Effects of Maize Dwarf Mosaic Virus on Water Relations of Corn

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ABSTRACT

Transpiration rates of maize dwarf mosaic virus (MDMV)-infected corn seedlings were reduced 15-40%, compared to healthy seedlings grown in soil or liquid culture without water stress or under increasing water stress in sealed pots. Onset of reduced transpiration was coincident with appearance of symptoms in infected leaves. Leaf water potentials of MDMV-infected seedlings were equal to, or higher than, those of healthy seedlings, which indicated that reduced transpiration was not caused by increased water stress in infected plants. Measurements of diffusive resistances of leaves, and direct observation of epidermal impressions, indicated that stomatal apertures were reduced in leaves of MDMV-infected plants. Movement of potassium ions into guard cells of healthy and MDMV-infected leaves was correlated with stomatal opening; potassium content of guard cells of MDMV-infected leaves was lower than that of guard cells of healthy leaves. Reduced transpiration by MDMV-infected corn seedlings appeared due to restriction of stomatal opening that was associated with reduced uptake of potassium by guard cells of infected leaves.

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Additional key words: transpiration, diseased plant physiology.

Reduced transpiration by the host plant has been associated with a number of viral infections (7, 9, 15, 17, 18, 21). We have frequently observed that corn plants infected with maize dwarf mosaic virus (MDMV) retained turgidity longer under periods of water stress than did healthy plants, suggesting an effect of the virus on the water status of infected plants. This particular aspect of diseased-plant physiology had not been investigated, although effects of MDMV on a number of other processes including photosynthesis (5, 26, 27) have been determined. Results of studies on the effect of MDMV on water relations in corn plants and possible

nature of the effect are reported here. A preliminary report (16) has been published.

MATERIALS AND METHODS.—*General.*—The virus used throughout this investigation was an Alabama isolate of MDMV-A. All studies were conducted on corn plants, *Zea mays* L. 'Hy × C103,' that, unless stated otherwise, were grown from seed in a steam-sterilized soil-peat-vermiculite mixture (2:1:1, v/v) in plastic pots in a controlled environment chamber. The chamber was programmed for: 13-hour day at 32 C and 60% relative humidity (RH), and 11-hour night at 16 C and 60% RH. A light intensity of 26,900 lx (2,500 ft-c) was supplied by a mixture of fluorescent and incandescent bulbs. Air turbulence within the chamber was not enough to cause leaf movement. Plants were watered as needed, and fertilized weekly with a complete fertilizer.

Virus inoculum was the crude sap obtained by trituration of leaves from corn plants infected 10-20 days with MDMV. Inoculations were made by the Carborundum/gauze-pad technique on leaves of corn seedlings in the two-leaf stage; the typical mottle indicative of MDMV infection usually appeared in the expanding third leaf 3-5 days after inoculation. Seedlings so treated with sap from healthy corn plants, and those receiving no treatment, were used as healthy-rubbed and healthy controls, respectively.

Transpiration.—Transpiration of corn seedlings was measured gravimetrically in three experiments. In the first experiment, seedlings were grown in individual plastic pots [906 ml (32 oz)] filled with approximately 1,000 g soil, and daily water loss from each pot was determined. Leaf areas were measured with a planimeter and transpiration rates were calculated as mg per day per 100 cm^2.

For the second experiment, healthy and MDMV-infected corn seedlings, grown in vermiculite until 5 days after inoculation, were transferred to liquid culture. Root systems were rinsed gently with tap water to remove vermiculite and each plant was placed in a light-proof plastic cup [226 ml (8 oz)] containing Hoagland's solution #2(10). Plants were supported by foam stoppers with care that the stopper did not touch the solution; the latter was replaced every third day. Beginning at 2 days after plants were transferred, water loss by each plant between 0900 and 1300 hours was determined daily; transpiration rates were calculated as mg per hour per cm^2.

