Rev. Biol. Trop., 28(1): 121-133, 1980

Morphological changes in bean leaves (*Phaseolus vulgaris* L.) induced by rugose mosaic virus infection*

by

Eugenia M. Flores** and Walter A. Marín***

(Received for publication December 6, 1979)

Abstract: Electron and light microscopy show that the foliar tissues of beans affected by the Bean Rugose Mosaic Virus (BRMV) disease undergo strong histologic and cytologic changes. The leaf blade is rugose and irregular, the stomata are malformed, the cuticle is striated, the mesophyll is packed and the intercellular spaces are missing. There are few, small and clear-colored chloroplasts; occasionally, they are degenerated. Sclerotic cells filled with phenolic substances are frequently found in severely affected areas. There is a general lack of correlation between growth of the organ and development of the tissues. Hypertrophy, hyperplasia, hypoplasia and eventual necrosis are the outstanding characteristics of this viral infection.

Rugose mosaic virus disease of beans (BRMV) was observed from 1964 to 1968 by Gámez (1972) in Turrialba, Costa Rica and described later in Guatemala (Gámez, 1971) and in El Salvador (Granillo *et al.*, 1975). The causal virus (rugose mosaic virus: BRMV), has polyhedric particles of approximately 28 nm (Bancroft, 1962; Gálvez *et al.*, 1974) and is a comovirus transmitted by *Cerotoma ruficornis* Oliver, *Diabrotica balteata* Le Conte and *Diabrotica adelpha* Harold (Cartín 1973); these last two species are the most important beetle vectors in the Americas (Fulton & Scott, 1977).

Little information is available on the morphological changes caused by BRMV infection in bean leaves. Kitajima *et al.*, (1974) report the results of an electron microscopic study of bean plants infected by either strain A_1 or A_2 of BRMV. They found crystalline virus particles, cell wall outgrowths, and laminated inclusions in the stromas of the chloroplasts associated with infection by strain A_1, while in tissues infected with strain A_2 numerous vesicles were observed in the cytoplasm. The cells studied were mainly mesophyll cells, although some observations were made on the vascular tissue. No reports were found on the changes at tissue level nor descriptions of epidermal alterations. In this study we describe

* This study received financial support from the Consejo Nacional de Investigaciones Científicas y Tecnológicas de Costa Rica (CONICIT).

** Escuela de Biología y Centro de Investigaciones en Biología Celular y Molecular, Universidad de Costa Rica.

*** Escuela de Biología, Universidad de Costa Rica.

striking and consistent changes that take place at tissue level in leaves infected with BRMV as observed under a Scanning Electron Microscope (SEM) or a compound light microscope.

MATERIAL AND METHODS

Expanding primary leaves (protophylls) of 8 to 10 day-old bean (*Phaseolus vulgaris* L., 27R) seedlings grown in a greenhouse (22–25C) were mechanically inoculated with purified BRMV, or crude sap specimens for microscopic examination were taken from infected leaflets of the first and second trifoliate leaves above the protophylls every 3 days commencing 8 to 20 days after inoculation. Healthy leaves of the same age were collected from control plants at the same time.

Leaf samples, 1 to 2 cm long were fixed in 4% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.0 for 2 hr at room temperature. After washing with buffer, the material was washed with water and dehydrated through a graded series of alcohol-amyl acetate solutions and stored in 100% amyl acetate. Samples were sonicated for 2 or 3 seconds and later dried by the critical point method using CO_2. They were then mounted on aluminum grids, gold-coated and examined in a Hitachi HHS–2R Scanning Electron Microscope (SEM), at 20 Kv.

Material for light microscopy was fixed in FAA (Johansen, 1940), Craft III (Sass, 1958) or formaldehyde-glutaraldehyde (Karnovsky, 1965), washed in water and dehydrated through a tertiary butyl alcohol series (Jensen, 1962), and infiltrated in paraffin. Transverse, tangential and median longitudinal serial sections were made at 8–12 μm and then stained using the Sharman (1943) technique or Harris hematoxylin, safranin and fast green (as modified by I. Morrow, personal communication).

