Titer Variation in Infected Sorghum Differing in Resistance to Maize Dwarf Mosaic Virus Strain-B

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ABSTRACT

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Variation associated with sampling for enzyme-linked immunosorbent assay (ELISA) and conditions necessary to make meaningful titer determinations to access resistance to maize dwarf mosaic virus strain-B (MDMV-B) in sorghum (*Sorghum bicolor*) were investigated. ELISA and infectivity assays were highly correlated. In ELISA, maximum sample variation was associated with leaf 3 and the least sample variation with leaves 5 and 6. ELISA values at a given leaf position increased with time after infection at all temperatures except 35 C, at which they decreased. ELISA values progressively decreased in each leaf above leaf 4, except at 35 C, where the opposite was true. ELISA values were different for equivalent leaves with various lengths from plants of the same age. A gradient in ELISA values was present in leaves, with values increasing from the leaf base to the tip. The resistant cultivar (Colt) had lower ELISA values at 7 days postinoculation (DPI), but not at 14, 21, and 28 DPI for leaves 4, 5, and 6. At 28 DPI, the resistant cultivar showed attenuation of symptoms and a large decrease in ELISA values for leaves 7 and 8. At 35 C, MDMV-V was not detected in the resistant cultivar.

Maize dwarf mosaic of sorghum (*Sorghum bicolor* (L.) Moench), caused by maize dwarf mosaic virus (MDMV), is recognized as an important disease of sorghum in the United States and other countries (13,20). Studies of MDMV-sorghum interactions have been confined to phenotypic responses to temperature changes. In these studies, susceptibility to MDMV infection was linked to the red-leaf reaction (4,12,18). This refers to the pigmentation associated with necrotic plant tissue (necrosis of whole or large portions of leaves). The term is also used to describe the reaction in genotypes that produce tan pigments during necrosis (10,18). The red-leaf response to infection by MDMV has been considered an indication of susceptibility, because sorghum cultivars having this reaction also sustain greater yield reduction (5,15,19,20). Thus, susceptibility and resistance are keyed to symptom expression, with susceptible and resistant plants developing mosaic symptoms and susceptible genotypes also developing necrosis. Although the criteria for resistance and susceptibility in MDMV-infected sorghum were established in these papers, no insight on MDMV interactions in infected sorghum was provided.

To better understand this host-pathogen interaction, we need information concerning MDMV titer in both susceptible and resistant (i.e., plants are infected but have only a mosaic reaction) sorghum cultivars. Enzyme-linked immunosorbent assay (ELISA) has been used to quantitate virus concentration (3,9,11,17) and should be a promising alternative to infectivity assays to study MDMV titer, because of its sensitivity and applicability to processing relatively large numbers of plants (1).

To effectively use ELISA to examine MDMV titer in sorghum, we need information concerning potential sources of variation in experimental procedures and inherent variation of MDMV in infected sorghum. Therefore, investigations were initiated to study these variations with MDMV-B in sorghum cultivars differing in resistance.

In this paper, we report titer variation intrinsic to leaf tissue in MDMV-B-infected sorghum. We have correlated the use of ELISA with infectivity assays and established conditions necessary to make meaningful comparisons within and between cultivars differing in resistance to MDMV.

MATERIALS AND METHODS

Virus isolate and source plant maintenance. The B strain of MDMV was obtained from S. G. Jensen (University of Nebraska) and maintained in sorghum seedlings (Colt) in a greenhouse. Colt sorghum was used for source plants because it does not develop necrosis to MDMV-B, providing a stable source of inoculum. Virus isolate identity was verified by host range tests and serological reactions. Maintenance of virus-source plants for inoculum production and inoculation procedures were as described by Seifers (14). Phenotypic response of cultivars used in this study was previously determined (16).

Antiserum. Antiserum to MDMV-B was prepared by injecting rabbits intramuscularly with preparations of MDMV-B purified from sorghum (8). Approximately 1 mg of virus emulsified with

Freund's incomplete adjuvant (1:1) was injected at weekly intervals for 4 wk. Serum was collected 1 wk following the last injection of antigen.

The gamma globulin (Ig) fractions of the antisera were precipitated with 40% ammonium sulfate. The precipitate was resuspended in distilled water and reprecipitated with a 35% concentration of this salt. The Ig was resuspended and exhaustively dialyzed against 0.01 M phosphate-buffered saline (pH 7.4) at 5 C.

