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Effect of Inoculation Pressure on Maize Dwarf Mosaic Virus Strain-A Disease Incidence, Severity and Titer in Sorghum

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Abstract

Use of a spray gun for mechanical inoculation of viruses could result in different pressures during inoculation, affecting disease ratings of two sorghum cultivars. This experiment evaluated the effect of mechanical inoculation pressure on virus titer as measured by double antibody sandwich method of enzyme-linked immunosorbent assay (DAS-ELISA), disease incidence and severity in sorghum cultivars differing in reaction to maize dwarf mosaic virus strain-A (MDMV-A). Positive correlations (r=0.58 and r=.0.53 for RTx430 and BTx378 respectively), were obtained between disease incidence (percentage infection) when inoculation pressure was in the range of 5.28 to 7.06 kg/cm^2. Neither disease severity nor titer was correlated to inoculation pressure. However, inoculations resulted in higher virus titer compared to uninoculated plants.

Introduction

Maize dwarf mosaic virus (MDMV) occurs world wide in gramineous hosts and causes significant economic loss in several crops, including grain and forage sorghum, millet, and maize. Host plant resistance has reduced the losses caused by this virus disease.

Artificial inoculation (using an airgun) of sorghum with MDMV has led to the release of tolerant and resistant inbreds and hybrids. When a spray gun is used for mechanical inoculation, differences in pressures during inoculation may result in selectively affected disease ratings among cultivars. Different field inoculation techniques have been used (6, 7, 8, 9), and there is no consistency in the protocol followed. Inoculation pressures ranging from 4 kg/cm^2 to 8.5 kg/cm^2 (6, 8) have been used for field inoculations. There has been no mention of the effect of inoculation pressure on virus titer and yield. Toler and Miller (15), using four different inoculation pressures (0, 1.4, 4.2, and 7.3 kg. cm^2), found increased cultivar susceptibility with increased pressure, and disease severity varied under different pressures. In their work, no mention was made of the effect of different pressures at inoculation on virus titer and yield. To better understand the host pathogen interaction, data on MDMV titer in both susceptible and resistant cultivars is required. Enzyme-linked immunosorbent assay (ELISA) has been used to quantify virus (2, 3, 10, 11, 12, 13, 14) and is a promising alternate to infectivity assay to study MDMV titer.

The objectives of this study were to evaluate the relationship among inoculation pressure virus titer, disease incidence, and disease severity in sorghum cultivars differing in reaction to MDMV-A.

Materials and Methods

Two sorghum cultivars, RTx430 (tolerant) and BTx378 (susceptible) were planted in randomized block designs replicated four times. Experimental units were single rows, 6 meters long. Each cultivar was subjected to six different pressures (Table 1).

Table 1. Air-gun pressures used for mechanical inoculation of sorghum with MDMV-A.

Treatment number	Description
1	not inoculated (control)
2	1.76 kg/cm^2 (25 lb/sq inch)
3	3.50 kg/cm^2 (50 lb/sq inch)
4	5.28 kg/cm^2 (75 lb/sq inch)
5	7.06 kg/cm^2 (100 lb/sq inch)
6	8.81 kg/cm^2 (120 lb/sq inch)

The different air pressures were provided to the spray gun by a portable 3000 watt (4Hp) gasoline powered air compressor.

Inoculum Source

MDMV – infected Johnsongrass (*Sorghum helepense*) was used as the virus source. Infected Johnsongrass leaves were combined with 0.1M (mol/litre) potassium phosphate buffer, pH 7.2, at a 1:4 (w/v) ratio and homogenized in a Waring blender. The resulting extract was expressed through four layers of cheesecloth. All operations were carried out on ice. One percent by volume of 600 mesh carborundum was added to the extract to insure wounding of the leaf epidermis during inoculation. The abrasive was kept in suspension by periodical shaking during inoculation.

Inoculation Procedure

Plants were inoculated at the three leaf stage using an artist airbrush (DeVillbis MGA spray gun). Plants were sprayed at a distance of approximately 5 cm with an inoculum flow rate of 20 ml/minute.

Disease incidence due to virus infection was recorded 14 days after inoculation and thereafter at weekly intervals until booting. Each plant in the plot was rated for disease severity on a 1 to 5 scale (Table 2).

Table 2. Maize dwarf mosaic virus strain-A disease rating system.