In the third experiment, seedlings were grown individually in 400-500 g of soil in plastic cups [226 ml (8 oz)] in a controlled environment chamber programmed as

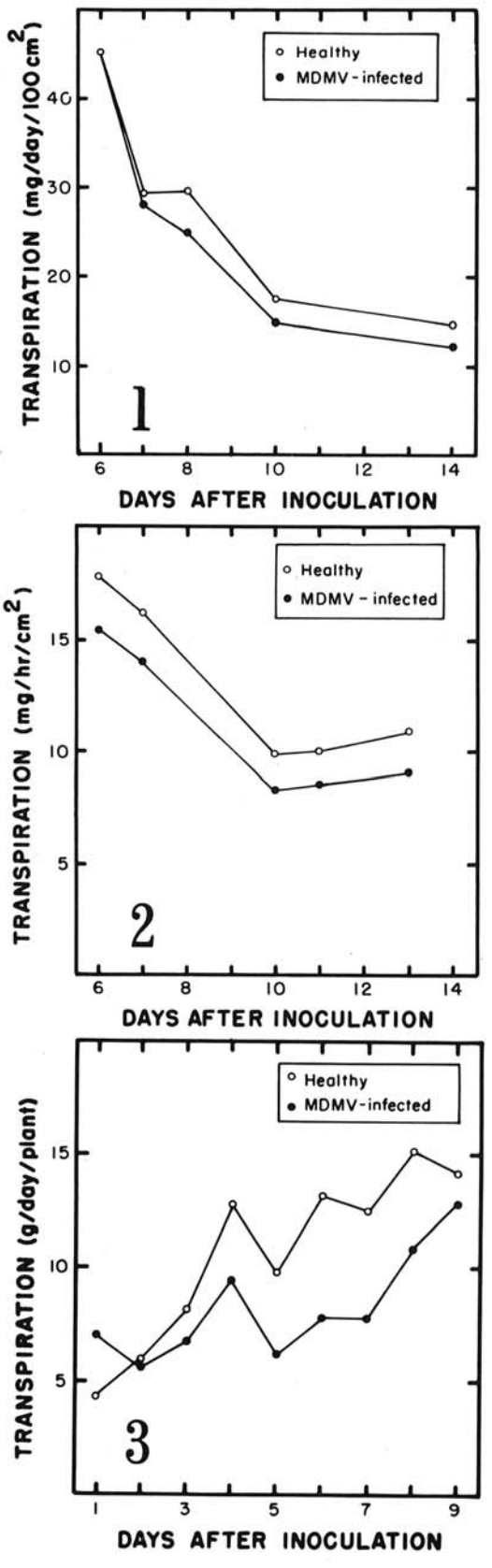

Fig. 1-3. Transpiration rates of healthy and maize dwarf mosaic virus-infected corn seedlings. **1)** Seedlings were growing without water stress in soil. Each value is an average from 13-15 plants; rates for MDMV-infected plants were significantly different ($P = 0.01$) from healthy at the 8th through 14th day after inoculation. **2)** Seedlings were growing in liquid culture. Each value is an average from nine plants; rates for MDMV-infected plants were significantly different ($P = 0.01$) from healthy at all days shown. **3)** Seedlings were growing under increasing water stress in sealed pots of soil. Each value is an average from 10 plants; rates for MDMV-infected plants were significantly different ($P = 0.01$) from healthy at the 4th through 8th day after inoculation.

