Effect of Nitrogen and Moisture Stress on Severity of Maize Dwarf Mosaic Virus Infection in Corn Seedlings

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ABSTRACT

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The impact of nitrogen and moisture stress on maize dwarf mosaic virus strain A (MDMV-A) in corn (*Zea mays*) was examined in a greenhouse experiment. The factors were MDMV-A, moisture, and nitrogen arranged in a factorial design, with each stress either present or absent. Moisture × nitrogen interaction was significant for number of leaves, plant height, and dry weight. MDMV × moisture was significant for dry weight. Ranking effect of stresses on MDMV titer, lowest to highest, was: adequate nitrogen and adequate moisture (no stress), and adequate nitrogen and inadequate moisture (moisture stress) < inadequate nitrogen and inadequate moisture (dual stress) < inadequate nitrogen and adequate moisture (nitrogen stress). Weeds that can increase moisture and nitrogen stress may need to have thresholds adjusted for cornfields in which the incidence of MDMV is high.

Maize dwarf mosaic virus strain A (MDMV-A) is an economically important virus of corn (*Zea mays* L.), capable of reducing height, biomass, and yield (2,4,9). This virus is widespread in the southeastern United States and is primarily found in fields infested with johnsongrass (*Sorghum halepense* (L.) Pers.).

We have observed MDMV infection to be more severe in fields where growing conditions are less than optimum. Olson et al (4) reported that the effects of moisture stress and MDMV on sweet corn were additive and also that no significant differences in MDMV titer were observed when the plots were subjected to drought stress. Tu and Ford (6) found MDMV concentrations to be low in corn plants grown in nutrient solutions lacking nitrogen.

There is a strong association between soil moisture and nitrogen availability (3,8). Nitrogen in the form of NO_3 is very water-soluble and moves through the soil via mass flow. Transport of nitrogen to plant roots is reduced when soil moisture is low. Reduced stomatal openings and transpiration stream due to moisture stress can also slow movement of nitrogen within a plant.

This study was designed to determine if nitrogen and/or soil moisture influenced the severity of MDMV infection in corn seedlings and also the impact of both moisture and nitrogen stress on MDMV titer in young tissue.

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MATERIALS AND METHODS

Greenhouse. Eight to 10 seeds of Pioneer Brand 3147, a corn hybrid moderately resistant to MDMV (Pioneer Hybrid International, *personal communication*), were planted in pots 22 cm tall and 22 cm in diameter. Each pot contained 16 kg of a loamy sand soil (86% sand, 10% silt, 4% clay). Plants were thinned to three per pot when the first leaf collar was visible. The plants were maintained in a greenhouse at day/night temperatures of 30/15 C, with supplemental light (metal halide lamps, 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetic photo flux density) to extend day length to 14 hr.

Soil pH was adjusted to 6.2, and all nutrients, except nitrogen, were amended to satisfactory levels by the addition of $Ca(OH)_2$, K_2HPO_4, and $MgSO_4$ (G. S. Miner, *personal communication*). Level of available nitrogen in the soil prior to initiating the experiment was 29 ppm of NO_3 and 35 ppm of NH_4.

The experiment was arranged as a randomized complete block design with eight treatments and three replications and was repeated once. The treatment design was a 2 × 2 × 2 factorial. The factors were: 1) plants inoculated with MDMV-A or not inoculated, 2) adequate moisture or moisture stress, and 3) added nitrogen or no added nitrogen.

Inoculations with MDMV began when the collar of the second leaf was visible and were repeated every second day for 1 wk. Tissue infected with MDMV-A was initially obtained from R. Toler (Texas A&M University, College Station). The corn plants infected with MDMV were rubbed with tissue from johnsongrass plants infected with MDMV-A and homogenized in buffer (KH_2PO_4, pH 7.0) at 1 g/5 ml and Carborundum at 1 g/100 ml. Noninfected plants were rubbed only with buffer and Carborundum. All pots were watered daily until completion of MDMV inoculations (third-leaf stage) to ensure that moisture stress did not affect the inoculation process.

Treatments with adequate moisture were watered daily to approximately field capacity, with minimal drainage. When leaves of moisture-stressed plants showed signs of leaf roll, they were watered to approximately −1 bar. A moisture retention curve was determined for the soil by North Carolina State University Soil Physics Laboratory and was used to calculate −1 and −5 bars. Soil and leaf samples were randomly selected from pots prior to watering, to help determine the maximum moisture stress. Leaf disks (6 mm in diameter) were weighed from moisture-stressed and non-moisture-stressed plants. Leaf disks were then placed in distilled water for a minimum of 12 hr, after which rehydrated leaves were weighed and percent change in moisture content was calculated. Soil samples were weighed, air-dried, then reweighed. The moisture retention curve indicated that soils were approximately −5 bars when corn leaves began to roll. Moisture-stressed plants were watered when soil moisture dropped to −5 bars, and percent moisture in leaf tissue was 38% lower than that in plants grown with adequate moisture.