The concentration of Ig to be used for each phase of ELISA was determined by reacting a 1:600 dilution of infected (14 days postinoculation [DPI]) or healthy plant material against combinations of nonlabeled and labeled Ig at 10, 5, 2.5, 1.25, and 0.75 $\mu g \cdot ml^{-1}$. The serum did not react to healthy plant extract.

ELISA. Enzyme conjugation (alkaline phosphatase type VIIS, Sigma Chemical Co., St. Louis, MO), ELISA conditions, and procedures were as described by Clark and Adams (2), with Ig at 1 $mg \cdot ml^{-1}$. For each phase of ELISA, 250 μl of a 1:400 dilution of the appropriate Ig was applied to flat-bottomed, polystyrene, microelisa plates (Dynatech Laboratories, Alexandria, VA) and incubated for 4 hr at 37 C. Test samples (250 μl) were applied to triplicate wells for each sample with randomly assigned plate locations and incubated overnight at 4 C. Antibody-antigen reactions were assessed by adding 300 μl of *p*-nitrophenyl phosphate (0.07 $mg \cdot ml^{-1}$) to each well and incubating for 30 min at room temperature. Reactions were stopped by adding 50 μl of 3 M NaOH to each well. Absorbance was measured at 405 nm (ELISA values) using a Titertek Multiskan microelisa plate reader (Flow Laboratories, Inc., McLean, VA).

ELISA-infectivity correlation. A susceptible cultivar (Bugoff) and a resistant cultivar (Colt) of *S. bicolor* were used. Seeds were planted in metal flats at a rate of 15 seeds/30-cm row. At 6 days following planting, the plants were thinned to 10 plants per row so that plants were of uniform size. To standardize inoculum among studies, source plants were inoculated 6 days following planting on the second leaf with a 1:20 dilution of inoculum and harvested at 10 DPI (14). Immediately following inoculation, the inoculated sorghum seedlings and control (inoculated with buffer and abrasive only) were placed in the same growth chamber (Warren/Sherer model E138-15) at 30 C, with a 12-hr photoperiod of fluorescent light (approximately 500 $\mu E \cdot sec^{-1} \cdot m^{-2}$).

At 14 DPI, whole plants were harvested, and the roots and leaves 1 and 2 were removed and discarded. The plant tissue was weighed and ground in a mortar and pestle in a 1:20 (w/v) dilution of 0.02 M potassium phosphate buffer, pH 7.0, and then two-fold serial dilutions (1:20–1:2,560) were made in the same buffer. Aliquots of the dilution were used for ELISA, and the remainder was used to inoculate 200 Colt sorghum seedlings as previously described (14). Inoculated plants were maintained in a greenhouse at 28 ± 4 C for 2 wk, and the number of systemically infected plants was recorded. The experiment was repeated three times.

Infectivity data from all three experiments were combined for analysis of variance (ANOVA). Simple correlation coefficients were generated from infectivity data and ELISA values.

Temperature experiment. Immediately following inoculation, seedlings (inoculated and healthy) were placed in growth chambers at 15, 20, 25, 30, and 35 C. Photoperiod and light intensity were as described for ELISA-infectivity correlation. Leaf length measurements on 25 plants from each treatment were taken every 3 days and on sampling days. Equivalent leaves of uniform size from each treatment were harvested 7, 14, 21, and 28 DPI and bulked within each treatment. The tissue was weighed and ground in a mortar and pestle in a 1:20 dilution of extraction buffer (EB) (phosphate-buffered saline containing 0.05% Tween 20 and 2% polyvinylpyrrolidone, average molecular weight 40,000) (pH 7.4). An aliquot of this was then diluted with EB to a 1:600 dilution for ELISA. The experiment was repeated twice with four replications per experiment with four leaves/replication/cultivar. Healthy plant tissue was treated identically in all experiments. Data presented are means of the two experiments, which were subjected to ANOVA.

Asynchronous plant growth effects. The lengths of leaves 5 and 6 were measured in systemically infected and healthy sorghum treatments. Temperatures for the greenhouse phase of this experiment were 23–27 C and growth chamber experiments were at 30 C. Leaf ratios (length of leaf 5/length of leaf 4 for leaf 5 and the length of leaf 6/length of leaf 5 for leaf 6) varied from 0.15 to 0.85 within a single replication because of normal, asynchronous plant growth. The equivalent leaves from plants that had leaf ratios of 0.25, 0.50, and 0.75 were harvested and bulked for each ratio for each of four replications for each cultivar (four leaves/ratio/replication). The experiment was repeated twice with four replications per experiment, and the data were subjected to ANOVA.

