# HILGARDIA

*A Journal of Agricultural Science Published by the California Agricultural Experiment Station*

VOLUME 13 - JANUARY, 1941 NUMBER 8

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UNIVERSITY OF CALIFORNIA · BERKELEY, CALIFORNIA

# H I L G A R D I A

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VOL. 13 JANUARY, 1941 No. 8


# INCLUSIONS IN GUARD CELLS OF TOBACCO AFFECTED WITH MOSAIC[1]


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LITERATURE contains conflicting reports on the occurrence of inclusions in stomatal guard cells of virus-diseased plants. Sheffield (1936),[3] studying the effect of many viruses upon their appropriate hosts, reported that guard cells never contained inclusion bodies. Certain of the plants—tomato, tobacco, *Hyoscyamus niger,* and *Solanum nodiflorum*—were checked for the distribution of plasmodesmata in leaf epidermis. Although these structures were abundant in ordinary epidermal cells, they were not observed in the walls between the guard cells and the adjacent epidermal cells. Sheffield therefore suggested that the absence of plasmodesmata prevented the virus from reaching the guard cells.

Hirayama and Yuasa (1937), however, found inclusions in guard cells of mosaic tobacco, which they studied in fresh sections treated with acetocarmine. Under the influence of the acetic acid the inclusions tended to become obliterated about an hour after treatment.

According to Kassanis (1939), plants (*Nicotiana Tabacum, sylvestris,* and *glutinosa; Solanum, Datura,* and *Hyoscyamus*) affected by the severe-etch virus disease invariably contain intranuclear inclusions in the guard cells but no cytoplasmic inclusions of the x-body type common in other cells.

In the present study, leaves of *Nicotiana Tabacum,* Turkish variety, affected by the Johnson tobacco mosaic virus, strain 1, were examined for distribution of inclusion bodies, with special regard to the guard cells. The material was killed and fixed in a chrom-acetic-formalin and a formalin-acetic-alcohol mixtures, then imbedded, sectioned, and stained by ordinary procedures used in the paraffin method.

In sections containing abundant mosaic inclusions throughout the


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[3] See "Literature Cited" for complete data on citations, which are referred to in the text by author and date of publication.

mesophyll, these structures invariably occurred in the guard cells of the upper and lower epidermis of both mesophyll and veins. In plate 1, inclusions—the ameboid vacuolated x-bodies (*x*) and the striated material (*str*)—are shown in several guard cells. Plate 1, *A* and *B*, shows two successive sections, 10 microns apart, of the same stoma from the epidermis of a comparatively large vein. The left guard cell contains in *A* a nucleus (*n*), an x-body, and striated material; in *B* some more striated material. The guard cell to the right contains a nucleus and striated material in *A;* an x-body and some more striated material in *B*. Plate 1, *D*, shows guard cells with striated material from the mesophyll area of a leaf. The guard cells in plate 1, *C*, are still immature. The one to the right shows a nucleus, an x-body, and striated material.

In view of these findings, it seemed pertinent to check the occurrence of plasmodesmata between the guard cells and the adjacent epidermal cells. The older botanical literature refers several times to this matter,[4] most authors agreeing that plasmodesmata, though obscure, are present in guard-cell walls.

Kohl (1897) apparently was the first to demonstrate plasmodesmata in guard cells by using *Viscum album*. Later (Kohl, 1902), examining other species of plants, he found the fern *Anemia Phyllitidis* particularly favorable for demonstrating cytoplasmic strands in guard-cell walls. He observed that the walls of guard cells swelled comparatively little when treated with acid. Because of this behavior and also because of the usually peculiar shape of guard cells, the plasmodesmata, according to Kohl, are visible only in most successful sections of these cells. The *Anemia Phyllitidis* guard cells have a very simple structure, showing the plasmodesmata readily.

Kuhla (1900) agreed with Kohl with regard to the presence of plasmodesmata and to the difficulty of demonstrating them in guard cells of *Viscum*.

In his first paper on plasmodesmata, Kienitz-Gerloff (1891) reported absence of these structures in guard cells; later (1902) he confirmed the conclusions of Kuhla and of Kohl.