At least once a week, two vertical channels (0.5 × 3 × 15 cm) were randomly made in each pot to facilitate water movement throughout the soil. Channels were made in all pots so the effect of root pruning would not confound with moisture stress. Moisture-stressed plants were watered to approximately −1 bar when soil moisture was −5 bars (onset of leaf roll). On the basis of the moisture retention curve and weight of soil, 80 ml of water was added to return soil moisture to −1 bar. All moisture-stressed treatments were watered immediately after nitrogen application to the −1 bar level.

The amount of nitrogen applied was based on preliminary experiments and determined as 4% dry weight of healthy plant tissue 45 days after planting (DAP) (E. J. Kamprath, *personal communication*). One-third of the nitrogen (ammonium nitrate, 34-0-0) was added at planting and the remaining thirds at 22 and 33 days after planting. Nitrogen was mixed into the top 2 cm of soil.

Height from soil to last visible collar

and severity of maize dwarf mosaic symptoms were measured approximately 7 wk after planting (WAP). Severity of symptoms was rated as: 1 = no symptoms, 2 = mosaic pattern covered 1–10% of leaf area, 3 = 11–25%, 4 = 26–50%, 5 = 51–75%, and 6 = >75% of leaf area. At 7 WAP in the second run of the experiment, the number of leaves with visible collars was counted.

At 7 WAP, two leaf disks (6 mm in diameter) were collected from the center of the first two leaves above the last visible collar to determine MDMV-A titer (four disks per plant). Leaf disks from the three plants in each pot were combined for analysis of MDMV titer. Leaf disks were stored at −4 C until the virus titer was determined. Enzyme-linked immunosorbent assay (ELISA) was performed as a modified double antibody sandwich (1,7). The plants were then cut at soil level and placed in a drying oven (70 C), and weights were recorded 5 days later.

The first run of the experiment was terminated 44 DAP and the second run, 47 DAP. The average of the three plants per pot was used for data analysis. Homogeneity of variances was determined before the analysis of variance (ANOVA). ANOVA was conducted with replications nested within runs. Treatment means for two-way interactions were separated with *t* tests utilizing SAS (5).

Determination of MDMV-A titer. Immunolon 1 plates were coated for the modified ELISA with polyclonal MDMV-A antiserum (0.3 μg/ml) at 37 C for 2 hr and then washed with PBS-Tween (0.15 M phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20). Plant tissues were prepared by pulverizing leaf disks in liquid nitrogen and grinding them in PBS-Tween (1:25, w/v). Extracts were incubated overnight in plates at 4 C, washed in PBS-Tween, and incubated in the second MDMV-A antibody (0.3 μg/ml), conjugated with bovine intestinal alkaline phosphatase, for 2 hr at 37 C. After a standard wash, *p*-nitrophenyl phosphate substrate was added, and plates remained at 25 C for 1 hr for color development. Absorbance at 405 nm was measured to determine virus titer (4).

RESULTS

Moisture-stressed plants with adequate nitrogen required more frequent watering than plants that were both moisture- and nitrogen-stressed. The texture of the soil and the use of preferential flow channels facilitated the movement of water throughout the soil profile to near equilibrium levels in the moisture-stressed treatments.

Plants with adequate nitrogen and adequate moisture (nonstressed) had the most leaves, 8.6 (Table 1). The number of leaves was similar in plants with inadequate moisture and adequate nitrogen (moisture stress) and inadequate moisture and inadequate nitrogen (dual stress). Plants with adequate moisture and inadequate nitrogen (nitrogen stress) and those with dual stress also had a similar number of leaves, 7.0 and 7.3, respectively.

Nitrogen × moisture interaction was also significant for plant height. Nonstressed plants were the tallest, 31.4 cm (Table 1). Plants that were nitrogen-stressed and those that were moisture-stressed had similar heights (24.3 and 22.7 cm, respectively), and plants with both stresses (dual-stressed plants) were the shortest (19.7 cm).

Two interactions were significant for dry weight. MDMV × moisture was significant, with nonstressed plants having the highest weight, 24.9 g (Table 1). Plants with adequate moisture but infected with MDMV weighed 17.9 g. Moisture-stressed plants, with or without MDMV infection, had similar weights, averaging 7.6 g.

Nitrogen × moisture interaction was also significant for dry weight (Table 1). Nonstressed plants weighed approximately twice as much as nitrogen-stressed plants, 28.8 and 13.9 g, respectively. Moisture-stressed and dual-stressed plants had similar dry weights, 8.3 and 7.0 g, respectively.

The percentage of leaf tissue showing mosaic symptoms was similar for all plants infected with MDMV (Table 2). The interaction of MDMV × moisture was significant for MDMV titer (Table 2). MDMV titer was lowest in plants with sufficient nitrogen, regardless of moisture level. Ranking effect of stresses on MDMV titer from lowest to highest was: adequate nitrogen and adequate moisture (no stress), and adequate nitrogen and inadequate moisture (moisture stress) < inadequate nitrogen and inadequate moisture (dual stress) < inadequate nitrogen and adequate moisture (nitrogen stress).

