes with Big Bud.

ESAU (1961) studying the anatomic effects of Curly Top and Aster Yellows in order to seek means of distinguishing between the two diseases in the tomato plant found in plants with Aster Yellows, the development of adventitious roots, hyperplasia of internal and external phloem of stems and chromophily of the phloem cells. The corresponding symptoms in tomato plants with «Mal Azul» are more accentuated (fig. 3-7).

COUSIN *et al.* (1966) found abnormal fluorescence in the phloem of plants with Stolbur. Using several staining techniques they observed chromophily in the phloem cells of *Solanum melongena*, *Datura stramonium* and *Vinca rosea* but not in *Lycopersicon esculentum* and *Solanum nigrum*.

Under the electron microscope mycoplasma-like organisms have been observed (fig. 8 and 9) in the phloem cells of adventitious roots, petioles and sepals from naturally and experimentally infected tomatoes.

The Mycoplasma were observed in the central vacuoles of the phloem cells. Usually the cells were so numerous that they filled completely those vacuoles. Due to the absence of a cell wall, the mycoplasma cells are pleomorphic and in the sections show circular or irregular elongated forms. The circular ones are about 300 to 600 m μ in diameter. Their limiting membrane, 90 m μ thick, has two electron dense layers separated by a less dense one. In some zones the outer layer seems thicker.

Inside the cells an electron dense substance is irregularly distributed close to the membrane. Ribosome-like granules could be recognised. In areas of low electron opacity a net of filaments, usually diverging from a dense bar, have been observed and interpreted as the fine threads of DNA described in Mycoplasma (DIENES *et al.*, 1967, DOMERMUTH *et al.*, 1964).

Besides the elements we just described, smaller cells, the so-called elementary bodies, have also been recognised. They have a limiting membrane and are completely filled with a granular dense material. No threads are visible in those elements.

Fig. 9 shows near a sieve plate mycoplasma cells with round or elongated protrusions. Some of the cells have been blocked by callose on the way to the next phloem cell through the pores of the sieve plate.

We have not yet succeeded in cultivating outside the host the Mycoplasma observed in the infected plants in order to fulfill Koch's postulates. However, the absence of Mycoplasma cells in healthy plants and their presence in organs of plants of different species with similar diseases in different countries (Table I), could be considered as good evidence to accept them as the causative agent.

The effects of antibiotics of tetracycline group in suppressing or, at least, retarding symptoms development of mulberry dwarf disease (ISHIIE *et al.*, 1967), also support this opinion.

The great number of Mycoplasma cells present in the phloem is sufficient to explain some of the alterations, characteristic of «Mal Azul». The development of lateral

TABLE I

Diseases related with the presence of Mycoplasma-like organisms

Country of origin	Disease	Plant host	Author
Czechoslovakia	Parastolbur	—	MARAMOROSCH <i>et al.</i> , 1968
France	Clover Phyllodie	<i>Trifolium repens</i> L. ¹	GIANNOTTI <i>et al.</i> , 1968a
»	Aster Yellow	<i>Vinca rosea</i> L.	MAILLET <i>et al.</i> , 1968
»	Clover Phyllodie	» » »	» » »
»	Stolbur	» » »	» » »
»	»	<i>Nicotiana Tabacum</i> L.	» » »
»	Apple Proliferation	<i>Malus sylvestris</i> L.	GIANNOTTI <i>et al.</i> , 1968b
»	Stolbur	<i>Solanum lycopersicum</i> L.	» » 1968c
Japan	Aster Yellows	<i>Petunia</i> sp.	DOI <i>et al.</i> , 1967
»	Mulberry Dwarf	<i>Morus</i> sp.	» » »
»	Potato Witches'Broom	<i>Solanum tuberosum</i> L.	» » »
»	Paulownia Witches'Broom	<i>Paulownia</i> sp.	» » »
Philippines	Rice Yellow Dwarf	<i>Oriza sativa</i> L.	SHIKATA <i>et al.</i> , 1968
Portugal	«Mal Azul»	<i>Lycopersicon esculentum</i> Mill.	in the present paper
Rumania	Stolbur	—	MARAMOROSCH <i>et al.</i> , 1968
Taiwan	Sugar Cane White Leaf	<i>Saccharum</i> sp.	SHIKATA <i>et al.</i> , 1968
U. S. A.	Aster Yellows	<i>Calistephus chinensis</i> Nees	MARAMOROSCH <i>et al.</i> , 1968
»	» »	<i>Nicotiana rustica</i> L.	» » »
»	Maize Stunt	<i>Zea mays</i> L. ²	» » »
»	» »	» » » ³	» » »
U. S. S. R.	Crimean Yellows	—	GRANADOS <i>et al.</i> , 1968
			MARAMOROSCH <i>et al.</i> , 1968