Within-leaf virus distribution. For greenhouse experiments (23–34 C), only the cultivar Colt was used, because Bugoff would have produced a red-leaf reaction under greenhouse conditions. For controlled-temperature experiments (30 C), both cultivars were used. Leaves 5 and 6 were harvested at three different times: 1) when they were half the length of the leaf immediately below them (0.5 ratio), 2) when they were as long as the leaves below them (1 ratio), and 3) 7 days following the occurrence of the 1 ratio (1 + 7 ratio).

On the day of harvest, the length of each leaf was measured and the leaf was divided into three sections of equal length (base, middle, tip). The equivalent sections from each of a minimum of four plants per replication were bulked and processed as previously described. Data presented here are the means of three experiments (four replications/experiment), which were subjected to ANOVA.

RESULTS

ELISA-infectivity assay correlation. Both infectivity assay (a biological test to quantitate virus titer) and ELISA curves showed the 1:640 dilution to be in the linear response range and that at this dilution, the two assays were most highly correlated. The correlation coefficients for Colt and Bugoff were 0.99 and 0.98, respectively (Fig. 1).

Temperature experiment. No data were obtained for Bugoff at 15 and 20 C because of leaf necrosis in this cultivar.

MDMV in Colt sorghum could not be detected at 15 C until 21 DPI. ELISA values for leaf 4 were 0.033 and 0.358 at 21 and 28 DPI, respectively.

At all temperatures, ELISA values and symptom expression were highly variable for leaf 3 from plants and the remaining leaves had marked symptoms. The coefficient of variation in ELISA values for each leaf was least at 25 C, with leaves 5 and 6 having the least variation at all temperatures (Table 1). Leaf 3 was not included in any data analysis because of the large variation among samples.

ELISA values of plants grown at 20 C increased throughout the study, except for leaf 4, which showed a decrease at 28 DPI (Table 2). This increase occurred in leaves that were fully expanded or still growing. Leaf 3 reached maximum fresh weight by 7 DPI, leaf 4 at 14 DPI, leaf 5 at 21 DPI, and the fresh weights of leaves 6 and 7 still increasing at 28 DPI. ELISA values were different among leaves at all sampling periods. ELISA values for each leaf above 4 were lower than the value for the leaf below it, with leaf 4 again being the exception at 28 DPI. Symptom attenuation and a large decrease in ELISA values were observed for leaf 7.

At 25 C, ELISA values for all leaves increased throughout the study (Table 3). This increase occurred in leaves that were fully expanded or still growing. Maximum leaf weights occurred at the same time for both cultivars (i.e., at 7, 14, and 21 DPI for leaves 3, 4, and 5, respectively). The fresh weights of leaves 6, 7, and 8 were still increasing at 28 DPI. ELISA values for each leaf above leaf 4 were progressively lower than the value of the leaf below it. Within cultivars, ELISA values among leaves were different, with the exception of the 28-DPI sample. ELISA values for Colt leaves 4 and 5 were not different, nor were Bugoff leaves 7 and 8. Symptoms were attenuated and ELISA values showed large decreases in Colt leaves 7 and 8, with leaf 8 having faint to no symptoms and very low absorbance values. This was not observed for Bugoff. Colt leaves

had lower ELISA values at 7 DPI than did Bugoff. At 14 DPI, ELISA values for Colt leaf 4 were still lower than leaf 4 values of Bugoff, with the opposite true for leaf 5 values. At 21 DPI, the ELISA values in Colt were higher than those of Bugoff for all leaves except leaf 7. At 28 DPI, Colt had higher ELISA values than did Bugoff, except for leaf 8. Values for leaf 4 were not different between cultivars at 28 DPI, but those for leaves 5, 6, 7, and 8 were different.