RESULTS

The control plants have well-developed protophylls followed above by a pair of opposite trifoliate megaphylls. The leaflets show an expanded blade with reticulate venation. Externally, leaves are as described by Flores, Espinoza & Kozuka (1977). In the infected leaves, the leaflet blades are rugose or warty, and the shape is irregular; different zones become chlorotic and occasionally the epidermis above the veins becomes necrotic.

The epidermis of healthy leaves is quite smooth and the stomata are sunken (Fig. 1). The cuticle has only moderate striations as reported by Flores, Espinoza & Kozuka (1977) and as observed in Fig. 2. In diseased leaves the cuticle is intensely striated (Figs. 3, 4, 5) and in some areas there is a deposition of fine epicuticular wax (Figs. 3, 5).

Fig. 1. A SEM view of the epidermis of *Phaseolus vulgaris* L. in healthy leaves.

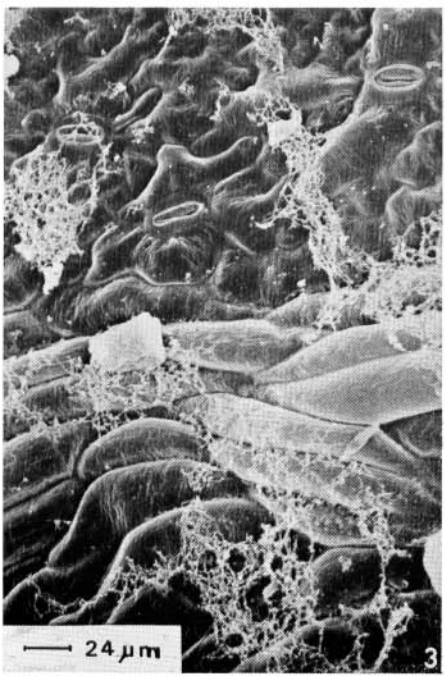
Fig. 2. Magnification of the epidermal cells of *Phaseolus vulgaris* in healthy plants.

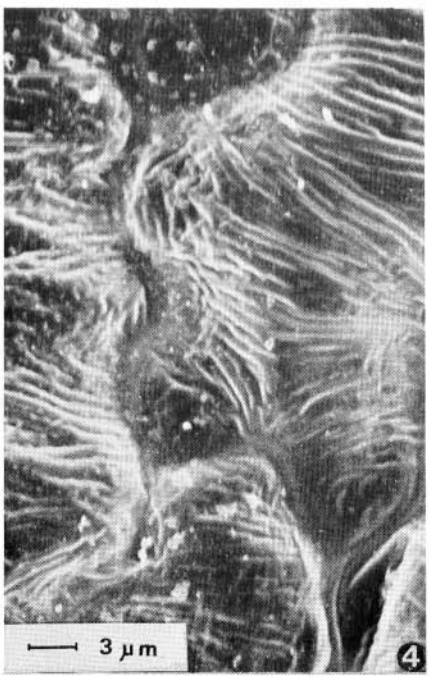
Fig. 3. Epidermis of diseased leaves.

Fig. 4. Striated cuticle of the epidermis in diseased leaves.


18 µm
1


8 µm
2


24 µm
3


3 µm
4

The stomata of control leaves are paracytic (Fig. 6), the guard cells crescent-shaped and the pore aperture is well defined. Slightly infected leaves show anomocytic stomata with the pore filled with waxy substances (Figs. 7, 8). Severely affected leaves have exposed stomata of the anomocytic type exhibiting a malformed pore; the aperture is sometimes partially filled with waxy substances (Figs. 9, 10). The straight veins characterizing healthy leaves become undulated and irregular in diseased leaves (Figs. 11, 12). Trichomes are frequently malformed.

