Comparison of Inoculation with Maize Dwarf Mosaic Virus on the Abaxial and Adaxial Leaf Surfaces in Corn

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Journal Series Paper 6411 of the Mississippi Agricultural and Forestry Experiment Station.
Accepted for publication 27 February 1987.

ABSTRACT

Rosenkranz, E., and Scott, G. E. 1987. Comparison of inoculation with maize dwarf mosaic virus on the abaxial and adaxial leaf surfaces of corn. Phytopathology 77:1243-1246.

The effect of inoculating abaxial (lower) versus adaxial (upper) leaf surfaces of corn with maize dwarf mosaic virus (MDMV) was investigated in the field for 2 yr to determine which surface was more susceptible to infection. In both years, plants of three corn hybrids (Ab28A × T226, T226 × T232, and Mo12 × Tx601) had significantly higher disease incidence when inoculated with MDMV strain A (MDMV-A) on the lower than on the upper leaf surfaces. Of 72 comparisons (three hybrids × four replications × six evaluation days) made during 1984, 67 had a higher percentage of diseased plants for inoculation on the lower than upper leaf surfaces. In 1985, 82 of 84 comparisons (three hybrids × four replications × seven evaluation days) had a higher disease incidence for lower- than for upper-surface inoculation. The range in the increase of disease incidence resulting from inoculation of the lower over the upper leaf surfaces amounted to 15–140% in 1984 and 18–130% in 1985. The time of day when inoculation was made, however, affected the magnitude of the difference in disease incidence between the two inoculation sites, being smallest when inoculation was performed at midday. In fact, there was also no statistical difference in disease incidence between plants inoculated, on the same day, on the lower leaf surfaces at midday and plants inoculated on the upper leaf surfaces at sunset. It is suggested that inoculation of the lower surfaces of corn leaves with MDMV-A in the field leads to increased disease incidence because reduced light intensity and enhanced relative humidity cause a high turgor pressure in the abaxial epidermal cells facilitating their wounding for virus entry.

Additional key words: inoculation technique, maize, *Zea mays*.

Many factors pertaining to the inoculation procedure, host plant, and environment influence the rate of infection by viruses. The age of corn (*Zea mays* L.) plants at the time of inoculation with maize dwarf mosaic virus strain A (MDMV-A) affected the subsequent disease incidence, with plants inoculated in the five-leaf stage producing a higher disease rate than plants inoculated in either the three- or seven-leaf stage of growth (4). Individual leaves on the same plant also vary in susceptibility to virus infection. For example, center leaves (8th–11th leaves) on plants of *Chenopodium amaranticolor* Coste & Reyn. with 13–19 leaves produced the highest number of local infections when inoculated with pea streak virus (3).

In some types of field experiments with MDMV, such as those dealing with genetics of resistance, it is essential to infect as many of the susceptible corn plants as possible. As one of the factors that could possibly contribute to this goal, we investigated the effect of inoculation of the abaxial (lower) versus adaxial (upper) surfaces of corn leaves with MDMV-A on subsequent disease incidence. To our knowledge, there is no published information on such an effect involving systemic infection by any plant virus. The underlying assumptions for this work were: 1) Virus in the inoculum deposited on the upper leaf surfaces is exposed to direct solar radiation and possibly to inactivation, while mechanically injured cells into which virus has already gained entry may be killed by desiccation; 2) plant tissues subjected to reduced light intensity on the lower surfaces of leaves become more susceptible to virus infection; and 3) differences in the anatomy of the two leaf surfaces seem to favor the lower surface for virus entry during mechanical inoculation.

MATERIALS AND METHODS

Three experimental corn hybrids (Ab28A × T226, T226 × T232, and Mo12 × Tx601), each a cross between a resistant (Ab28A, T232, and Tx601) and a susceptible inbred, were grown in double-row plots in a randomized complete block design with four replications in each of 2 yr. In 1984, the planting was made on 25 April, and the plants were inoculated at 8:30–9:15 a.m. on 24 May. In 1985, the seed was planted on 22 April, and the plants were inoculated on 16 May. In the latter year, the experiment was doubled in size and was divided into two equal parts. One half of the plants of each hybrid were inoculated at midday (12:30–1:15 p.m.) and the other half of the plants were inoculated in the evening (6:45–7:30 p.m.) of the same day. At each inoculation time, one half of the plants were inoculated on the upper and the other half on the lower leaf surfaces. Inoculation each year was performed on a bright, sunny day. The experiments were conducted in the same field on the Plant Science Farm at Mississippi State in both years.