Category	Description
1.0	Symptomless or healthy plants
2.0	Mild to moderate mosaic symptoms
3.0	Strong to severe mosaic symptoms, yellowing with possible stunting.
4.0	Slight necrotic reaction (red leaf response), strong to severe mosaic symptoms, usually with stunting.
4.5	Necrosis over 20 to 50% of the leaves surface area and growing tip.
5.0	Necrosis over 50% of the leaves surface area and growing point, little or no useable yield expected, or dead plant.

A disease severity index (DSI) was calculated using the following equation:

$$DSI = (a2 + b3 + \text{------} e5)/N$$

where a, b, ------ e represent the number of infected plants within each symptom severity category and N is the total number of infected plants.

Data also were collected for percent infection and yield and analyzed by ANOVA and regression. Tukey's studentised range test was used for mean separations.

Enzyme-linked Immunosorbent Assay (ELISA)

Titer of MDMV-A was assessed using the double antibody sandwich (DAS)-ELISA. Half leaf samples from the top-most fully expanded leaf from each of 10 tagged plants in a plot were collected and combined. Plant tissue was macerated in plastic sleeves using a hammer and the sap was expressed with a Banttaris's device and diluted 1:10 in 0.1M potassium phosphate buffered saline solution (PBS) pH 7.2. Microliter plates were coated with 100 µl of MDMV-A immunoglobin (IgG) and incubated overnight at 4 C.

Coating of microliter plates with IgG, adding of antigen, biotinylated secondary antibody (conjugate), enzyme and substrate were done as described by Clark and Adams (1). Antigens (100 µl), including known concentrations of purified virus, buffer, and healthy control were added to ELISA plant wells according to a randomized block design with four replications. Antibody-antigen reactions were assessed by adding 50 µl of p-nitrophenyl phosphate substrate and incubating for 30 minutes at room temperature in the dark. Reactions were stopped by adding 50 µl of 3M sodium hydroxide to each well. The absorbency of each sample at 410 nm was read on a Minireader II (Dynatech Laboratories, Alexandria, VA), ELISA plate reader.

Virus concentration, measured as optical density (OD) values, was analyzed by regression and ANOVA, and paired comparisons of treatment means within and between cultivars were done according to Tukey's studentised range test.

Results

The relationship between percentage infection and mechanical pressure used for inoculating MDMV-A was best described by a quadratic equation. For RTx430, the equation was:

$$Y = 26.576 + 1.146\ X1 - 0.0074\ X2$$

with $R2 = 0.67$ and $Pr > F = 0.0001$

For Btx378

$$Y = 38.911 + 0.823\ X1 - 0.\text{--}5\ X2$$

with $R2 = 0.18$ and $Pr > 0.0001$

where Y = observed percentage infection

X1 = inoculation pressure

X2 = inoculation pressure squared

All parameter estimates were highly significant with $p < 0.0001$.

Disease incidence was positively correlated ($r = 0.58$ and $r = 0.53$ for RTx430 and BTx378, respectively) with mechanical pressure used for inoculation. Paired comparisons of mean percentage infections showed no difference for inoculation pressures greater than 5.28 kg/cm^2 for both RTx430 and BTx378. All inoculations resulted in significantly higher percentage infection compared to the non-inoculated controls (Table 3).

Table 3. Mean percent infection of two sorghum cultivars following use of different inoculation pressures.

Inoculation pressure (kg/cm^2)	Cultivar	
	RTx430	BTx378
0.00	28.11^{c}	30.92^{b}
1.76	47.14^{bc}	67.36^{a}
3.50	66.17^{ba}	66.89^{a}
5.28	74.38^{a}	68.92^{a}
7.06	64.46^{ba}	65.40^{a}
8.81	58.47^{b}	70.82^{a}
F value	8.03	6.33
Pr > F	0.0004	0.0017
STD	11.699	10.714
R^2	0.69	0.65

Least squares regression for disease severity index against inoculation pressure used were not significant for either cultivar, and no differences were observed for DSI among inoculation pressures (Figure 1). There was no interaction between inoculation