Gardiner and Hill (1901) succeeded in demonstrating plasmodesmata in guard cells of *Pinus Pinea* and *Pinus sylvestris*. These workers, having thoroughly surveyed all types of tissues in these species, concluded that, with regard to plasmodesmata, "the guard cells of the stomata are quite the most difficult cells to investigate."

In the present study, cytoplasmic connections between cells of epidermis were examined in leaves of *Nicotiana Tabacum* and *Helianthus*

[4] The writer acknowledges the assistance of Dr. L. G. Livingston of Swarthmore College in locating references on plasmodesmata in guard cells.

*annuus* by Crafts's (1931) method of demonstrating plasmodesmata—the same method used by Sheffield (1936). Convincing views of plasmodesmata were obtained only after young differentiating leaves were employed. In mature leaves of tobacco and sunflower the guard cells project above the surface, and the wall connecting these cells with the adjacent epidermal cells is somewhat inclined with regard to the surface of the leaf. This position of the wall must partly cause the difficulty encountered in demonstrating plasmodesmata in guard cells in face views. Transverse sections of mesophyll are, on the other hand, difficult to make freehand. Young, not fully differentiated guard cells are not raised above the surface and clearly show the deeply stained lines connecting the protoplasts of the guard cells with those of the adjacent cells.

The two drawings in plate 2 depict at *a* to *e* five stages in the development of guard cells. Judging by plate 2, *A*, early and late stages in stoma differentiation occur side by side. At *a* and *b* recently divided cells have given rise to guard-cell mother cells (cells marked by *a* and *b*) and to their sister cells. The guard-cell mother cell later rounds off (plate 2, *A*, at *c*) and then divides into two equal cells—the two guard cells (plate 2, *A*, at *dd*). The stomatal opening is just forming between *dd*, whereas it is well developed between the two cells marked by the letter *e*.

In many cells in plate 2 the continuity between protoplasts and plasmodesmata was broken, and the latter appeared as discrete dots and lines between the protoplasts. In some cells, however, the deeply stained strands were continuous from protoplast to protoplast of contiguous cells. Such continuity occurs between the guard-cell mother cell and two adjacent cells at *c*, and between a guard cell and two adjacent cells at *d*.

Although the results of this study do not refute the concept that plasmodesmata are necessary for the passage of virus from cell to cell, they invalidate Sheffield's (1936) conclusions that cell inclusions are absent from guard cells and that this absence is due to the lack of plasmodesmata in guard cells. The present study shows that both, the inclusions and the plasmodesmata occur in these cells.

Virus entry does not, however, necessarily depend on the presence of plasmodesmata in mature cells. Viruses invade differentiating organs and could enter the future guard cells before they separate as such from their sister cells.

## SUMMARY

The inclusions characteristic of tobacco mosaic—the x-bodies and the striated material—occur in the guard cells of stomata of *Nicotiana Tabacum*. The guard cells and the adjacent epidermal cells are connected with each other by plasmodesmata—structures that could serve as paths for virus entry into guard cells.