The virus used was MDMV strain A, which had been isolated from johnsongrass of a corn field in Yazoo County, MS, in 1965. This isolate has been maintained in johnsongrass and sweet corn cultivar Seneca Chief, which also served as the source material for the inoculum. The preparation and handling of the inoculum were essentially the same as described earlier (5).

All plants were inoculated with MDMV-A at the four- to five-leaf stage either on the upper or lower leaf surfaces with an artist's air brush operated from a tractor-mounted air compressor that supplied a constant pressure of 100 psi (7.0 kg/cm^2). Two adjacent rows of seedlings, which constituted one plot, were inoculated simultaneously by two persons sitting behind the tractor driven at a speed of 1.6 km/hr. To ensure that only one or the other leaf surface was being inoculated, the nozzle of the artist's air brush was kept less than 1 cm from the leaf surface while as much leaf surface was being sprayed as time permitted.

Beginning 7 days after inoculation, each plant was evaluated for the presence or absence of maize dwarf mosaic symptoms every 3–6 days until the increase in the number of diseased plants became negligible. The proportions of diseased to total number of plants inoculated used for statistical analyses were based on an average of 125 plants/treatment/hybrid in 1984 and 180 plants/

treatment/hybrid/time of day of inoculation in 1985. Combining the results from the three hybrids over the 2 yr, a comparison between the two inoculation sites (upper vs. lower leaf surfaces) could be made on about 900 plants per treatment.

RESULTS

The first distinct differences in disease incidence between plants inoculated on the upper and those inoculated on the lower leaf surfaces were observed in all three hybrids 15 days after inoculation in the 1984 test. At that time, hybrids T226 × T232 and Mo12 × Tx601 had more than twice as many diseased plants in the group inoculated on the lower leaf surfaces as in the group inoculated on the upper leaf surfaces, whereas Ab28A × T226 had 31% more diseased plants in the former than the latter group of plants (Table 1). On subsequent evaluation days, these differences in disease incidence decreased somewhat but still remained statistically significant 1 mo after inoculation. Of 72 comparisons (three hybrids × four replications × six evaluation days) made between the two inoculation sites, 67 had a higher percentage of diseased plants for abaxial inoculation than adaxial inoculation. The increase of disease incidence resulting from inoculation of the abaxial leaf surfaces among the three hybrids on all evaluation days ranged from 15 to 140%. When the disease data for all three hybrids were combined, the group of plants inoculated on the abaxial leaf surfaces had 68, 47, 39, and 41% more diseased plants 15, 20, 25, and 30 days after inoculation, respectively, than the group of plants inoculated on the adaxial surfaces of leaves.

In 1985, the symptoms in inoculated plants of the same hybrids developed 5–10 days later than in 1984, possibly because deficient soil moisture retarded plant growth. Distinct differences in disease incidence between plants inoculated on the adaxial and those inoculated on the abaxial leaf surfaces were first noticeable in all three hybrids 20 days after inoculation. However, results were similar to those recorded in 1984. The initially large differences in disease incidence in favor of abaxial leaf surface-inoculation tended to become smaller on later evaluation days but remained statistically significant (Table 1). Of 84 comparisons (three hybrids × four replications × seven evaluation days) made between abaxial and adaxial inoculation sites, 82 had a higher percentage of diseased plants when inoculated abaxially. Whereas Ab28A × T226 had much lower disease incidence and Mo12 × Tx601 had higher disease incidence in 1985 than 1984, T226 × T232 had a similar amount of disease in both years. The increase of disease incidence from inoculation of the abaxial over the adaxial leaf surfaces among the three hybrids on all seven evaluation days ranged from 18 to 130%. The combined disease incidence data for all three hybrids showed the 541 plants inoculated abaxially had 82, 42, 34, and 34% more diseased plants 20, 25, 30, and 35 days after inoculation, respectively, than the 544 plants inoculated adaxially.

By pooling the data for all hybrids in both years, a comparison between the frequency of disease occurrence among plants inoculated on the lower side of leaves and that among plants inoculated on the upper side of leaves could be made on about 900 plants per inoculation site. The former group of plants had 94, 66, 41, 38, and 37% more diseased plants than the latter group 15, 20, 25, 30, and 35 days after inoculation, respectively (Table 2). These differences in the proportions of diseased plants for the two treatments were highly significant ($P = 0.01$, 1 df) on all recording days (Table 2).