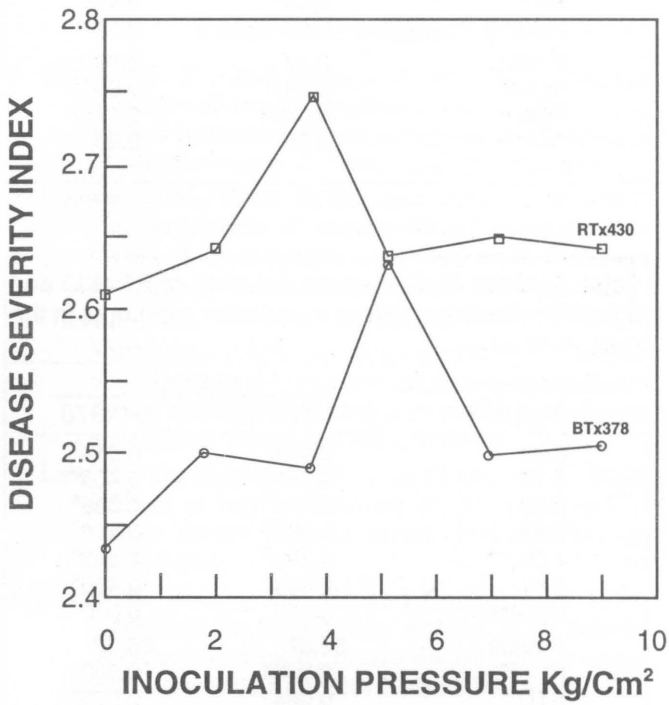

Figure 1. Relationship between disease severity and inoculation pressure used for inoculating maize dwarf mosaic virus strain - A in two sorghum cultivars.

pressure and cultivar for disease severity and percentage infection. No correlations were observed among inoculation pressure, disease severity, and yield.

ELISA

Differences were observed for mean ELISA values for the different inoculation pressures used at inoculation for RTx430. For BTx378, no differences were observed for ELISA values among the different pressures used for inoculation. All inoculated plots had significantly higher mean ELISA values compared to the non-inoculated plots (Tables 4, 5). A high coefficient of determination (R = 0.96) was obtained between OD values and known concentrations of purified MDMV-A (Figure 2). Significant correlations (r = 0.60 for RTx430 and BTx378, respectively) were obtained between inoculation pressure and OD values for field data (Table 6). However, low nonsignificant correlations (r = 0.145 and 0.246 for RTx430 and BTx378, respectively) were obtained between OD and mechanical inoculation pressure for greenhouse data (Table 7).

Table 4. Mean ELISA values (OD410) following use of different inoculation pressures in the field.

Inoculation pressure (kg/cm^2)	Cultivar	
	RTx430	BTx378
0.00	0.28^{b}	0.28^{b}
1.76	0.34^{ba}	0.43^{a}
3.50	0.30^{ba}	0.44^{a}
5.28	0.39^{a}	0.35^{a}
7.06	0.36^{ba}	0.38^{a}
8.81	0.31^{ba}	0.43^{a}
Healthy	0.02	0.02
F value	2.18	3.93
Pr > F	0.041	0.0035
STD	0.0876	0.1127
R^2	0.18	0.23

Table 5. Mean ELISA values (OD410) of RTx430 and BTx378 following different inoculation pressures in the greenhouse.

Inoculation pressure (kg/cm^2)	Cultivar	
	RTx430	BTx378
0.00	0.014^{b}	0.014^{b}
1.76	0.340^{a}	0.400^{a}
3.50	0.374^{a}	0.388^{a}
5.28	0.382^{a}	0.412^{a}
7.06	0.393^{a}	0.402^{a}
8.81	0.388^{a}	0.400^{a}
Healthy	0.010	0.010
F value	52.37	66.02
Pr > F	0.0001	0.0001
STD	0.0847	0.07531
R^2	0.73	0.80

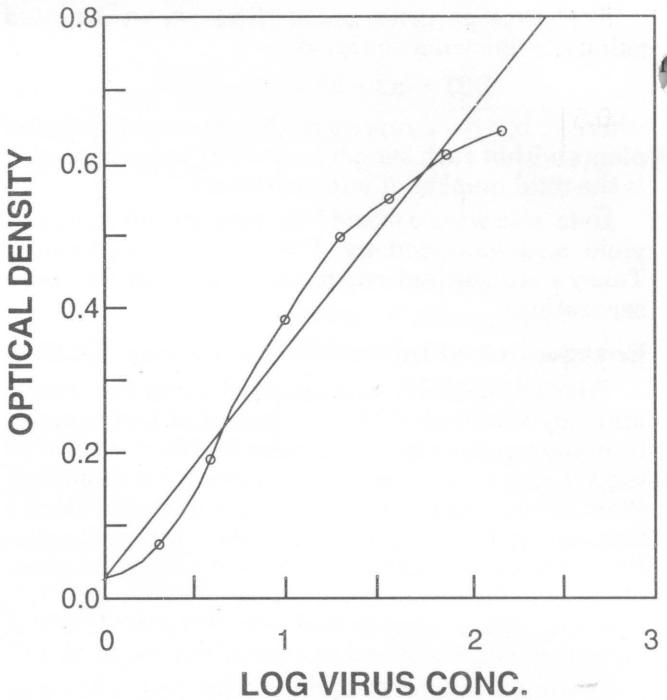

Figure 2. Maize dwarf mosaic virus strain A standard curve.