In both cultivars, ELISA values at 30 C also increased throughout the study (Table 3). This increase occurred in leaves that were fully expanded or still growing. Maximum fresh weights occurred at the same time for both cultivars (i.e., at 7 DPI for leaf 3 and at 14 DPI for leaves 4 and 5). The fresh weights of leaves 6, 7, and 8 were still increasing at 28 DPI. ELISA values for each leaf above leaf 4 were progressively lower than the value of the leaf below it. Within cultivars, ELISA values among leaves were different at 7, 14, and 21 DPI. At 28 DPI, all Colt leaves except 4 and 5, were different, and in Bugoff, leaves 4 and 5 and leaves 7 and 8 were not different. Colt leaves 7 and 8 had attenuated symptoms and large decreases in ELISA values, with leaf 8 having faint to no symptoms and very low ELISA values. Leaves 7 and 8 of Bugoff had prominent symptoms and similar ELISA values. When ELISA values were compared between cultivars, Colt had a lower value at 7 DPI. At 14 DPI, the value for Colt leaf 4 was not different from that of Bugoff leaf 4. Leaf 5 values for Colt were higher than those of Bugoff at 14 DPI. At 21 DPI, values from all Colt leaves were higher than those of Bugoff, except for leaf 7. The 28-DPI leaf values were not different between cultivars, except for leaf 8. At 28 DPI, Colt leaves 7 and 8 had attenuated symptoms and large decreases in ELISA values when compared to leaf 6. Colt leaf 8 had faint to no symptoms and an absorbance value significantly lower than that of leaf 7. Bugoff leaves 7 and 8 had no attenuation of symptoms and similar ELISA values.

At 35 C, MDMV was not detected in Colt at any sampling period, but it was detected in Bugoff. At 35 C, ELISA values in Bugoff decreased after the first sample, except for leaf 7 at 28 DPI (Table 4). Maximum fresh weight occurred at 7 DPI for leaf 3 and at 15 DPI for leaves 4 and 5. The fresh weights of leaves 6 and 7 were still increasing at 28 DPI. ELISA values progressively increased in each leaf above leaf 4. At 14 DPI, ELISA values among leaves were different. At 21 and 28 DPI, certain leaf comparisons proved to be different, whereas others were not. The upper leaves had the most pronounced symptoms and symptom expression was attenuated in each subtending leaf.

Asynchronous plant growth effects. Leaves 5 and 6 were used in this experiment because they were shown to have the least sample variation under controlled temperature.

In all replications, ELISA values increased from the 0.25 to the 0.75 ratio (Table 5). ELISA values were different among ratio classes depending on leaf and environment, with the 0.25 ratio class almost always having a lower value than the other ratio classes.

Within leaf virus distribution. At 30 C, the 0.5, 1, and 1 + 7 samples occurred at 10, 14, and 21 DPI and 15, 20, and 27 DPI for leaves 5 and 6, respectively. In the greenhouse, the 0.5, 1, and 1 + 7 samples occurred at 16, 18, and 25 DPI and 17, 22, and 29 DPI for leaves 5 and 6, respectively.

In all leaves at 30 C, the ELISA values increased from the leaf base to the tip (Table 6). This gradient existed in all sampling periods, with the significance between ELISA values from parts of leaf 5 varying with sample date. For both cultivars, ELISA values for leaf 6 were significantly different at all samplings.

Under greenhouse conditions, ELISA values also increased from the leaf base to the tip (Table 6). The values for each part of leaves 5 and 6 were different at all samplings.

DISCUSSION

If ELISA is to be used to study relative virus titer, it must correlate well with infectivity tests to ensure that infective virus and not just coat protein is being measured. The high correlation between our ELISA and infectivity studies indicate that the ELISA is measuring infective virus particles. The 1:600 dilution has been