DISCUSSION

MDMV infection had no influence on either the number of leaves per plant or weight per plant. The presence of MDMV was significant for height at α = 0.1 (Table 1). Stunting is a common symptom of MDMV when plants are infected early. However, stunting occurs during the period of internodal elongation (9), and 7 wk was not sufficient time for internodal elongation to occur. Olson et al (4) reported that MDMV infection reduced the final height of sweet corn; had we continued our experiment, height differences may have developed. Neither moisture nor nitrogen stress had an additional impact on the severity of maize dwarf mosaic symptoms in corn seedlings.

MDMV × moisture interaction was significant for dry weight (Table 1). Dry

Table 1. Treatment means and analysis of variance for number of leaves, height, and dry weight of corn grown under presence or absence of nitrogen stress, moisture stress, and maize dwarf mosaic virus (MDMV) infection[a]

Stress factor[b]		Leaves/plant (no.)		Height (cm)		Dry weight (g)	
Moisture	Nitrogen	No virus	Virus	No virus	Virus	No virus	Virus
−	−	8.4	8.8	31.5	31.3	32.9	24.7
−	+	6.9	7.0	25.7	22.9	16.8	11.0
+	−	7.7	7.8	23.6	21.8	8.8	7.8
+	+	7.1	7.6	20.3	19.1	7.4	6.6
ANOVA values ($P > F$)[c]							
Nitrogen (N)		0.001		0.001		0.001	
Moisture (M)		0.124		0.001		0.001	
N × M		0.001		0.023		0.001	
MDMV			0.122		0.090		0.004
MDMV × N			0.851		0.569		0.621
MDMV × M			0.859		0.991		0.023
MDMV × M × N			0.376		0.364		0.674

[a]Treatments were arranged as a 2 × 2 × 2 factorial. Data were collected 7 wk after planting.
[b]− = Absence, + = presence of factor.
[c]Values are alpha levels for each treatment; levels ≤0.05 are considered significant.

Table 2. Treatment means and analysis of variance for maize dwarf mosaic symptom ratings and maize dwarf mosaic virus titers in mechanically inoculated corn plants[a]

Stress factor[b]			Titer
Moisture	Nitrogen	Rating[c]	(A_{405})
−	−	4.6	0.63
−	+	5.2	1.24
+	−	5.4	0.70
+	+	5.2	0.99
ANOVA values ($P > F$)[d]			
Moisture (M)		0.331	0.136
Nitrogen (N)		0.572	0.001
M × N		0.275	0.008

[a]Treatments were arranged as 2 × 2 factorial. Data were collected 7 wk after planting.
[b]− = Absence, + = presence of factor.
[c]1 = No symptoms, 2 = mosaic pattern covered 1–10% of leaf area, 3 = 11–25%, 4 = 26–50%, 5 = 51–75%, and 6 = >75% of leaf area.
[d]Values are alpha levels for each treatment; levels ≤ 0.05 are considered significant.

weight was reduced when corn was infected with MDMV and moisture was not limiting. MDMV had no additional impact on moisture-stressed corn. Olson et al (4) reported no MDMV × drought stress interaction in final height, yield, or leaf area and concluded that the effects of these two stresses were additive. Comparison of our results with those of Olson et al (4) is difficult because of differences in soil type, methods of maintaining moisture stress, and corn variety (dent vs. sweet). Also, our experiment was terminated 7 wk after planting, before maximum biomass was attained; full-season growth of the corn in the study by Olson et al (4) may account for some inconsistencies between the two studies.

MDMV titer was similar for all plants receiving adequate nitrogen, regardless of moisture level. Similarly, Olson et al (4) found moisture stress did not influence MDMV titer in sweet corn grown with sufficient nitrogen. In our study, corn with adequate moisture but nitrogen-stressed had the highest MDMV-A titer. There was no correlation between virus titer and any of the growth parameters measured. Tu and Ford (6), however, reported a decrease in MDMV titer in MDMV-susceptible field corn plants grown in nutrient solutions without nitrogen and concluded that MDMV concentration is positively correlated with growth of the plant. Tu and Ford (6) completely excluded nitrogen from their solution cultures, whereas our soils contained a limited supply of nitrogen. In a field setting, it is likely that the soil will contain residual nitrogen. Nitrogen stress was not visibly evident in our study until 4 wk after planting. Differences in initial levels of nitrogen and corn's susceptibility to MDMV may explain some inconsistencies between our study and that of Tu and Ford (6).

Greater understanding of the interaction of multiple stresses on crop development will aid in implementing management decisions for a grower. Nitrogen and moisture stresses are commonly encountered in situations of weed/crop competition. The interaction of MDMV × moisture was significant for titer and dry weight, indicating that these stresses are not additive. These types of interactions need to be thoroughly studied to accurately predict their impact on yield loss. Weed thresholds for a cornfield may need to be altered when there is a high incidence of MDMV in the field.

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