Table 6. Correlation coefficients (r) for data obtained from field studies.

Variable	RTx430	BTx378
Pre x OD	0.308	0.391
Pre x DSI	0.168	0.120
DSI x OD	0.024	0.059
INF x OD	0.160	0.598**
INF x Pre	0.582**	0.527**

Pre - pressure at inoculation
OD - optical density (& ELISA)
DSI - disease severity index
INF - % infection
** - significance at $p < 0.001$

Table 7. Correlation coefficients (r) for data obtained from greenhouse studies.

Variable	RTx430	BTx378
Pre x OD	0.732***	0.668***
Pre x DSI	0.682***	0.810***
DSI x OD	0.868***	0.781***
INF x OD	0.904***	0.948***
Pre x INF	0.732***	0.712***

Pre - pressure at inoculation
OD - optical density
DSI - disease severity index
INF - % infection
*** - significance at $p < 0.0001$

There was no association between virus titer and mechanical inoculation pressure (Figure 3).

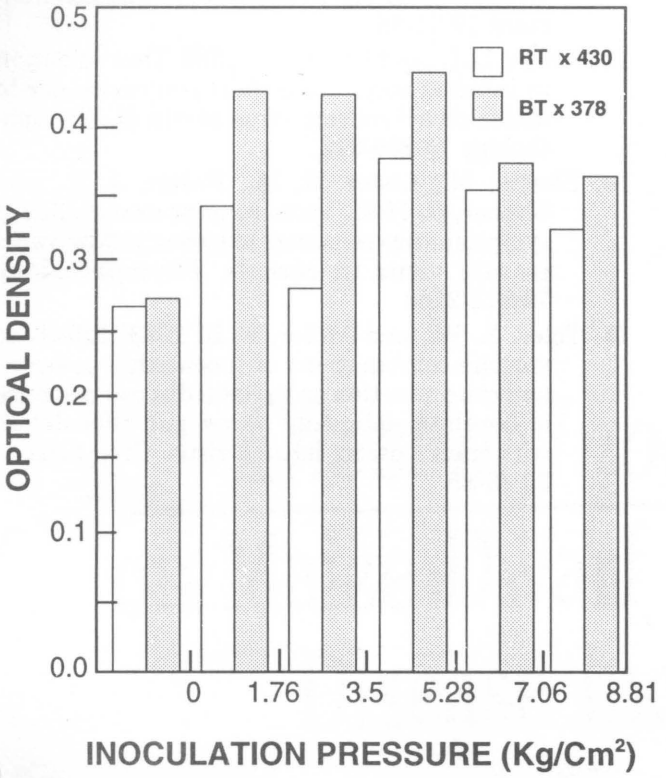

Figure 3. Mean ELISA values (OD410) obtained following use of different pressures when inoculating with MDMV-A (Field).

Discussion

Disease severity varied with mechanical inoculation pressure. Toler and Miller (15) reported that "cultivars responded uniformly to inoculation pressure and that rankings of cultivars by DSI remain unchanged". Results from this study show that there is no correlation between inoculation pressure and disease severity, but symptom expression was delayed when using pressures lower than 3.50 kg/cm^2.

Positive correlation (r = 0.58 and r = 0.53 for RTx430 and BTx378,respectively) were observed between percentage of infection and inoculation pressure. The relationship was best described by a quadratic equation. However, pressures above 7.06 kg/cm^2 were associated with somewhat reduced disease incidence. This could be a result of lethal-cell damage by the high pressures during inoculation, which resulted in necrotic tissues and thereby impeding virus multiplication and spread. For successful virus infection, non-lethal wounding of the leaf cells is essential.

Weak negative correlations were obtained between inoculation pressure and yield. The weak associations could have resulted from the effect of the MDMV tolerant RTx430 plants that were asymptomatic and the high yielding capability of BTx378 plants that escaped infection. Titer, symptoms severity, yield, and pressure used at inoculation were not associated within the susceptible cultivar BTx378.