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# PLATES

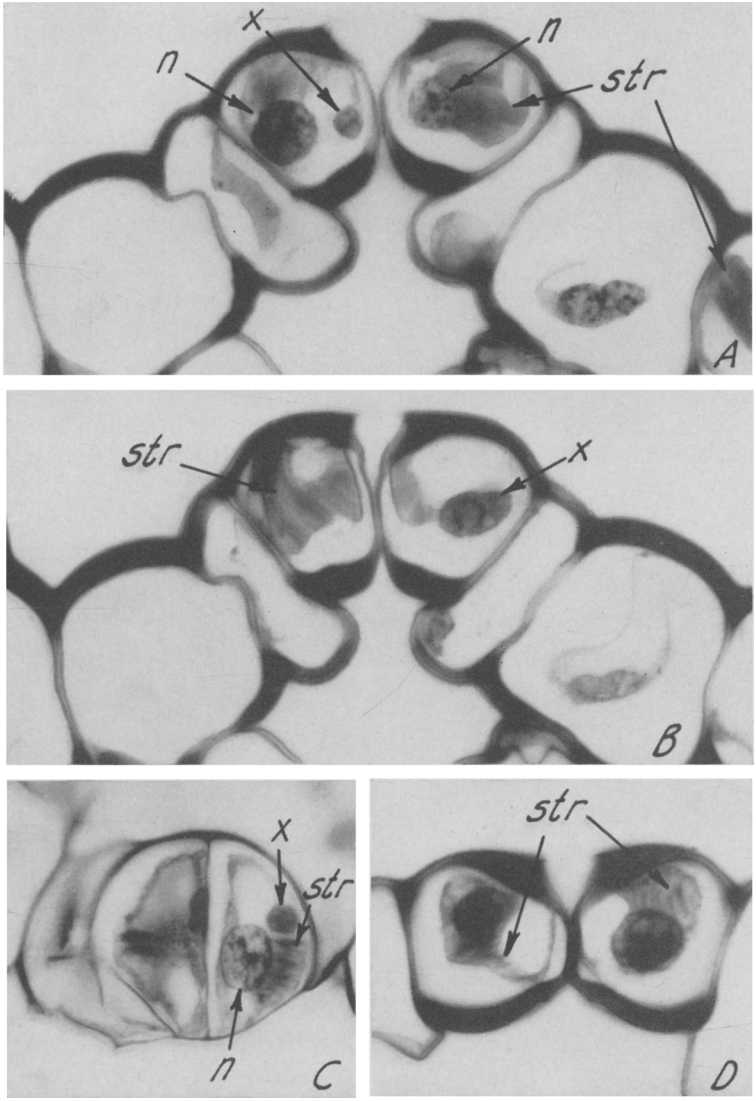


Plate 1.—Photographs of stomatal guard cells and adjacent cells of leaves of *Nicotiana Tabacum* showing inclusions characteristic of tobacco mosaic. *A*, *B*, *D*, Mature guard cells in sectional views; *C*, immature guard cells in surface view. Details are: *n*, nucleus; *x*, x-body; *str*, striated material. (*A–C*, × 1,220; *D*, × 1,560.)

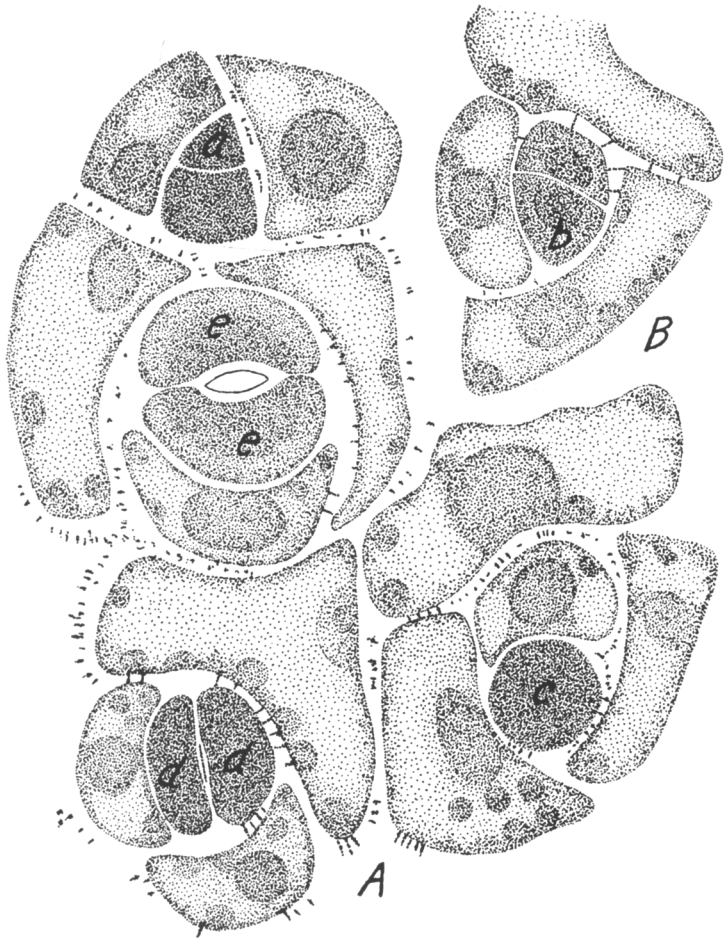


Plate 2.—Drawings of surface views of epidermis of young leaves of *Nicotiana Tabacum* showing distribution of plasmodesmata between the protoplasts. Cells marked *a* to *e* are guard cells in different stages of development. Plasmodesmata in guard-cell mother cell and in a young guard cell are shown at *c* and *d*. (× 1,460.)