¹ Names according to MARTYN (1968), except «Mal Azul»² Mycoplasma has been also found in the insect vector *Euscelis plebejus* Fall³ Idem in *Dalbulus elimatus* (Ball)

shoots, great number of adventitious roots, rolling and thickness of the leaves and lower resistance to humidity changes are typical symptoms of a disease of the conducting tissues.

CONCLUSIONS

Mycoplasma-like organisms have been consistently observed in the phloem of adventitious roots, petioles and sepals of tomatoes naturally and experimentally infected with «Mal Azul». No similar microorganisms have been found in healthy plants. Mycoplasma are tentatively accepted as the causative agent of the disease.

The demonstration of Mycoplasma in plants affected with Stolbur, Parastolbur, Aster Yellows, Clover Phyllodie, Potato Witches' Broom and «Mal Azul» supports the previous suggestion of a relationship amongst those diseases. The differences observed in the severity of symptoms and host range implies the possibility that different strains or species of Mycoplasma are involved.

SUMÁRIO

O «Mal Azul», enfermidade do tomateiro, frequente em Portugal, é transmissível por enxertia e supõe-se ter como vectores naturais *Cicadellidae*. A sua etiologia tem sido até agora atribuída a vírus.

A observação ao microscópio electrónico de cortes de raízes adventícias, pecíolos e sépalas de tomateiros com infecções naturais e experimentais revelou, nas células do floema, a presença de microorganismos morfológicamente idênticos a Mycoplasmas. Observações semelhantes foram feitas no Japão, França e Estados Unidos da América em enfermidades idênticas em diversos hospedeiros provenientes de vários locais e também até agora atribuídas a vírus.

Estas observações, assim como o facto de ter sido conseguida no Japão a eliminação de doenças deste grupo após tratamento com antibióticos do grupo das tetraciclínas, levam a considerar os Mycoplasmas como agentes patogénicos para as plantas. Tendo em conta estes dados é necessária

c urgente a revisão da etiologia das enfermidades até agora atribuídas a vírus e apenas transmissíveis por enxertia ou por enxertia e vectores do tipo dos *Cicadellidae*.

BIBLIOGRAPHY

ALBOUY, J., COUSIN, M. T. & GRISON, C.

- 1967 Etude comparée de trois maladies à virus souche «californienne» de l'Aster yellow du Glaieul, Phyllodie du Trèfle et Stolbur de la Tomate sur *Vinca rosea*. *Annls. Epiphyt.*, **18**: 157-171.

CICCARONE, A.

- 1951 Sintomi di «Virescenza ipertrofica» (Big bud) del pomodoro nei pressi di Roma. *Boll. Staz. Patol. Vég. Roma* **7** (Série 3): 1-5.

COUSIN, M. T. & GRISON, C.

- 1966 Premières observations concernant une fluorescence anormale dans le liber interne de plusieurs Solanées infectées par le virus du Stolbur et d'une Apocynacée atteinte de Phyllodie. *Annls. Epiphyt.*, **17**: 93-98.
- 1966 Quelques observations et essais concernant le Stolbur de la tomate. *Annls. Epiphyt.*, **17**: 99-111.

DIENES, L. & BULLIVANT, S.

- 1967 Comparison of the morphology of PPLO and L-forms of Bacteria with light and electron microscopy. *Ann. N. Y. Acad. Sci.*, **143**: 719-733.

DOI, Y., TERANAKA, M., YORA, K. & ASUYAMA, H.

- 1967 Mycoplasma — or PLT group — like Microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches'broom, Aster Yellows or Paulownia Witches' broom. *Ann. Phytopath. Soc. Japan*, **33**(4): 359-266.

DOMERMUTH, C. H., VIELSEN, M. H., FREUNDT, E. A. & BIRCH-ANDERSEN, A.

- 1964 Ultrastructure of *Mycoplasma* Species. *J. Bacte.*, **88**(3): 727-744.

GIANNOTTI, J., DEVAUCHELLE, G. & VAGO, C.