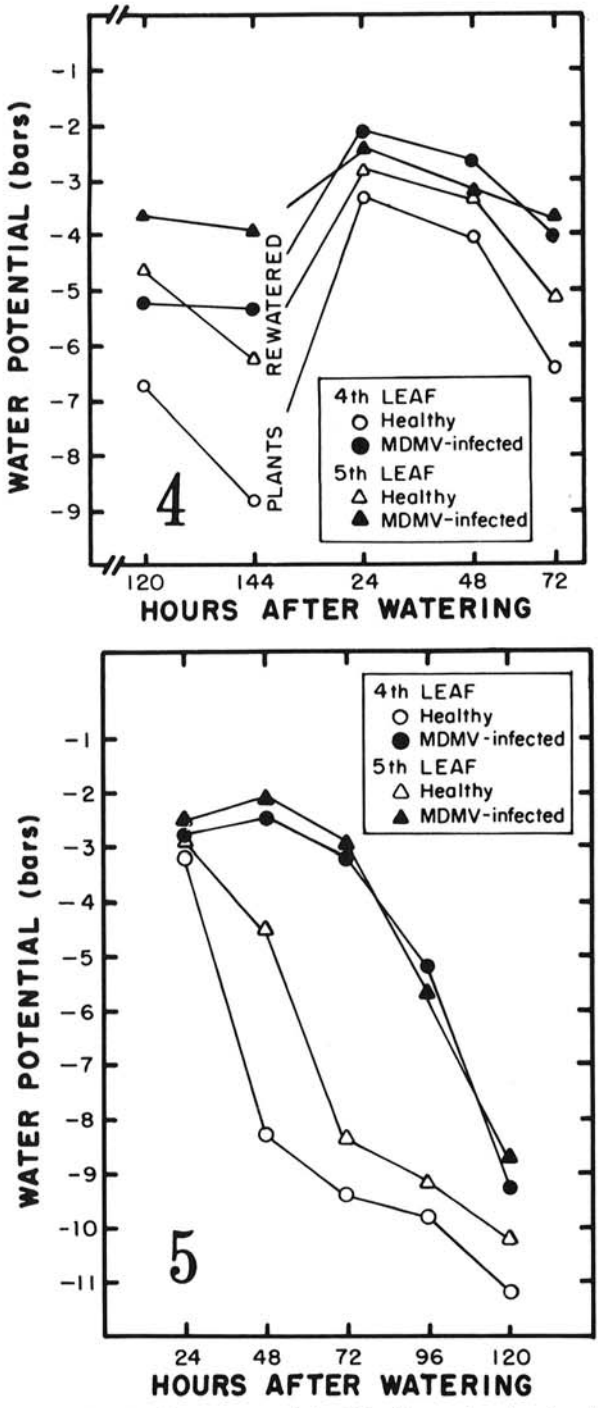

Fig. 4-5. Leaf water potentials of healthy and maize dwarf mosaic virus-infected corn seedlings. **4)** Seedlings were growing in soil during periods of water stress and recovery. Each value is an average from 12 plants in two experiments; potentials for MDMV-infected leaves were significantly different ($P = 0.01$) from corresponding healthy leaves at all times except for the fifth leaves at 48 hours after watering. **5)** Seedlings were growing under increasing water stress in vermiculite. Each value is an average from 12 plants in two experiments; potentials for MDMV-infected leaves were significantly different ($P = 0.01$) from corresponding healthy leaves at all times beginning at 48 hours after watering.

above except that air turbulence within the chamber was sufficient to cause leaf movement. Immediately after inoculation, each pot was enclosed in a plastic bag which was sealed around the base of the plant. Transpiration was calculated as g per day per plant.

Leaf water potential.—Leaf water potential in healthy and MDMV-infected corn seedlings was determined by the pressure-chamber method, which has been reported to give close approximation of leaf water potential in corn, sorghum, and wheat (2, 3, 4). Soil in plastic pots containing healthy and MDMV-inoculated seedlings was saturated daily until symptoms appeared in the latter; thereafter, water was withheld. Measurements of water potential in the fourth and fifth leaves were begun when no visible differences in turgor of healthy and MDMV-infected plants were apparent and continued until the day wilting was first observed. The soil was then saturated with water and water potentials of the fourth and fifth leaves were determined at 24, 48, and 72 hours after watering.

Leaf water potentials of older plants under stress were measured in seedlings grown in vermiculite and watered daily until 16 days after inoculation when watering was discontinued. Beginning 24 hours later, water potentials of fourth and fifth leaves were determined daily until all plants were wilted.

Stomatal response.—Diffusive resistances of leaves of well-watered healthy and MDMV-infected corn seedlings were determined with an Alvim porometer (1); data were expressed as seconds required for pressure to fall from 120 to 100 mm Hg as measured with a 100 cm^3 reservoir. All measurements were made midway between the base and tip of fourth leaves at least 30-45 minutes after lights were on and day temperature attained in the environment chamber. Diffusive resistances of leaves were checked each morning prior to onset of lighting and day temperature to confirm stomatal closure (resistance $>$ 120 seconds).

Stomatal frequency was determined from Duco (DuPont) cement impressions taken midway between the base and tip on upper and lower surfaces of healthy and MDMV-infected fourth leaves 16 days after inoculation. All counts were of stomata occurring between the third and fourth veins from the midrib.