Internally, the control leaves show the classical leaf structure of beans: both epidermal layers, a vascular system and well developed mesophyll (Fig. 13). Stomata are present on both surfaces but the number is higher abaxially (Fig. 13). Hooked trichomes and long uniseriate trichomes of healthy leaves have a thick cuticle, especially at the distal end (Fig. 14). The mesophyll differentiates into palisade and spongy parenchyma and contains a large volume of intercellular spaces (Fig. 13).

The slightly infected leaf epidermis shows a thinner cuticle than that of healthy leaves. Stomata are exposed and quite irregular (Fig. 15); the palisade parenchyma becomes more compact and the chloroplasts are grouped close to the plasmalemma. The spongy parenchyma is more compact also, and the intercellular spaces are reduced (Figs. 15, 16). Stomatal chambers are reduced, and sometimes hardly visible; long uniseriate trichomes are usually straight, narrow and long and the cuticle is very thin (Fig. 17). The hooked hairs are shorter and the basal cell changes its morphology (Fig. 18), as occurs in the distal cell. The cuticle is again very thin.

Severely diseased plants show different degrees of alteration in the leaf blade. In the green rugose zones close to the chlorotic areas, the damage is similar to that found in slightly infected leaves (Figs. 19, 20, 21); however, the stomata may be more altered (Figs. 19, 21) and quite exposed (Fig. 21). Some of the epidermal cells are bigger (Figs. 20, 21) than the normal cells; the vascular bundles are difficult to observe (Fig. 19) and the phloem and vascular parenchyma are sometimes obliterated (Figs. 20, 22). The parenchyma cells surrounding the vascular bundle have few chloroplasts and some are almost empty (Fig. 22). In the chlorotic areas, the compactation of tissue is general and no intercellular spaces are formed (Figs. 23, 24). At the abaxial surface, the epidermis above the veins becomes very irregular and there is a proliferation of parenchyma cells between the vascular bundle and the epidermis, which produces a protuberance (Figs. 23, 24). The cell walls of these parenchyma cells are very thin and the cytoplasm contains few chloroplasts. Some of the vascular bundles lack xylem or are mostly formed by phloem (Figs. 23, 24).

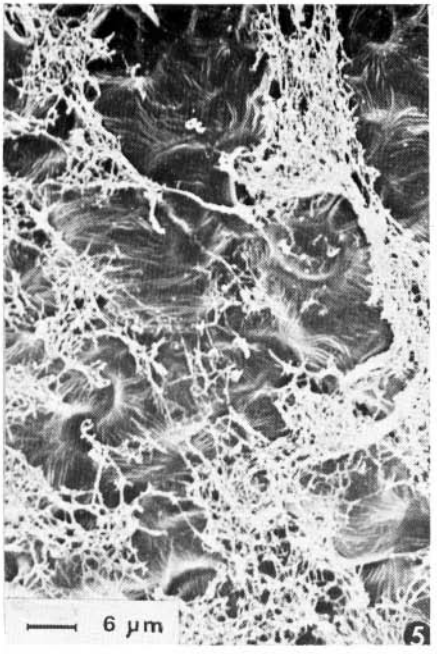
Thick, irregular epidermal cells are occasionally observed in the chlorotic zones as well as dark spaces with phenolic substances (Fig. 25). The neighbouring tissues are absolutely abnormal (Fig. 26).

Fig. 5. Irregular deposition of epicuticular wax in diseased leaves.

Fig. 6. Healthy stoma of the epidermis in control plants.

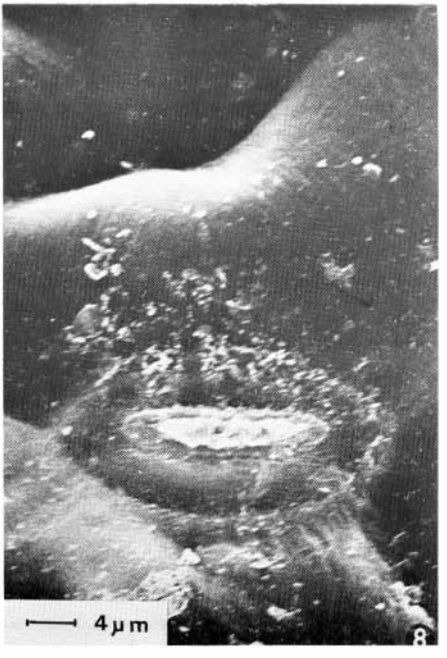
Fig. 7. Slightly damaged stoma of an infected plant.