- 1968a Micro-organismes de type mycoplasme chez une cicadelle et une plante infectées par la phyllodie. *C. r. hebd. Séanc. Acad. Sci., Paris* **266** (Série D): 216-2170.

GIANNOTTI, J., MORVAN, G. & VAGO, C.

- 1968b Micro-organismes de type mycoplasme dans les cellules libériennes de *Malus Sylvestris* L. atteint de la maladie des proliférations. *C. r. hebd. Séanc. Acad. Cci., Paris* **267** (Série D): 76-77.

GIANNOTTI, J., MARCHOU, G., VAGO, C. & DUTHOIT, J.-L.

- 1968c Micro-organismes de type mycoplasme dans les cellules libériennes de *Solanum Lycopersicum* L. atteinte de Stolbur. *C. r. hebd. Séanc. Acad. Sci., Paris* **267** (Série D): 454-456.

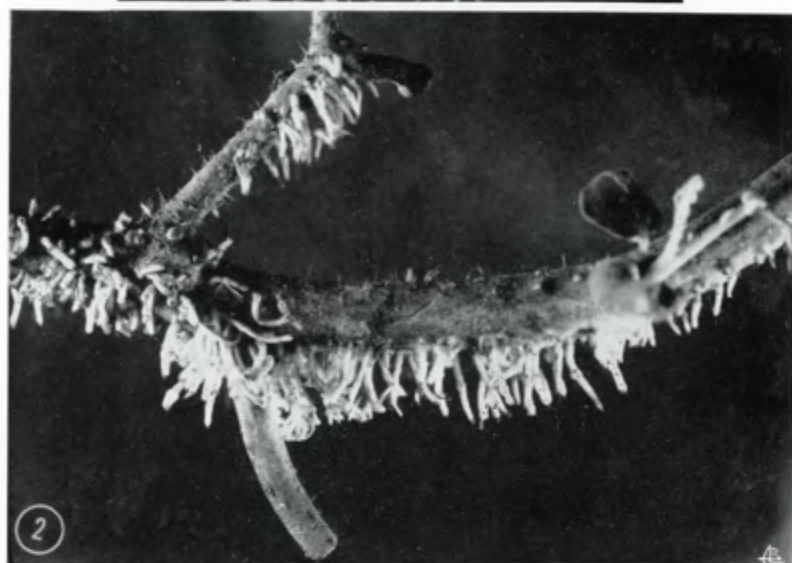
- GRANADOS, R., MARAMOROSCH, K. & SHIKATA, E.
1968 Mycoplasma: suspected etiologic agent of corn stunt. *Proc. natn. Acad. Sci., U. S. A.* **60**(3): 841-4.
- ISHIIE, T., DOI, T. Y., YORA, K. & ASUYAMA, H.
1967 Suppressive effects of antibiotics of tetracycline group on symptom development of mulberry dwarf disease. *Ann. Phytopath. Soc. Japan*, **33**: 267-275.
- KLINKOWSKI, M.
1958 Beiträge zur Kenntnis der Stolbur-Krankheit des Kartoffel. Proc. 3rd Conf. on Potato Virus Diseases. *Wageningen* (pp. 239-245).
- MAILLET, P., GOURRET, J.-P. & HAMON, C.
1968 Sur la présence de particules de type Mycoplasme dans le liber de plantes atteintes de maladies du type «jaunisse» (Aster yellow, phyllodie du Trèfle, Stolbur de la tomate) et sur la parenté ultrastructurale de ces particules avec celles trouvées chez divers Insectes Homoptères. *C. r. hebdom. Séanc. Acad. Sci., Paris* **266** (Série D): 2309-311.
- MARAMOROSCH, K., SHIKATA, E. & GRANADOS, R. R.
1968 Structures resembling mycoplasma in diseased plants and in insect vectors. *Trans. N. Y. Acad. Sci.*, **30**(6): 841-55.
- MARTYN, E. B.
1968 Plant Virus names. *Phytopath. paper 9*, Commonwealth Mycological Institute.
- MESSIAEN, C. M. & MARROU, J.
1967 Comparaison de la virulence sur diverses Solanacées de trois souches du virus du «Stolbur» et d'un virus attaquant le tomate en Tunisie. *Annls. Epiphyt.* **18**: 173-8.
- OLIVEIRA, MARIA DE L.
1965 Flower symptoms of a Virus disease of the Solanaceae. Livro de homenagem ao Professor Fernando Fonseca. Lisboa.
- RASA, E. A. & ESAU, K.
1961 Anatomic effects of Curly Top and Aster Yellows Viruses on Tomato. *Hilgardia*, **30**: 469-515.
- SAMUEL, G., BALD, J. G. & EARDLEY, M.
1933 «Big bud» a virus disease of the tomato. *Phytopathology*, **23**: 641-653.
- SHIKATA, E., MARAMOROSCH, K., LING, K. C. & MATSUMOTO, T.
1968 On the mycoplasma-like structures encountered in the phloem cells of American Aster Yellows, corn stunt, Phillipine rice yellow dwarf and Taiwan Sugar cane white leaf disease plants. *Ann. Phytopath. Soc. Japan*, **34**(2): (in MARAMOROSCH *et al.*).
- WRIGHT, N. S.
1958 Potato witches'broom in North America. Proc. 3rd Conf. on Potato Virus Diseases. *Wageningen* (pp. 264-277).