Although the three experimental corn hybrids used in this study possess different levels of susceptibility to MDMV-A, each reacted with significantly higher disease incidence in the field when inoculated on the abaxial than on the adaxial leaf surfaces in both 1984 and 1985. The most consistent results were obtained with hybrid T226 × T232, which also responded with the greatest difference in disease incidence between the abaxial and adaxial inoculations in both years.

Comparison of the disease incidence resulting from abaxial and adaxial inoculations made both at midday and at sunset of the

TABLE 1. Disease incidence in corn hybrids at different times after inoculation in the field with maize dwarf mosaic virus strain A on upper vs. lower leaf surfaces in 1984 and 1985[a]

Hybrid	Inoculation site[b]	Plants inoculated[c] (no.)	Percent diseased plants on days after inoculation: 15			20			25			30		
			1984	1985	$\bar{x}$[d]	1984	1985	$\bar{x}$	1984	1985	$\bar{x}$	1984	1985	$\bar{x}$
Ab28A × T226	U	235	28.7	0.0	14.9	41.0	0.0	21.3	44.3	5.3	25.5	45.9	7.1	27.2
	L	257	37.5	0.8	19.1	47.7	5.4	26.5	50.8	9.3	30.0	53.9	16.3	35.0
T226 × T232	U	322	8.2	6.6	7.1	23.6	13.7	17.1	29.1	18.9	22.4	29.1	25.9	27.0
	L	314	19.7	12.2	15.0	43.6	29.4	34.7	49.6	38.1	42.4	52.1	44.7	47.5
Mo12 × Tx601	U	332	11.5	1.4	4.8	31.0	37.9	35.5	40.7	58.5	52.4	46.9	64.8	58.7
	L	373	26.0	7.0	15.0	50.0	64.2	58.2	57.6	73.5	66.8	64.6	76.7	71.6
All hybrids	U	889	16.5	3.1	8.3	32.2	20.6	25.1	38.3	32.0	34.4	40.9	37.7	38.9
	L	944	27.8	7.4	16.1**[e]	47.4	37.5	41.7**	53.1	45.3	48.6**	57.6	50.7	53.6**

[a] In 1984, inoculation was performed at 8:30–9:15 a.m., whereas the 1985 data derive from inoculation performed at 6:45–7:30 p.m.
[b] U = Upper (adaxial), L = lower (abaxial) leaf surface.
[c] Total number of plants inoculated in 2 yr.
[d] Mean percent of diseased plants for the 2 yr.
[e] Differences in disease incidence between plants inoculated on the upper leaf surfaces and those inoculated on the lower leaf surfaces for all hybrids combined was highly significant ($P = 0.01$, 1 df) on each evaluation day as determined by the chi-square test.

TABLE 2. Proportion of diseased plants and mean percent increase in disease incidence in three corn hybrids resulting from inoculation of lower leaf surfaces over upper leaf surfaces at different times after inoculation in 2 yr

Inoculation site[a]	Plants inoculated (no.)	15 days	% increase L/U	20 days	% increase L/U	25 days	% increase L/U	30 days	% increase L/U	35 days	% increase L/U
U	889	0.083[b]		0.251		0.344		0.389		0.413	
L	944	0.161**[c]	94.0	0.417**	66.1	0.486**	41.3	0.536**	37.8	0.567**	37.3

[a] U = Upper (abaxial), L = lower (abaxial) leaf surface.
[b] Proportion of diseased plants/inoculated plants.
[c] Difference in proportion of diseased plants between the group of plants inoculated on the lower leaf surfaces and the group of plants inoculated on the upper leaf surfaces for all three hybrids combined was highly significant ($P = 0.01$, 1 df) on each evaluation day as determined by the chi-square test.

same day showed a significant difference in disease incidence between abaxial and adaxial leaf surface inoculations when plants were inoculated at sunset (Table 1) but not at midday (Table 3). Of 84 comparisons (three hybrids × four replications × seven evaluation days) made of disease data obtained for midday inoculation, only 49 had a higher disease incidence for plants inoculated on the abaxial than for plants inoculated on the adaxial leaf surfaces. The range in the increase of disease incidence resulting from inoculation on the abaxial leaf surfaces among the 49 "positive" comparisons amounted to 3–47%. When the group of plants inoculated on the abaxial leaf surfaces at midday was compared for disease incidence with the group of plants inoculated on the adaxial leaf surfaces in the evening, there was also no statistical difference 25, 30, 35, and 40 days after inoculation (Table 4).