Fig. 8. Stoma of a diseased leaf.


6 µm
5


8 µm
6


8 µm
7


4 µm
8

DISCUSSION

The foliar tissues of *Phaseolus vulgaris* L. affected by the rugose mosaic virus disease undergo profound histologic and cytologic changes. Among these, the irregular, rugose surface of the lamina, the alteration of the stomata and the compact mesophyll of leaves are the most striking symptoms. The rugose aspect of the lamina seems to be the result of a lack of correlation between growth of the organ and development of the tissues. This fact was observed earlier by Rokhlina (1931) and Clinch (1932) in potatoes infected with mosaic diseases.

The alteration of the epidermis, including the stomata, apparently is part of the degenerating changes affecting the tissues; sometimes, these changes are destructive and involve thickening of cell walls, necrosis and obliteration as reported in this research.

Clinch (1932) reported that in potato plants affected by the streak disease, the parenchyma surrounding the subsidiary veins is stimulated to abnormal growth and exhibits degenerated chloroplasts. Esau (1933) stated that the mesophyll cells of sugar beet leaves affected by curly top are stimulated to growth and division, becoming closely packed, with no intercellular spaces. Later, she found a similar alteration in grape leaves infected by Pierce's disease (Esau, 1948). As reported in this paper, rugose mosaic virus disease produces a similar compactation of the mesophyll due to hyperplasia and sometimes hypertrophy. This hyperplastic tissue is mainly found in the chlorotic areas; its light color is due to the small number of pale chloroplasts, some of which are degenerated.

Summarizing, we may state, as Esau (1967, 1968) pointed out, that hypertrophy (epidermis and mesophyll), hyperplasia (mesophyll), hypoplasia (stomata) and eventual necrosis, are usually associated to a viral infection. The character of the pathological changes differs according to the maturity of the tissue infected, the localization and the intensity of the processes.

RESUMEN

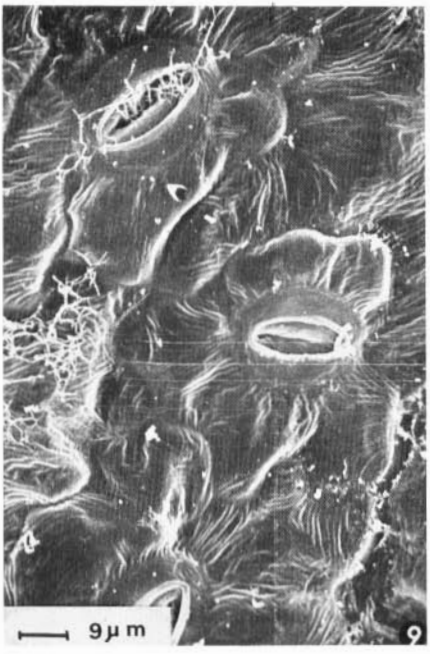
Observaciones con el microscopio de luz y electrónico de barrido muestran que los tejidos de las hojas de frijol (*Phaseolus vulgaris* L.) infectadas por el virus rugoso de mosaico (VRM) sufren cambios histológicos y citológicos. La lámina de la hoja es rugosa e irregular, los estomas son malformados, la cutícula es estriada, el mesofilo es compacto y no hay espacios intercelulares. Hay pocos cloroplastos pequeños de color claro; ocasionalmente están degenerados. Con frecuencia se encuentra células escleróticas llenas de sustancias fenólicas en las áreas severamente afectadas. Hay una falta general de correlación entre el crecimiento del órgano y el desarrollo de los tejidos. Las características sobresalientes de esta infección viral son la hipertrofia, la hiperplasia, la hipoplasia y la necrosis eventual.