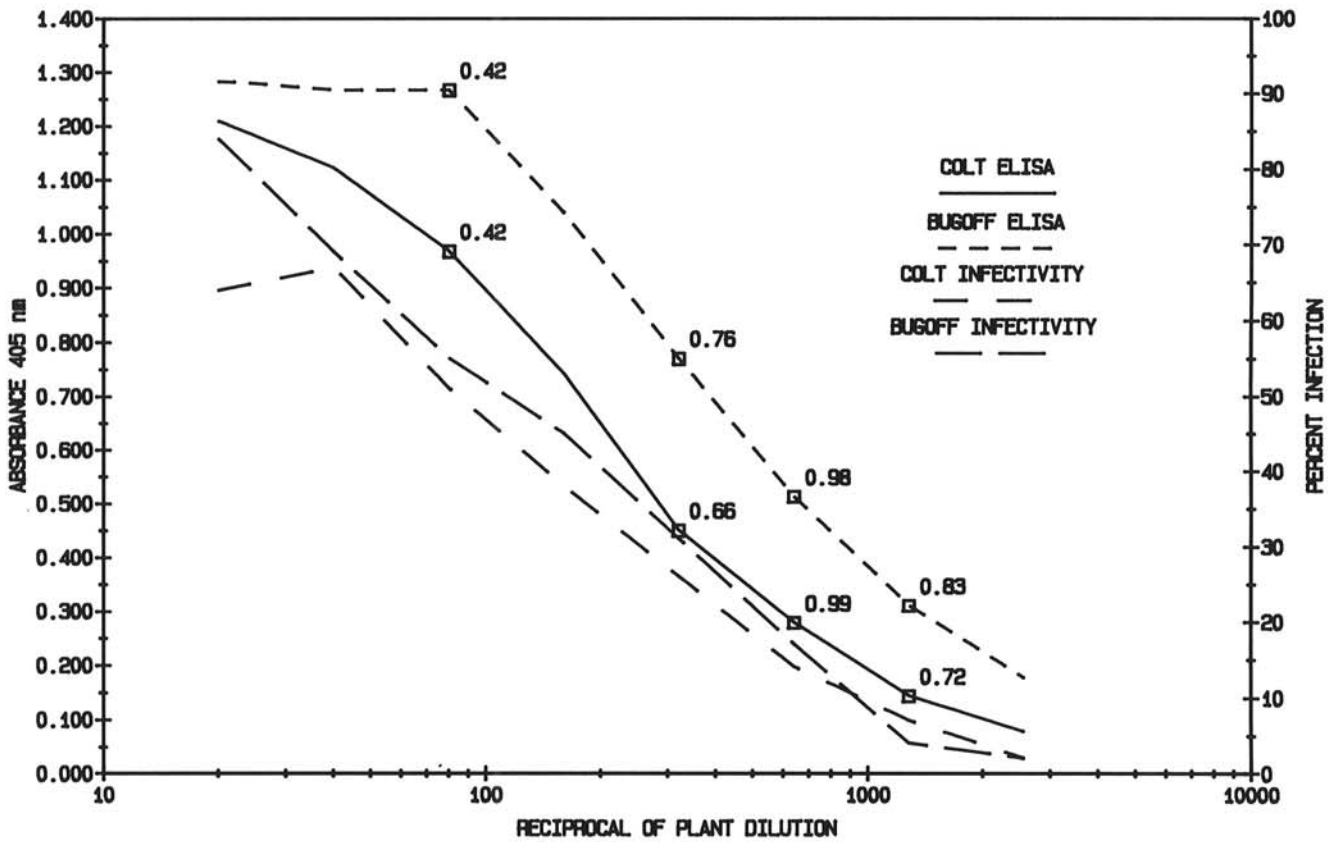

Fig. 1. Enzyme-linked immunosorbent assay (ELISA) dilution and infectivity curves with correlation coefficients from maize dwarf mosaic virus strain-B-infected sorghum (14 days postinoculation) incubated at 30 C.

shown to be in the linear response range for MDMV-B infectivity dilution curves (14), and its appropriateness for use as an assay dilution for ELISA is confirmed in this study (Fig. 1).

At 15 C, MDMV-B could not be detected until 21 DPI. Because leaf 3 was not used in the data analysis, no comparison was made between leaves 3 and 4 at 15 C.

At 20, 25, and 30 C, ELISA values increased throughout the study, which is in agreement with results of others (7). ELISA values from the first sample in each leaf above 4 progressively decreased, which correlates well with infectivity results in MDMV-infected corn (22). At 35 C, growth in both cultivars was severely reduced, as demonstrated by lack of internode elongation and much shorter leaves than at the other temperatures in which both cultivars were grown (25 and 30 C). The very low ELISA values in Bugoff at this temperature are supported by other results (7). No MDMV-B could be detected in Colt at the dilutions used in this study. Some ELISA results were atypical, because the values for a single leaf decreased after the first sampling, except for leaf 7, and values progressively increased in each leaf above leaf 4 (Table 4). However, regardless of the temperature at which sorghum was grown, differences in ELISA values existed among leaves on the same plant. Therefore, comparisons between cultivars must be made on equivalent leaves.

Maximum ELISA values and the least ELISA sample variation on a single leaf basis occurred at 25 C. The high ELISA values at this temperature agree with results of infectivity assays reported for MDMV-infected sorghum (14) and corn (21). Our results showed lower ELISA values at 15 C than at all other temperatures, except 35 C, within the time frame of this study. This is supported by previously reported infectivity assay data from sorghum (14).