DISCUSSION

Plants are usually inoculated with mechanically transmissible viruses on the upper leaf surfaces. Research results from inoculation of lower leaf surfaces are scarce and show no consistent trend. Sheffield (7) inoculated half of each of three leaves of *Nicotiana glutinosa* L. with aucuba mosaic virus on the upper surface and the other half of each of the three leaves on the lower surface. The total number of local lesions that developed on six such plants was 2,872 for upper leaf surface inoculation (160 lesions per half leaf) and 1,526 for lower leaf surface inoculation (85 lesions per half leaf). Working with tobacco mosaic virus and pepper plants, Boyle and McKinney (1) found no significant differences in the number of local lesions that developed when halves of leaves were inoculated either on the lower or upper sides; there were six and seven lesions per square centimeter on halves of leaves wiped with inoculum on the lower and upper surfaces, respectively. In an effort to transmit sugar beet yellows virus mechanically, Costa and Bennett (2) inoculated the lower leaf surfaces of sugar beet with this virus and found that more local lesions were produced on the inoculated lower than on the inoculated upper leaf surfaces. In one test in the greenhouse in which one-half of each of 200 leaves were inoculated on the upper side and the other half of each leaf on the lower side, the total lesion counts were 5,763 and 9,288 (61% increase) for the upper and lower sides, respectively. All of these studies involved local lesion tests that were performed in the greenhouse. Ours seems to be the first report on the effect of inoculation of lower leaf surfaces on systemic virus infection in the field.

Throughout this work, a general trend was observed for the difference in disease incidence between inoculations on the abaxial and adaxial leaf surfaces to be greatest in the early stage of disease development (10–20 days after inoculation), then to diminish and to remain fairly constant during the later period of disease development (25–40 days after inoculation). This trend implies a shorter incubation period in plants inoculated on the abaxial than in those inoculated on the adaxial leaf surfaces. The reason for this difference may be twofold. First, probably more infected sites per unit area result from inoculation of the lower than upper leaf surfaces because of the greater turgor pressure in the lower epidermal cells, which facilitates wounding of these cells and virus entry. This component of enhanced systemic infection would be due to a dosage effect. Second, the virus introduced via the lower epidermis needs to move a shorter distance to the vascular tissue than virus introduced via the upper epidermis because vascular bundles in the midrib and the adjacent blade are close to the lower surface (6).

The magnitude of the difference in disease incidence resulting from the introduction of MDMV-A through the adaxial and abaxial surfaces of leaves is influenced not only by the host genotype but also by subtle environmental changes at or near leaf surfaces, such as those associated with the time of day that inoculation takes place. This difference in disease incidence was smallest and statistically nonsignificant when inoculation was made at midday and was much larger and statistically significant when inoculation was performed either in the morning or in the evening. These results indicate that our first working assumption—the underside of leaves might offer the virus some protection from exposure to direct solar radiation and from possible inactivation—may not be appropriate.

The results of this study leave the second assumption intact. Because the underside of leaves receives less light than the upper surface, inoculating the lower leaf surfaces may be somewhat analogous, on the cellular or tissue level, to shading plants before inoculation, which generally increases susceptibility of plants to virus infection. A concomitant factor with reduced light intensity is often a greater relative humidity. Relative humidity would be higher at the lower surfaces than at the upper surfaces of leaves, especially in the morning and evening hours. High relative humidity causes high turgor pressure, which may facilitate the wounding of epidermal cells for virus entry during mechanical inoculation (2). Kimmins and Litz (3), who investigated the effect of leaf water balance on the susceptibility of bean plants to tobacco necrosis virus, found that turgor pressure and susceptibility of darkened plants decreased with decreasing humidity until, at a relative humidity of 20%, they were similar in magnitude in both darkened and illuminated plants.