PLATES

PLATE I

Lycopersicon esculentum L. with «Mal Azul»
Symptoms in plants experimentally infected by grafting
(fig. 1).

Prostrated stem of naturally infected plant showing a
great number of adventitious roots (fig. 2).



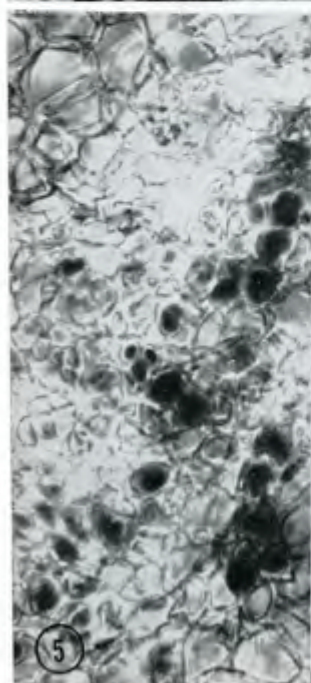
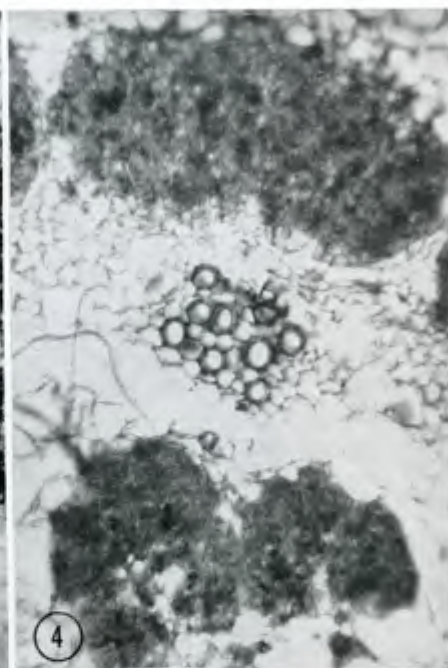
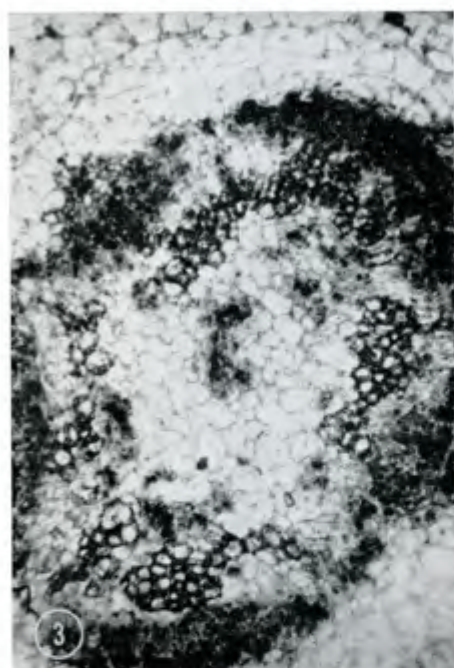


PLATE II

***Lycopersicon esculentum* with «Mal Azul»**

Abnormal development of phloem and presence of chromophilous substances inside phloem cells.

Transversal section of adventitious root (fig. 3). Detail (fig. 5). Transversal (fig. 4) and longitudinal (fig. 6 and 7) sections of stems.