Fig. 9. Abaxial epidermis of a diseased leaf.

Fig. 10. Altered stoma in an infected leaf.

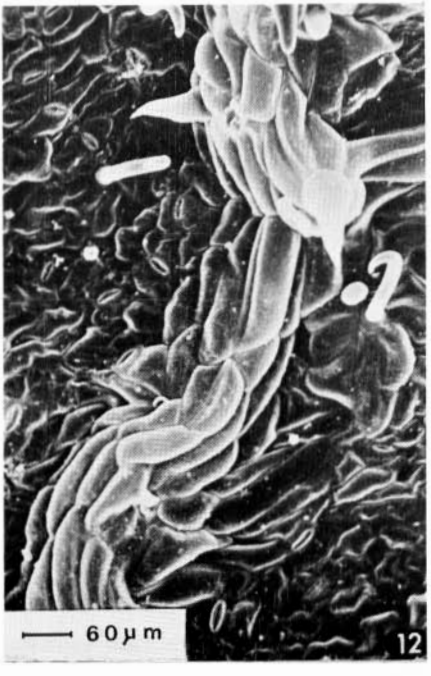
Fig. 11. General view of veins in a diseased leaf.

Fig. 12. Detail of an altered vein in an infected leaf.


9 µm
9


4 µm
10


110 µm
11


60 µm
12

LITERATURE CITED

Bancroft, J.B.
1961. Purification and properties of bean pod mottle virus and associated centrifugal and electrophoretic components. Virology, 16:419–427.

Cartín G., L.F.
1973. Caracterización de dos nuevas razas del virus del Mosaico Rugoso del Frijol (*Phaseolus vulgaris* L.). Tesis. Facultad de Agronomía, Universidad de Costa Rica. Costa Rica. 42 p.

Clinch, P.H.
1932. Cytological studies of potato plants affected with certain virus diseases. Sci. Proc. Roy. Dublin Soc., 20: 143–175.

Esau, K.
1933. Pathologic changes in the anatomy of leaves of the sugar beet, *Beta vulgaris* L. affected by curly top. Phytopathology, 23: 679–712.

Esau, K.
1948. Anatomic effects of the viruses of Pierce's disease and phony peach. Hilgardia, 18: 423–482.

Esau, K.
1967. Anatomy of plant virus infections. Ann. Rev. Phytopath., 5: 45–76.

Esau, K.
1968. Viruses in plant hosts: Form, distribution, and pathologic effects. The University of Wisconsin Press. Madison, Wisconsin.

Flores, Eugenia M., Ana M. Espinoza, & Y. Kozuka
1977. Estudio ultraestructural de la epidermis foliar de *Phaseolus vulgaris* L. Turrialba, 27: 117–124.

Fulton, J. P., & H.A. Scott
1977. Bean Rugose Mosaic Virus and related viruses and their transmission by beetles. Fitopatologia Brasileira, 2: 9–16.

Gálvez, G., M. Cárdenas, & A. Díaz
1974. Purificación del virus del ampollado del frijol. Proc. Amer. Phytopath. Soc., 1: 122.

Fig. 13. Transverse section of a healthy leaf.

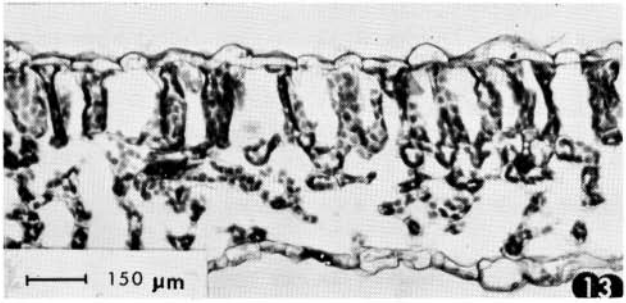
Fig. 14. Normal hooked trichome of a healthy epidermis.