Potassium content of guard cells was determined by the cobaltinitrite technique {6); the procedures of Pallaghy (19) were used to obtain functional stomata. Epidermal strips were taken 15-20 days after inoculation from the lower epidermis between the second and third veins from the midrib. Several strips were removed and floated on distilled water in small watch glasses under a light intensity of ca. 21,520 lx (2000 ft-c) and in CO_2-free air. After exposure for 2 hours, several strips were randomly selected for estimation of stomatal apertures and localization of potassium.

Root/shoot ratio.—Shoots of healthy and MDMV-infected plants were removed at soil level. Root systems were carefully removed from pots and the soil gently washed from them. Roots and shoots were dried for 10 days at 80 C and w/w ratios were calculated. Root weight/shoot surface area (cm^2, per planimeter measurements) ratios also were determined.

Data were subjected to analysis of variance and tested for significance by the F test; where applicable, means

were compared by Duncan's multiple range tests.

RESULTS.—*Transpiration.*—Beginning at 4-8 days after inoculation, transpiration rates of MDMV-infected plants were significantly lower than those of the healthy-rubbed and healthy controls in all three experiments (Fig. 1-3). Generally, rates for the two types of controls did not differ significantly, hence only data for the healthy controls are plotted in the graphs. Transpiration rates of MDMV-infected plants growing without stress in soil (Fig. 1) or liquid culture (Fig. 2) were reduced 15-20% through the 13th or 14th day after inoculation; rates for MDMV-infected plants in soil were reduced 17% at 20 days after inoculation (data not shown). Reductions in transpiration rates of MDMV-infected plants growing under increasing water stress in sealed pots were 25-40% lower than those of corresponding control plants (Fig. 3). In this experiment, all virus-free plants were wilted after 9 days without water; whereas, MDMV-infected plants remained turgid.

Leaf water potential.—Leaf water potentials of MDMV-infected seedlings were significantly higher than those of healthy plants throughout the 12-day period after inoculation, during which plants were subjected to water stress and recovery from it. At 9 days post-inoculation and 144 hours without additional water, healthy fourth leaves were rolled slightly at the margins while MDMV-infected leaves appeared turgid; average water potentials were -8.8 and-5.3 bars for healthy and MDMV-infected leaves, respectively (Fig. 4). Fifth leaves were not wilted, but water potentials of healthy leaves were lower than those of MDMV-infected leaves. Water potentials of fourth and fifth leaves at 10 days after inoculation and 24 hours after saturating the soil with water showed recovery by both healthy and infected tissues; however, water potentials of healthy leaves were still significantly lower than those of MDMV-infected leaves.

Leaf water potentials of vermiculite-grown plants under increasing water stress, beginning at 17 days post-inoculation and continuing until all plants were wilted, are shown in Fig. 5. Little difference in water potentials was observed after 24 hours without water; however, leaf water potentials of MDMV-infected plants were significantly higher than those of healthy plants throughout the remainder of the experimental period. After 72 hours without water, healthy leaves were wilted (completely rolled up); water potentials averaged -9.4 and -8.4 bars in fourth and fifth leaves, respectively. MDMV-infected leaves did not wilt until 120 hours after watering, at which time water potentials averaged -9.3 and -8.7 bars in fourth and fifth leaves.

Stomatal response.—Diffusive resistance of MDMV-infected leaves was significantly higher than that of healthy leaves during the period 10-21 days after inoculation (Fig. 6). Diffusive resistance of infected leaves was at least twice that of healthy leaves at each time of measurement; over all days, resistance for infected leaves averaged 32.5 seconds as compared to 15.5 seconds for healthy leaves. During the course of a day, MDMV-infected leaves had higher resistance at 0800 (30-45 minutes after lights on) and 1100 hours and 1300 and 1600 hours (Fig. 7). No differences in diffusive resistance of healthy and MDMV-infected leaves were observed 30-45 minutes after lights were turned off.

Duco cement impressions of fourth leaves revealed no differences in stomatal frequency between healthy and MDMV-infected leaves. Average numbers of stomata per mm^2 from 25 determinations were 90 and 125 for upper and lower epidermis of healthy leaves, respectively, and 95 and 135 for upper and lower epidermis of infected leaves.