Virus concentrations estimated by DAS-ELISA were not different among inoculation pressures between cultivars. Seifers and Hackerott (12), observed high virus concentrations in cultivars having red leaf reaction using MDMV-B. Giorda (2, 3), working with MDMV-A, obtained highest titer in BTx378, a susceptible cultivar with necrotic reaction; the present study confirms these observations. The low correlation obtained between OD values and air-gun pressure at inoculation could be a result of natural infection by aphids.

Low virus concentrations that were observed for the susceptible cultivar BTx378 compared to RTx430 (mosaic reactor) might be due to the age of the tissue used for assaying virus titer. Seifers and Caceres (13) found that the sorghum cultivar Colt, which was resistant to MDMV-B, had lower ELISA values at 7 days post-inoculation but not at 14, 21, and 28 days post-inoculation. Giorda (2, 3) found that RTx430 had 53 to 74 percent less MDMV-A accumulated than the susceptible cultivars RTx378, BTx3197 and RTx7000 within the first 10 days after inoculation. This proportion decreased thereafter depending on plant age and temperature. In this study, averages of three assessment times were used and this could have masked the differences between the two cultivars. BTx378 was observed to have numerous necrotic specks on the leaves and this could have impeded virus multiplication and reduced virus titer in the leaf, making titer variation with inoculation pressure difficult to ascertain.

Literature Cited

1. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
2. Giroda, L. M., Toler, R. W., and Miller, F. R. 1987. Concentration of maize dwarf mosaic virus strain A in sorghum cultivars with different reaction to the virus. Sorghum NL 30:83-87.
3. Giorda, L. M., Toler, R. W., and Miller, F. R. 1987. Virus concentration in sorghum cultivars inoculated at different growth stages with maize dwarf mosaic virus strain A under greenhouse conditions. Sorghum NL 30:87-89.
4. Law, M. D., Moyer, J. W., and Payne, G. A. 1989. Effects of host resistance on pathogenesis of maize dwarf mosaic virus. Phytopathology 79:757-761.
5. Louie, R., Findley, W. R., and Knoke, J. K. 1976. Variation in resistance within corn inbred lines to infection by maize dwarf mosaic virus. Plant Disease Reporter 60:838-842.
6. Louie, R. 1986. Effects of genotype and inoculation protocols on resistance evaluation of maize dwarf mosaic virus strains. Phytopathology 76:769-773.

7. Mackenzie, D. R., Anderson, P. M., and Wernham, C. C. 1966. A mobile airblast inoculator for pot experiments with maize dwarf mosaic virus. Plant Disease Reporter 60:363-367.
8. Martin, T. J., and Hackerott, H. I. 1982. Greenhouse seedling technique to determine the reaction of sorghum to maize dwarf mosaic virus strain A. Crop Science 22:1255-1256.
9. Miller, F. R., and Toler, R. W. 1977. Maize dwarf mosaic virus concentration, inoculation pressure and variety interactions. Pages 87-97 in: Development of improved High-yielding Sorghum Cultivars. 3rd Ann. Rept. USAID Tex Agric. Exp. Sta., College Station, TX 77843.
10. Pataky, J. K., Murphy, J. F., and D'Arcy, C. J. 1990. Resistance to maize dwarf mosaic virus, severity of symptoms, titer of virus, and yield of sweet corn. Plant Disease 74:359-364.
11. Reeves, J. T., Jackson, A. O., Paschke, J. D., and Lister, R. M. 1978. Use of enzyme-linked immunosorbent assay (ELISA) for serodiagnosis of two maize viruses. Plant Disease Reporter 62:667- 671.
12. Seifers, D. L., and Hackerott, H. L. 1987. Estimates of yield loss and virus titre in sorghum hybrids infected with maize dwarf mosaic virus strain B. Agric. Ecosystems and Environment 19:81-86.
13. Seifers, D. L., and Caceres, J. 1988. Titer variation in infected sorghum differing in resistance to maize dwarf mosaic virus strain B. Phytopathology 78:208-212.
14. Skaria, M., Lister, R. M., Foster, J. E., and Shaner, G. 1985. Virus content as an index of symptomatic resistance to barley yellow dwarf mosaic virus in cereals. Phytopathology 75:212-216.
15. Toler, R. W., and Miller, F. R. 1983. Effects of various combinations of inoculation pressure and concentration on varietal disease response of sorghum following spray gun inoculation with maize dwarf mosaic virus. Crop Science 23:83-85.