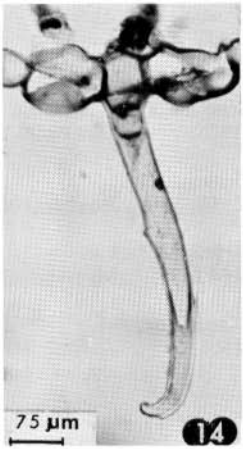
Fig. 15. Slightly altered leaf. Cross section.

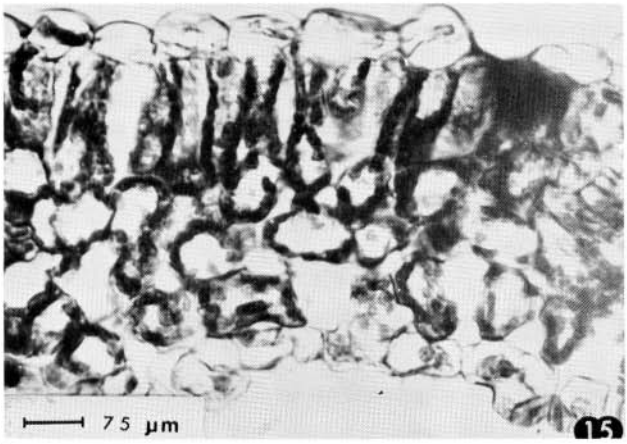
Fig. 16. Severely infected leaf. Cross section.

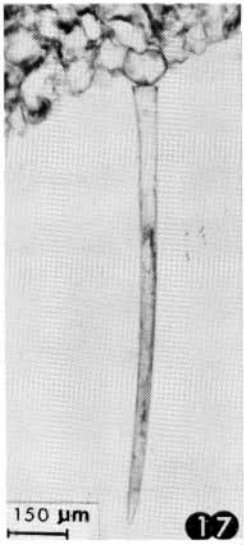
Fig. 17. Abnormal trichome of an infected epidermis.

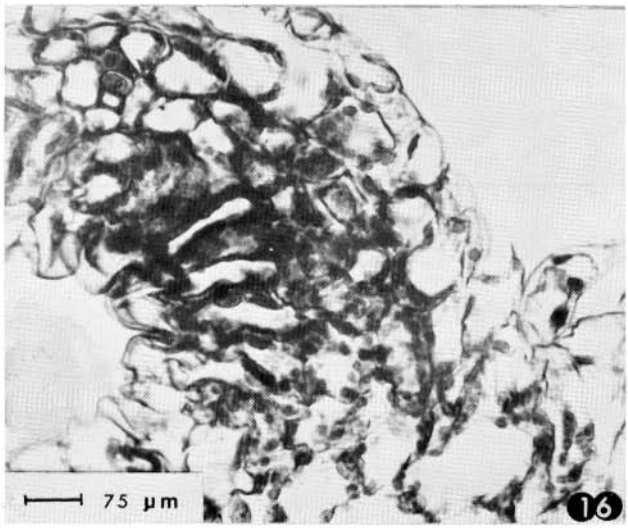
Fig. 18. Abnormal hooked trichome of an infected epidermis.

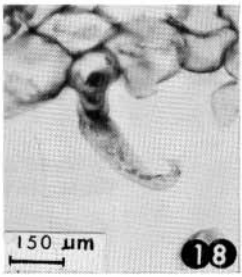

150 µm
13


75 µm
14


75 µm
15


150 µm
17


75 µm
16


150 µm
18

Gámez, R.
1971. Observación y estudio preliminar sobre virus del frijol en Guatemala. *In* Instituto Interamericano de Ciencias Agrícolas. Resúmenes XVII Reunión Anual, Programa Cooperativo Centroamericano de Cultivos Alimenticios. Panamá, Panamá. 2 p.