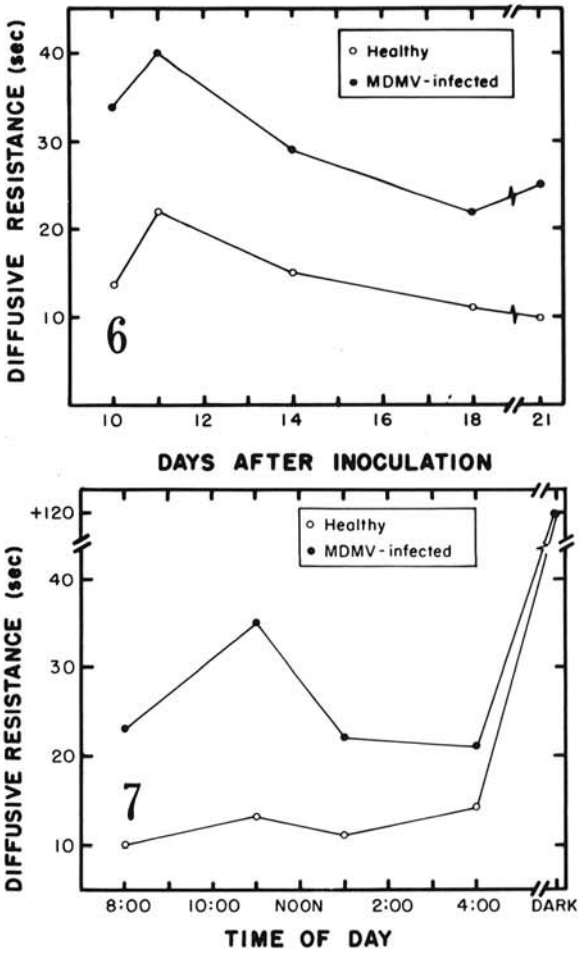

Fig. 6-7. Diffusive resistances of fourth leaves of healthy and maize dwarf mosaic virus-infected corn seedlings: **6)** at intervals after inoculation. Each value is an average from 15 plants; resistances of MDMV-infected leaves were significantly different ($P = 0.01$) from healthy leaves at all days shown. **7)** during the 14th day after inoculation. Each value is an average from 30 plants in two experiments; resistances of MDMV-infected leaves were significantly different ($P = 0.01$) from healthy leaves at all times shown except during dark.

Estimations of stomatal apertures and location of potassium in guard cells of healthy and MDMV-infected epidermal strips at 15-20 days after inoculation are shown in Table 1. Stomata were recorded as completely open, partially open, or closed as illustrated in Fig. 8-(A to C); potassium was scored on an arbitrary intensity scale of 0-5 such as used by Pallaghy (19). Epidermal strips from healthy leaves had more stomata completely open, and correspondingly fewer stomata partially open or completely closed, than strips from infected leaves. Potassium content of guard cells also was higher in

TABLE 1. Numbers of stomata at various apertures, and potassium content in guard and subsidiary cells in epidermal strips from healthy and maize dwarf mosaic virus-infected corn seedlings

Epidermal strip	Numbers of stomata[a]			Potassium content[b]	
	Closed	Partially open	Completely open	Guard cell	Subsidiary cell
Healthy	3.0	14.7	32.3	4.2	1.0
MDMV-infected	12.3	18.7	18.7	2.7	2.1

[a]Each value represents an average from three epidermal strips.
[b]Each value represents an average from 500 stomata in 10 epidermal strips; potassium content based on 0-5 rating: 0 = none, 5 = maximum potassium.

healthy epidermal strips [Table 1, Fig. 8-(D to G)]. Subsidiary cells in MDMV-infected strips tended to show higher potassium content than those in healthy strips. However, guard cells in both healthy and MDMV-infected strips had higher potassium content than their respective subsidiary cells. Overall, potassium content in the stomatal apparatus of MDMV-infected strips appeared reduced as compared to healthy strips.

Root/shoot ratio.—Average root/shoot ratios of 10 healthy and MDMV-infected seedlings at 21 days after inoculation were (g/g) 0.300 for healthy and 0.245 for infected. On a g/100 cm^2 basis, ratios were 0.181 for healthy and 0.128 for infected. Both ratios for MDMV-infected plants were significantly lower ($P = 0.01$) than those for healthy plants.