PLATE III

***Lycopersicon esculentum* with «Mal Azul»**

Transversal section of a phloem cell, showing the cell wall (w), marginal cytoplasm (c) and mycoplasma cells.

It is possible to recognise in the larger mycoplasma-cells the unit membrane, ribosomes and DNA threads (fig. 8).



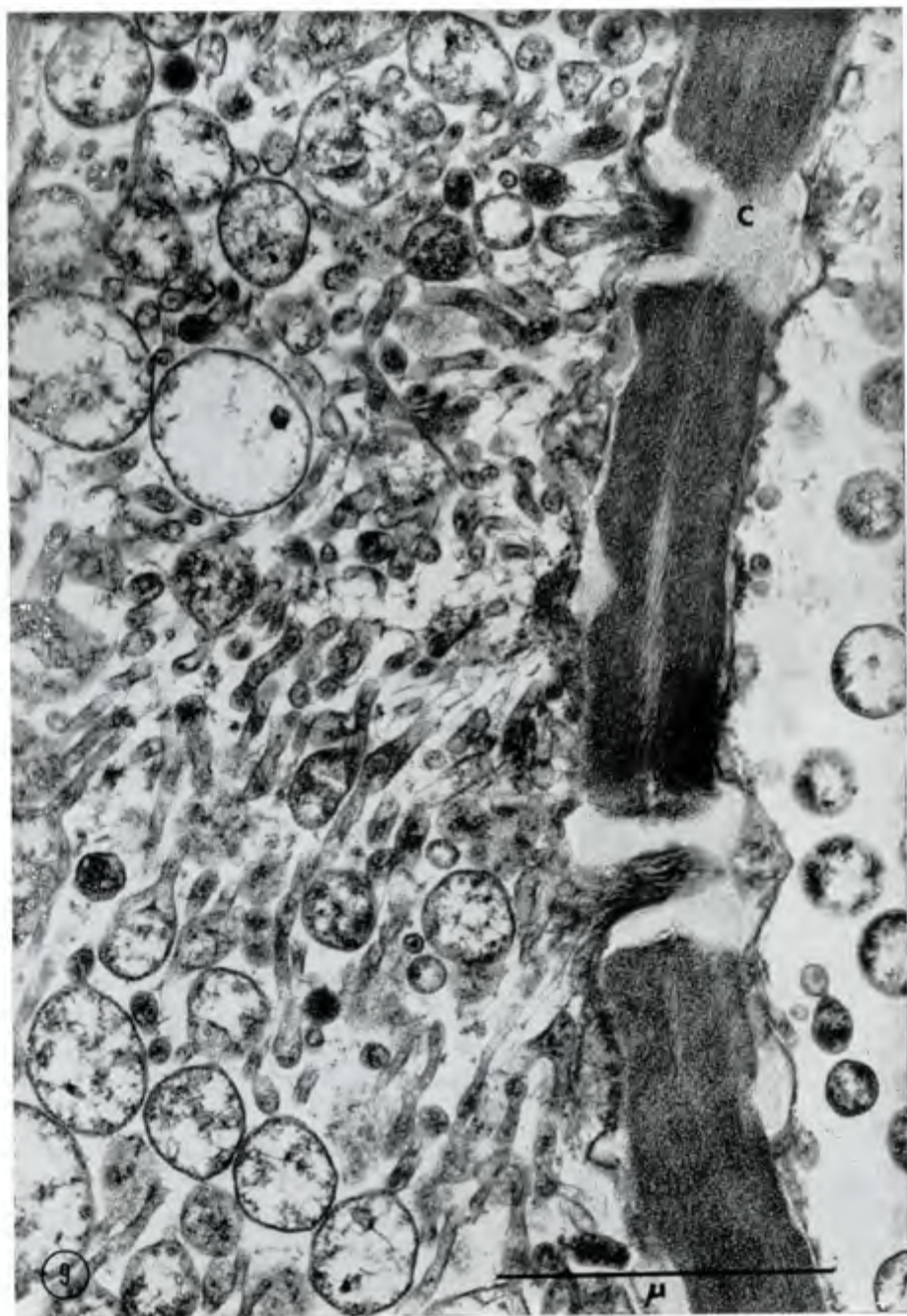


PLATE IV

Lycopersicon esculentum with «Mal Azul»
Longitudinal section of a sieve element. The sieve pores
of the plate are filled with callose (c).
Elongated forms of Mycoplasma have been blocked on
their way through the pores (fig. 9).