Gámez, R.
1972. Los virus del frijol en Centro América. II. Algunas propiedades y transmisión por crisomélidos del virus del mosaico rugoso del frijol. Turrialba, 22: 249–257.

Granillo, C. R., A., Díaz, M.A. Araya, & G.E. Jiménez
1975. Una nueva virosa del frijol transmitida por crisomélidos. Siades, 4: 3–4.

Jensen, A.W.
1962. Botanical histochemistry. W.H. Freeman & Co. Berkeley, California.

Johansen, D.A.
1940. Plant microtechnique. McGraw–Hill Book Co. New York.

Karnovsky, M.J.
1965. A formaldehyde-gluteraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell. Biol., 27: 137A– 138A.

Kitajima, E.W., A. Tascon, R. Gámez, & G.E. Gálvez
1974. Ultraestructural studies on bean leaf tissues infected with two strains of bean rugose mosaic virus. Turrialba, 24: 393–397.

Rokhlina, E.
1931. On the anatomy of the potato plant infected with mosaic disease materials. Mycol. Phytopath., 8: 145–154.

Sass, J. E.
1958. Botanical microtechnique. The Iowa State Univ. Press. Ames, Iowa.

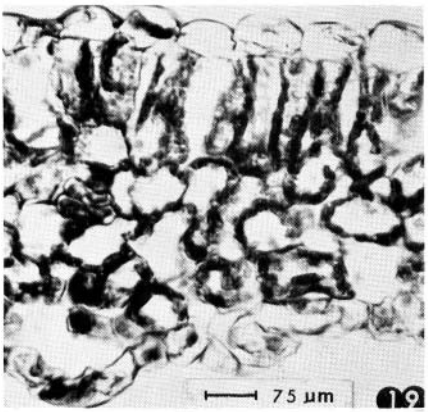
Sharman, B.C.
1943. Tanic acid and iron alum with safranin and orange G in studies of the shoot apex. Stain Tech., 18: 105–111.

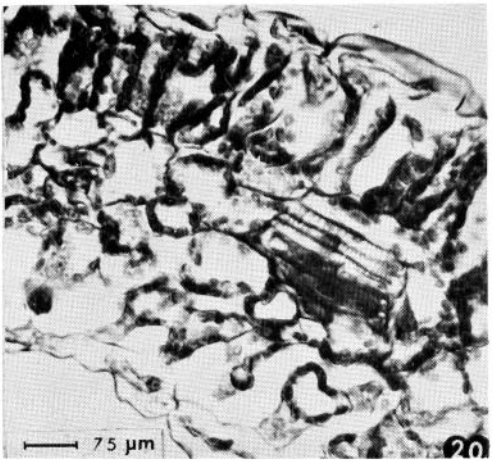
Fig. 19. Damaged area of the leaf close to the chlorotic areas showing exposed stomata. Cross section.

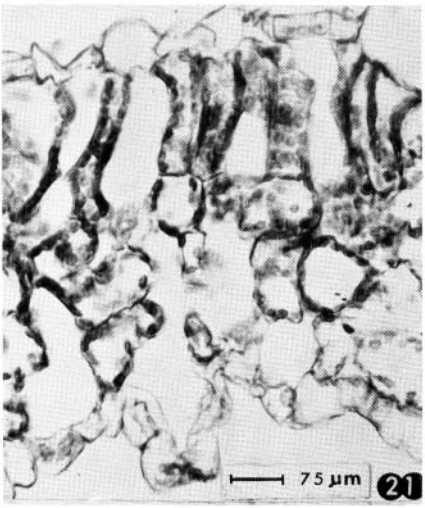
Fig. 20. Damaged area close to a chlorotic spot.

Fig. 21. Exposed stoma of an infected leaf. Cross section.

Fig. 22. Chlorotic tissue (abaxially) below the vascular bundle. Cross section.