DISCUSSION.—Reduction in transpiration rates of MDMV-inoculated plants was associated with appearance of symptoms. Usually, symptoms appeared and developed in the expanding third, fourth, and fifth leaves over the 3-8-day period after inoculation, which coincided with the onset of decreased transpiration. Although reduced transpiration by MDMV-infected plants was detected in all three experiments to measure water loss, results obtained from liquid culture are probably more reliable because of constant availability of water to all roots.

The reduction in transpiration rates of MDMV-infected plants was not caused by increased water stress in the plants, as indicated by the fact that water potentials were higher in MDMV-infected leaves than in healthy ones, especially when water was withheld for several days. Thus, any alteration in water relations in MDMV-infected leaves apparently was not caused by an interference in water uptake by leaf tissue as suggested for tomatoes infected with aspermy virus (13, 25). The little difference in water potentials observed when adequate moisture was available to roots of healthy and MDMV-infected plants would further indicate that water uptake was not limited in MDMV-infected leaf tissue. MDMV infection did not alter the water potential at which corn seedlings wilted, but did delay the onset of wilting. Sanchez-Diaz and Kramer (23) reported the average leaf water potential of well-watered Pioneer 309 corn to be -4.5 bars. Leaf water potentials of MDMV-infected plants were greater than -4.5 bars for 3 days without additional water, while healthy leaves showed an average water potential of -8.3 bars within 48 hours without additional water (Fig. 5).

Root/shoot ratios indicated that root systems were reduced in MDMV-infected corn seedlings. However, this was probably of little importance in the overall water status of infected plants since any reduced water uptake due to such increased root resistance was not reflected by a lowering of water potential in MDMV-infected leaves.

Stomatal frequency in healthy and MDMV-infected leaves did not differ; however, results from the studies of diffusive resistance of leaves and direct observation of epidermal impressions indicated that stomatal apertures were reduced in MDMV-infected leaves. Such reduction has also been observed with some other virus infections (8, 9, 15, 17). Krüger (15) suggested that reduced transpiration of leafroll virus-infected potatoes resulted from a slower opening of stomata. Generally, changes in diffusive resistances of both MDMV-infected and healthy leaves were about maximal within 30-45 minutes after lights on or off. Thus, there did not appear to be any slowing down of stomatal opening but rather the degree to which stomata opened was reduced in MDMV-infected leaves.

Stomatal closure during daylight can be caused by water stress; however, as discussed earlier, MDMV-infected leaves showed no indication of water stress. Water potentials of MDMV-infected leaves were equal to or higher than those of healthy leaves throughout most of the experimental periods employed; any reduction in stomatal apertures as a result of water stress would have occurred in healthy leaves before MDMV-infected leaves.

It was not determined if stomata specifically occurring in the chlorotic areas were less functional than those in greener areas of infected leaves. However, we did observe that stomatal apertures in some areas of infected leaves equalled those of healthy leaves suggesting that less functional stomata might be limited to certain portions of the leaf. In all likelihood, these would be the chlorotic areas (9, 14). Reductions in transpiration coincided with appearance of mosaic symptoms which may suggest some role for chlorophyll in the reduced transpiration by MDMV-infected leaves. Virgin (28) studied stomatal transpiration in chlorophyll-deficient mutants of barley and concluded that chlorophyll pigments were prerequisite for stomatal opening in light. It has been shown that chloroplasts in MDMV-infected leaves are reduced in number, size, and chlorophyll content compared to those in healthy leaves (5, 26, 27).

Reduction in chlorophyll content or efficiency could limit ATP generation and this may influence stomatal opening. ATP from photophosphorylation can be sufficient and may be required for pumping potassium into guard cells (11, 20); active potassium uptake by guard cells is regarded as a possible mechanism in stomatal