A similar situation may have existed in our field experiment in 1985 when plants were inoculated on the adaxial or abaxial leaf surfaces both at midday and at sunset. In the evening inoculation, when plants inoculated on the underside of leaves had a significantly higher disease incidence than plants inoculated on the upper leaf surfaces, the relative humidity and turgor pressure in epidermal cells at the lower leaf surfaces may have been higher than at the upper leaf surfaces. In the midday inoculation, on the other

TABLE 3. Disease incidence in corn plants in the field at different times after midday inoculation with maize dwarf mosaic virus strain A on upper vs. lower leaf surfaces (1985)

Hybrid	Inoculation site[a]	Plants inoculated (no.)	Diseased plants after inoculation (%) 15 days	20 days	25 days	30 days	35 days	40 days
Ab28A × T226	U	95	2.1	3.2	6.3	7.4	10.5	15.8
	L	86	0.0	1.2	4.7	5.8	8.1	12.8
T226 × T232	U	206	7.3	14.6	19.9	26.7	29.1	32.0
	L	210	6.2	15.2	20.5	27.6	30.5	34.8
Mo12 × Tx601	U	213	6.6	48.8	63.4	68.5	71.4	72.3
	L	217	9.7	56.7	66.4	71.9	76.9	78.3
All hybrids	U	514	6.0	26.7	35.4	39.7	43.2	45.7
	L	513	6.6[ns b]	30.4[ns]	37.2[ns]	42.7[ns]	46.4[ns]	49.5[ns]

[a] U = upper (adaxial), L = lower (abaxial) leaf surface.
[b] Difference in disease incidence between plants inoculated on the upper and those inoculated on the lower leaf surfaces was nonsignificant for all hybrids on all evaluation days as determined by the chi-square test.

TABLE 4. Disease incidence in corn hybrids at different times after inoculation in the field on the same day with maize dwarf mosaic virus strain A on lower leaf surfaces at midday (M-L) and on upper leaf surfaces in the evening (E-U) in 1985

Hybrid	Treatment	Plants inoculated (no.)	Percent plants diseased after inoculation 20 days	25 days	30 days	35 days	40 days
Ab28A × T226	M-L	86	1.2	4.7	5.8	8.1	12.8
	E-U	113	0.0	5.3	7.1	9.7	11.5
T226 × T232	M-L	210	15.2	20.5	27.6	30.5	34.8
	E-U	212	13.7	18.9	25.9	28.8	30.7
Mo12 × Tx601	M-L	217	56.7*[a]	66.4	71.9	76.9	78.3
	E-U	219	37.9	58.5	64.8	65.8	66.2
All hybrids	M-L	513	30.4**	37.2	42.7	46.4	49.5
	E-U	544	20.6	32.0	37.5	39.9	41.0

[a] Chi-square values were calculated by using the number of diseased and healthy plants for each treatment at each evaluation day; * = significant difference ($P = 0.05$, 1 df), ** = highly significant difference ($P = 0.01$, 1 df).

hand, when plants inoculated on the upper or lower leaf surfaces produced no significant difference in disease incidence, the relative humidity also probably did not differ significantly at the two leaf surfaces. In fact, when the disease incidence data for midday inoculation of lower leaf surfaces were statistically compared with those for evening inoculation of upper leaf surfaces, there was also no significant difference. For example, hybrid T226 × T232 had 29.1, 30.5, 28.8, and 45.7% diseased plants for inoculation of upper leaf surfaces at midday, lower leaf surfaces at midday, upper leaf surfaces at sunset, and lower leaf surfaces at sunset, respectively, 35 days after inoculation. These data were based on about 200 plants per treatment.

On the surface, it appears that our idea of high relative humidity contributing to increased MDMV infection contradicts Yarwood's (8) finding that quick drying of leaves after inoculation increases the number of infection sites. However, the fact that his was a postinoculation treatment and our notion of the importance of high relative humidity refers to the period preceding inoculation make this apparent contradiction disappear. The quick drying effect is on the infection process, diminishing the deleterious influence of water on infectivity, whereas reduced light intensity and high relative humidity before inoculation affect the host plant, rendering it more receptive to virus entry during inoculation.

Based on our field data and what we believe to be a reasonable analogy between our experimental setup and the system used by Kimmins and Litz (3), we offer the following hypothesis: The benefit of lower leaf surface inoculation with MDMV-A on maximum infection of corn derives primarily from a combination of reduced light intensity and increased relative humidity at the abaxial epidermis and, secondly, to a much smaller extent, from the specific anatomy of the corn leaf.

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