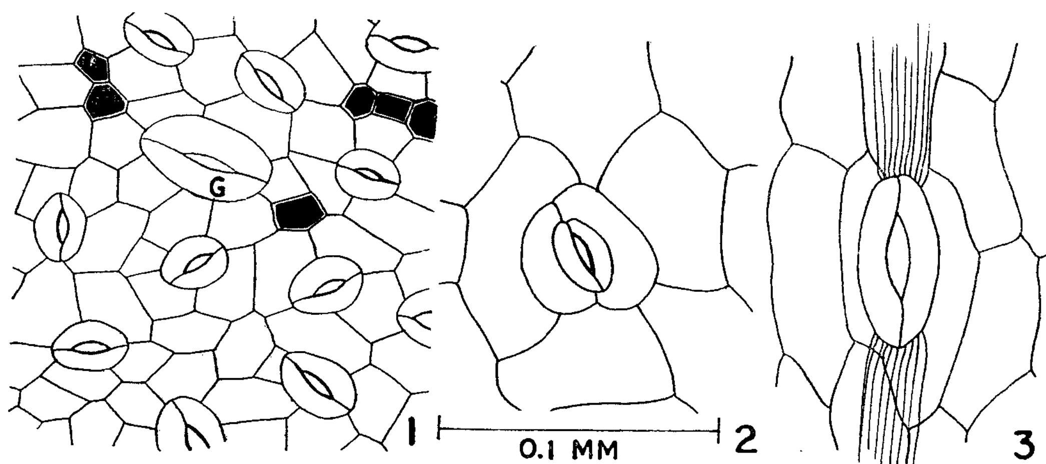
OCCURRENCE OF GIANT STOMATA IN CELASTRACEAE AND CONVOLVULACEAE

GIANT stomata occur on certain leaves along with the normalized stomata[3]. There are a few reports on the occurrence of giant stomata[1-6]. While studying the epidermal features in Celastraceae and Convolvulaceae, we observed abnormally large stomata on the leaves of *Celastrus stylosus* Wall., *Euonymus hamiltonianus* Wall. and *Hippocratea arborea* Roxb. (Celastraceae), and *Ipomoea aquatica* Forsk. (Convolvulaceae).

Stace[4] reported their rare occurrence on the veins and venules. The giant stomata on the midrib and larger viens are in the process of degeneration. However, the ones on the venules show distinct stomatal pores and guard cells comparable to normal stomata. In all the species studied, the giant stomata are almost twice as large as the normal stomata (Table I, Figs. 1–3). In addition to their size, in *I. aquatica*, the giant stomata can be distinguished from normal, by the presence of two lateral groups of striae (Fig. 3).



FIGS. 1–3. Fig. 1. A portion of lower epidermis of *Hippocratea arborea* in surface view showing a giant stomata (G) along with several stomata of normal size. Figs. 2, 3. A normal stomata from intercostal area and a giant stomata from costal area respectively in surface view from lower epidermis of *Ipomoea aquatica*.

TABLE I

Species	Size of stomata (L×B) in μ		Stomatal frequency per sq. mm	Stomatal index
	Normal	Giant		
1. *C. stylosus*	23·25 ×20·46	39·99 ×25·11	342	14·16
2. *E. hamiltonianus*	25·11 ×23·25	39·06 ×26·37	369	18·75
3. *H. arborea*	19·53 ×15·81	45·57 ×29·76	368	20·06
4. *I. aquatica*	18·52 × 7·63	41·16 ×18·52	U: 125 L: 250	9·23 16·62

U—upper surface of leaf, L—lower surface of leaf.

The leaves are hypostomatic in Celastraceae, and amphistomatic in Convolvulaceae. In the former, giant stomata occur on the lower surface of leaf and in the latter on both the surfaces. They occur on the midrib, veins, venules, and areoles, though

School of Plant Morphology, Meerut College, Meerut, *August* 5, 1974.

D. K. JAIN.
V. SINGH.

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FLUORESCENT ANTIBODY TEST FOR DETECTION OF CITRUS GREENING MYCOPLASMA

IN medical sciences the fluorescent antibody technique has been widely used in visualization and identification of bacteria and viruses[2], and to some extent of mycoplasma[3]. Also, some enzymes and

ıormones have been traced in animal tissues by his method[2]. However, very little work has been lone in the field of plant pathology[4,5,6]; none lealing with plant mycoplasmas.

In the present studies, culture of mycoplasma obtained earlier from greening affected citrus plants on PPLO broth[7], served as antigen. It was concentrated by one cycle of differential centrifugation which involved centrifugation at 10,000 rpm for 20 minutes in a Spinco Ultracentrifuge Model L using phosphate buffer (pH 7·0) to separate the suspended impurities, followed by high speed centrifugation at 25,000 rpm for 120 minutes and dissolving the pellet in a small quantity of M/30 phosphate buffer before final centrifugation at 5,000 rpm for 15 minutes. The concentrated antigen was injected in rabbits intramuscularly thrice at weekly intervals with Freund's adjuvant followed by one intravenous injection without the adjuvant. The blood, collected by bleeding the rabbits after 10 days following the last injection, was allowed to clot at room temperature for four hours and stored overnight in a refrigerator for clot to shrink. The serum was decanted and clarified by low speed centrifugation (7,000 rpm for 15 minutes). The antiserum reacted specifically with the mycoplasma culture and had a titre of 1 : 512. γ-globulins were extracted from antiserum by precipitating with equal volume of 3·2 N ammonium sulphate, dissolving the precipitate in 2 ml *tris*-HCl buffer (pH 7·2) and reprecipitating repeatedly until the precipitate was absolutely white. The γ-globulin solution was dialysed against the same buffer until all the sulphate ions were removed. The pH of the solution was raised to 8·5 with 0·5 M carbonate-bicarbonate buffer (pH 9·0) before stirring it with fluorescein isothiocyanate (FITC), added to the solution at the rate of 50 mg per g of protein, for 4–6 hours in cold (4° C). The preparation was then eluted with the *tris*-HCl buffer on a Sephadex (G–25) column to remove the unconjugated dye (FITC).

For detection of mycoplasma, sections of infected as well as uninfected leaves were cut and flooded with *tris*-HCl buffer. They were then transferred to slides and each one was covered with 1–2 drops of FITC conjugated antiserum. The slides were left in a moist chamber for 8–12 hours at room temperature. After draining off excess conjugated antiserum, the sections were washed with *tris*-HCl buffer and mounted in *tris*-HCl buffered glycerine. When examined under the fluorescent microscope, sections of healthy leaves showed dull blue autofluorescence all over except in the thick walled cells which fluoresced in light green range. In infected leaves, in addition to autofluorescence noted in the healthy leaves, brilliant apple green fluorescence in the form of dots was observed in phloem cells (Fig. 1). This indicated the presence of mycoplasma.

FIG. 1. Fluorescence photomicrograph of greening affected citrus phloem cell showing mycoplasma-like pathogen (arrows) stained with fluorescein-conjugated antiserum, × 1,200, approx.

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Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi 110012, *July* 3, 1974.

DINESH KUMAR.
S. K. GHOSH.
S. P. RAYCHAUDHURI.
T. K. NARIANI.

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