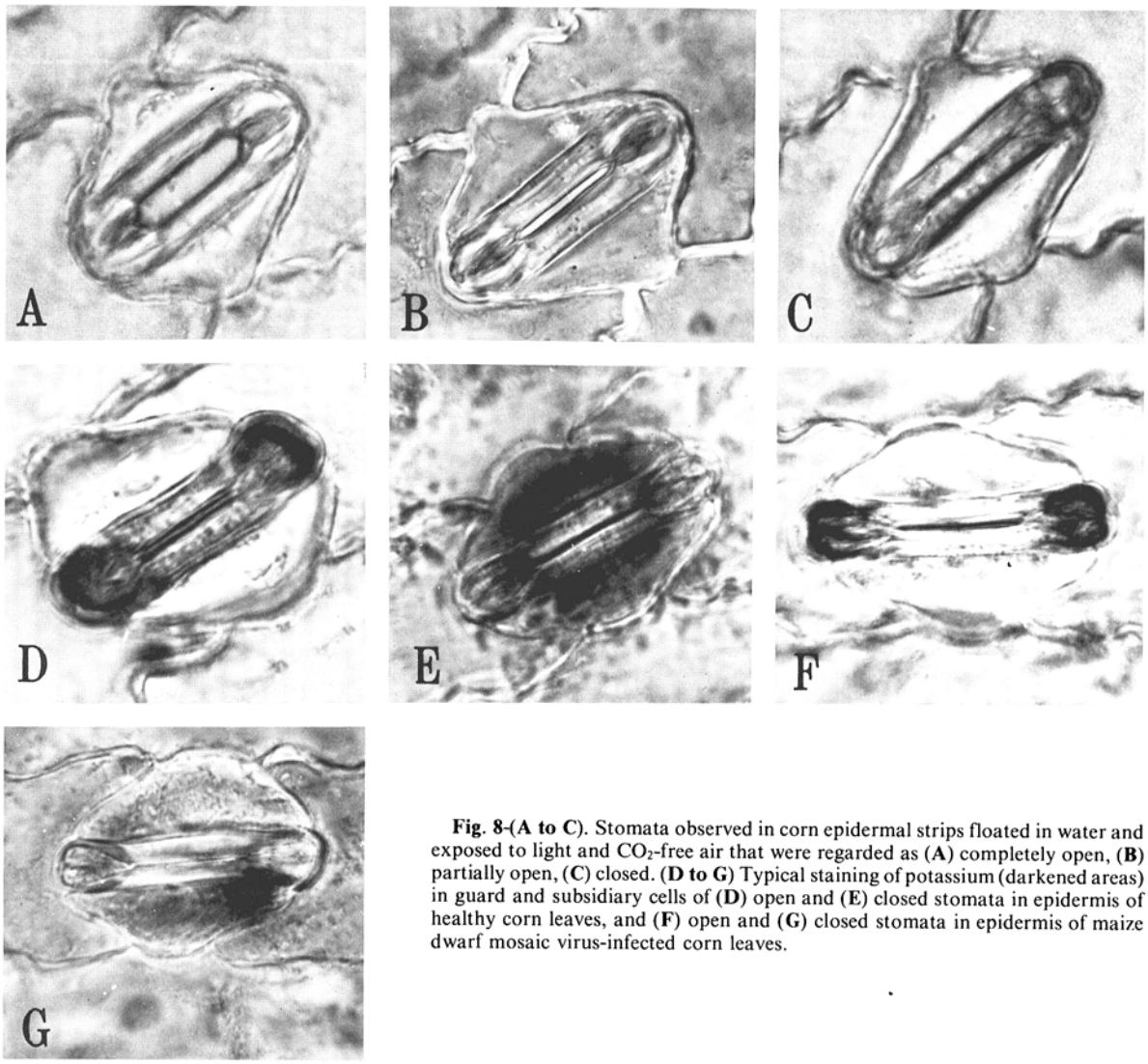

Fig. 8-(A to C). Stomata observed in corn epidermal strips floated in water and exposed to light and CO_2-free air that were regarded as (**A**) completely open, (**B**) partially open, (**C**) closed. (**D to G**) Typical staining of potassium (darkened areas) in guard and subsidiary cells of (**D**) open and (**E**) closed stomata in epidermis of healthy corn leaves, and (**F**) open and (**G**) closed stomata in epidermis of maize dwarf mosaic virus-infected corn leaves.

opening (11, 12, 19, 22, 24). There appeared to be a good correlation between stomatal opening and potassium movement into guard cells in this study.

Reduction of stomatal apertures in MDMV-infected leaves may account for some of the reductions in photosynthesis that apparently are not attributable solely to reduced chlorophyll content (5, 26, 27). Such an effect of stomatal closure has been suggested for the reduced photosynthesis associated with sugarcane mosaic and beet yellows diseases (8, 14).

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