75 µm
19


75 µm
20


75 µm
21

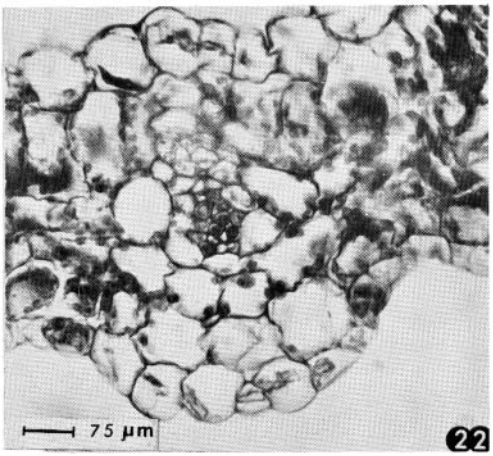
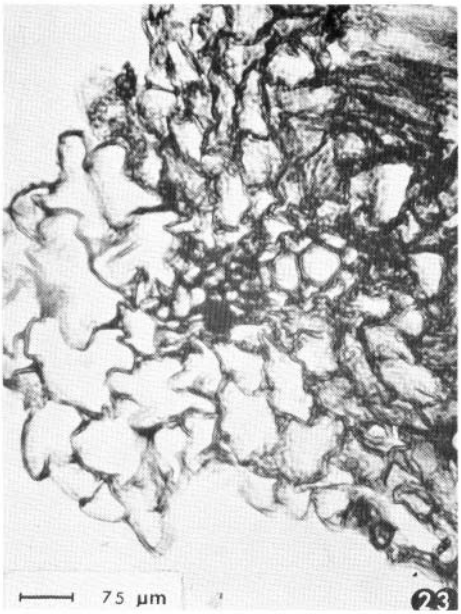

75 µm
22

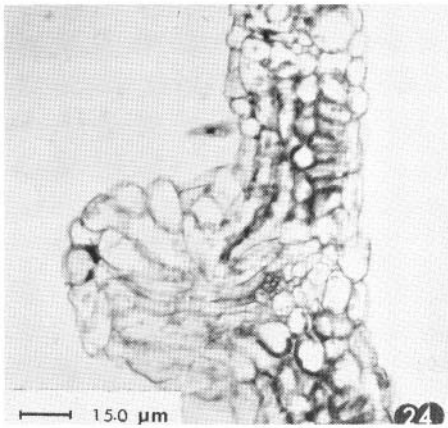
Fig. 23. Compactation and alteration of tissues of infected leaves.

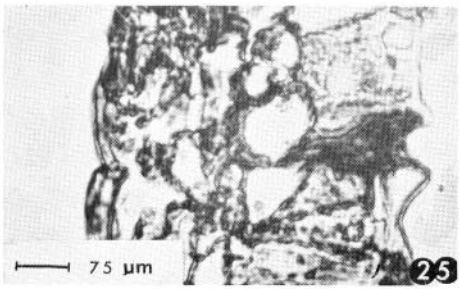
Fig. 24. Abaxial protuberance below the vascular bundle of an infected leaf. Cross section.

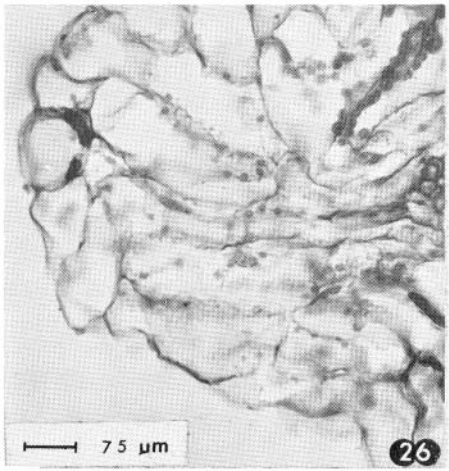
Fig. 25. Thick cells of an infected leaf. Cross section.

Fig. 26. Chlorosis of the abaxial protuberance below the vascular bundle.


75 µm
23


150 µm
24


75 µm
25


75 µm
26