Sorghum is defined as susceptible to MDMV if temperature-induced leaf necrosis (red-leaf) occurs and is considered resistant if the mosaic symptoms present remain after cool-temperature exposure (10,18). Using this definition, Colt is a resistant cultivar, because it does not show the red-leaf reaction. However, resistance to MDMV has been correlated with low virus titer in MDMV-infected corn (23) and with barley yellow dwarf-infected cereals (6,17). Using the titer criterion, Colt could be considered both resistant and susceptible, depending on when and on what leaf titer estimates are made. At 7 DPI, Colt had lower ELISA values than did Bugoff and, thus, fit the low-titer resistance definition at that stage (Table 3). However, at 14, 21, and 28 DPI, it did not meet the criterion, because the susceptible cultivar Bugoff had a lower virus titer (Table 3). A second unreported phenomenon in the resistant cultivar Colt is the rapid drop of ELISA readings and attenuation of symptoms in leaves 7 and 8, which again conform to the titer criterion for resistance. These trends for the upper leaves of Colt are supported by field results (15). A further differential response between the cultivars was the lack of symptoms and the inability to detect MDMV-B at the dilution used for assay in the resistant cultivar Colt at 35 C.

TABLE 1. The coefficient of variation[a] for enzyme-linked immunosorbent assay values on a single leaf basis from maize dwarf mosaic virus strain B-infected sorghum incubated at different temperatures

Cultivar	Temperature (C)	Leaf 3	4	5	6	7	8
Colt	20	92	34	12	14	26	···
Colt	25	34	12	8	10	33	40
Colt	30	86	21	15	18	37	48
Bugoff	25	20	12	9	8	19	20
Bugoff	30	26	15	15	13	24	25
Bugoff	35	50	45	45	47	47	41

[a] The coefficient of variation derived from data averaged over two experiments, with four replications per experiment.

TABLE 2. Enzyme-linked immunosorbent assay values from maize dwarf mosaic virus strain-B-infected Colt sorghum leaves incubated at 20 C

Leaf	Days postinoculation[a] 7	14	21	28
4	0.012	0.347 a	0.558 a	0.484 b
5	···	0.265 b	0.472 b	0.572 a
6	···	···	0.303 c	0.431 b
7	···	···	···	0.248 c

[a] Means within a column not followed by the same letter differ according to the Student-Newman-Keuls' multiple range test ($P < 0.05$).

TABLE 3. Enzyme-linked immunosorbent assay values from maize dwarf mosaic virus strain-B-infected sorghum leaves

Cultivar	Leaf	Days postinoculation[a] 7	14	21	28
		Incubated at 25 C			
Colt	4	0.236 b	0.481 b	0.649 a	0.681 a
Bugoff	4	0.386 a	0.525 a	0.563 b	0.658 a
Colt	5	···	0.413 c	0.563 b	0.640 a
Bugoff	5	···	0.344 d	0.426 c	0.555 b
Colt	6	···	···	0.412 c	0.539 b
Bugoff	6	···	···	0.281 d	0.339 c
Colt	7	···	···	0.201 e	0.313 c
Bugoff	7	···	···	0.162 e	0.190 d
Colt	8	···	···	···	0.030 e
Bugoff	8	···	···	···	0.143 d
		Incubated at 30 C			
Colt	4	0.187 b	0.435 a	0.497 a	0.532 a
Bugoff	4	0.291 a	0.448 a	0.451 b	0.561 a
Colt	5	···	0.333 b	0.410 b	0.479 a
Bugoff	5	···	0.278 c	0.308 c	0.492 a
Colt	6	···	···	0.269 c	0.338 b
Bugoff	6	···	···	0.205 d	0.383 b
Colt	7	···	···	0.104 e	0.123 c
Bugoff	7	···	···	0.127 e	0.188 c
Colt	8	···	···	···	0.056 d
Bugoff	8	···	···	···	0.120 c

[a] Means within a column not followed by the same letter differ according to the Student-Newman-Keuls' multiple range test ($P < 0.05$).

TABLE 4. Enzyme-linked immunosorbent assay values from maize dwarf mosaic virus strain-B-infected Bugoff sorghum incubated at 35 C

Leaf	Days postinoculation[a] 7	14	21	28
4	0.074	0.062 b	0.019 b	0.018 b
5	···	0.129 a	0.031 b	0.027 b
6	···	···	0.066 a	0.048 b
7	···	···	0.065 a	0.087 a
8	···	···	···	0.114 a

[a] Means within a column not followed by the same letter differ according to the Student-Newman-Keuls' multiple range test ($P < 0.05$).

TABLE 5. Enzyme-linked immunosorbent assay values from sorghum leaves of different lengths on plants of the same age

Cultivar	Leaf ratio[b]	30 C[a] Leaf 5[c]	Leaf 6[d]	Greenhouse Leaf 5[c]	Leaf 6[d]
Colt	0.25	0.414 b	0.265 b	0.249 b	0.227 c
Colt	0.50	0.451 a	0.334 a	0.336 a	0.277 b
Colt	0.75	0.467 a	0.336 a	0.337 a	0.323 a
Bugoff	0.25	0.344 d	0.296 a	···	···
Bugoff	0.50	0.367 c	0.312 a	···	···
Bugoff	0.75	0.394 c	0.325 a	···	···

[a] Means within a column not followed by the same letter differ according to the Student-Newman-Keuls' multiple range test ($P < 0.05$).
[b] The length of the sample leaf divided by the length of the leaf subtending it.
[c] Leaf 5 sampled at 14 days postinoculation.
[d] Leaf 6 sampled at 18 days postinoculation.

TABLE 6. Enzyme-linked immunosorbent assay values from different parts of maize dwarf mosaic virus strain-B-infected sorghum leaves incubated under various conditions

Cultivar	Leaf part	Leaf 5[a]			Leaf 6[a]		
		0.5[b]	1[c]	1 + 7[d]	0.5[b]	1[c]	1 + 7[d]
Colt[e]	Base	0.213 c	0.367 c	0.532 b	0.195 c	0.313 c	0.420 c
Colt[e]	Middle	0.332 b	0.572 a	0.714 a	0.319 b	0.522 b	0.680 b
Colt[e]	Tip	0.410 a	0.594 a	0.749 a	0.395 a	0.659 a	0.820 a
Bugoff[e]	Base	0.138 d	0.261 d	0.450 c	0.115 d	0.344 c	0.484 c
Bugoff[e]	Middle	0.309 b	0.503 b	0.666 a	0.281 b	0.536 b	0.698 b
Bugoff[e]	Tip	0.430 a	0.573 a	0.685 a	0.361 a	0.673 a	0.817 a
Colt[f]	Base	0.228 c	0.405 c	0.237 c	0.143 c	0.157 c	0.050 c
Colt[f]	Middle	0.434 b	0.772 b	0.314 b	0.336 b	0.411 b	0.113 b
Colt[f]	Tip	0.513 a	0.922 a	0.361 a	0.527 a	0.592 a	0.147 a

[a] Means within a column not followed by the same letter differ according to the Student-Newman-Keuls' multiple range test ($P < 0.05$).
[b] 0.5 = Sampled leaf one-half the length of the subtending leaf.
[c] 1 = Sampled leaf the same length as the subtending leaf.
[d] 1 + 7 = Leaf sample taken 7 days after the 1 sample.
[e] Incubated at 30 C.
[f] Incubated under greenhouse conditions. Data analyzed separately from the 30 C results.

The normal variation in leaf size that occurs within cultivars planted at the same time has a significant effect on the validity of comparisons within and between cultivars. At 30 C, in both leaves 5 and 6, a gradient in ELISA values existed so that the 0.25 ratio class had the lowest values and the 0.75 ratio class the highest (Table 5). The values for leaves 5 and 6 in the shortest leaves (0.25 ratio) were lower in most instances than the values in the 0.50 and 0.75 leaf lengths. This difference is amplified under greenhouse conditions, so that all leaf ratio classes were different. This intrinsic variation of titer in tissue from leaves of different lengths has not been reported for MDMV-infected sorghum. Thus, this variation precludes the comparison of equivalent leaves within and between cultivars that are not the same size.

Our studies demonstrated a further unreported source of variation in titer: the differences in ELISA values between parts of the same leaf (Table 6). A gradient in absorbance values was measured in all leaves and under all conditions. Highest values were measured in the leaf tips and the lowest values in the leaf bases. These values were often different at 30 C in the leaves studied (Table 6), and were always different under varying temperature conditions (Table 6). Thus, valid comparisons can be made only between whole leaves or identical leaf parts within and between cultivars.

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