Ozone Sensitivity in Sweet Corn (Zea mays L.) Plants: A Possible Relationship to Water Balance

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ABSTRACT

Stomatal characteristics affecting gas exchange were compared in two sweet corn hybrids (var. Bonanza and Monarch Advance) which differ in foliar ozone sensitivity. No significant differences were observed in stomatal frequencies, guard cell lengths, or conductances to water vapor diffusion.

When plant water status was compared, leaf-water potentials for the ozone-resistant cultivar, Bonanza, were lower. A comparison of the relationship between soil- and leaf-water potential indicated that the leaf-water potential of Bonanza was more sensitive to declines in soil-water potential. Additionally, a comparison of stomatal conductance to water vapor diffusion as soil moisture declined and following root detachment indicated that stomata of Bonanza were, likewise, more sensitive to increasing water stress. Data suggest that these differences are attributable to a greater shoot-to-root fresh weight ratio and higher resistance to water movement in the water-conducting tissues for Bonanza.

Our observations suggest that root and water delivery system characteristics play major contributory roles in the determination of foliar ozone sensitivity in this species.

Differential ozone sensitivity in sweet corn (Zea mays L.) cultivars has been reported (6). However, the mechanisms responsible for heritable differences in foliar sensitivity have not been elucidated. Two commercially available F1 hybrids, Monarch Advance and Bonanza, represent opposite extremes in sensitivity (24). Field impressions of both high ozone levels and high temperatures result in acute injury expressed as chlorotic and necrotic foliar patterns on the sensitive cultivar, Monarch Advance. Under identical conditions, leaves of resistant cultivar, Bonanza, are not appreciably affected.

The differential response is observed in plants grown under a variety of conditions (7, 24) (J. Cameron, M. Kaufmann, and C. Thompson, unpublished data); however, expression of differential injury is accentuated by conditions of high evaporative demand (i.e., high temperature and irradiance in concert with low RH). Elevated ozone levels in the field produce few or no visible symptoms if ambient air temperatures during and after exposure are below 32 C (7). Because these conditions might also be expected to induce water stress, the present study was initiated to evaluate a possible relationship between factors related to plant water status and differential ozone sensitivity in these two cultivars.

MATERIALS AND METHODS

Plant Material. Zea mays L. (var. Monarch Advance [Northrup, King and Co., Los Angeles, CA] and Bonanza [Ferry Morse Seed Co., Mountain View, CA]) plants were grown from seed in the field and in a glasshouse equipped with evaporative coolers and activated charcoal filters from May through August, 1978. Planting conditions in the field were as described previously (5). Glasshouse plants were grown in 8-liter containers in a peat: sand: soil (1:1:1; v/v/v) potting mix. Nutrient was supplied daily in 500-m1 aliquots as a modified Hoagland solution (16) made up as follows: 1.0 mM KH2PO4; 0.25 mM K2SO4; 4.0 mM MgSO4; 5.25 mM KNO3; 2.6 mM Ca(NO3)2; 2.6 mM Mg(NO3)2; 1.4 mM CaCl2; 10.0 mM H2BO3; 2.0 mM ZnSO4·H2O; 0.1 mM CuSO4·5H2O; 0.03 mM NaMoO4·2H2O; and Sequestrene 330 (Ciba-Geigy Chemical Co.) 0.26 g/l. The solution was adjusted to pH 6.0 with H2SO4. Average day/night temperatures and RH were 32/20 ± 2 C and 55/70 ± 5%, respectively. The photosynthetically active radiation (400 to 700 nm), as measured with a Quantum Sensor (Model LI-1908, Lambda-LICOR Instrument Co., Inc., Lincoln, NE), was approximately 70% of the natural light with an average maximum of 1300 ± 100 μE/m2·s.

All measurements were made at the midpoint (± 5 cm) between the leaf insertion point and the tip, lateral to the midrib, of the 6th leaf (numbered from the base of each plant) of 30-day-old plants. Cultivar differences in foliar ozone sensitivity have been observed for sweet corn plants at this age and leaf position (Cameron, personal communication).

Field root systems were determined from soil blocks cut 30 cm from the shoot axes and at a soil depth of 30 cm.

Microscopy Samples. Stomatal frequency per unit leaf area (1 mm2) and guard cell lengths (μm) were determined from cellulose acetate positives of silicon rubber (RTV-11, General Electric Co., Inc., Wilmington, MA; hardened with Nuocure 28 catalyst) impressions (27).

Gas Exchange Measurements. Gas exchange assessments were made with a portable dual isotope (3H2O and 14CO2) porometer (17) on the abaxial leaf surfaces. A small area (about 1.3 cm2) was exposed to an airstream containing known concentrations of 3H2O vapor and 14CO2 for 30 s. Leaf discs (1-cm diameter) were excised from the exposed area and immersed in 2 ml 80% methanol in water. Alcohol extraction for 24 h removed 95% of the radiotracer from the discs. Chl was bleached from the alcohol extract by sunlight, and the amount of 3H and 14C present was determined by liquid scintillation counting (Beckman LS-9000; Beckman Instruments, Inc., Fullerton, CA) in Handifluor cocktail (Mal-linckrodt, Inc., St. Louis, MO). Conductance to water vapor diffusion and CO2 uptake was calculated and used to compute transpiration (g H2O/dm2·h) and CO2 uptake (mg CO2/dm2·h), according to the methods of Hanscom and Ting (14).

Stomatal resistance (s/cm) to water vapor diffusion was also measured with a diffusion porometer (Model LI-65, Lambda-LICOR Instrument Co., Inc.) on the abaxial and adaxial leaf surfaces. Data were expressed in terms of conductance (cm/s), the reciprocal of the measured resistance.

Leaf temperatures were measured with an IR field thermometer.
(Model PRT-10, Barnes Engineering Co., Stamford, CT) and/or a portable microprobe thermocouple sensor (Model BTA-4, Bailey Instruments, Inc., Saddle Brook, NJ). Humidity measurements were determined with a wet and dry bulb psychrometer (Model CP-147, Environmental Tectonics Corp., Southampton, PA).

**Soil- and Leaf-Water Potentials.** Soil-water potentials were measured with ceramic-capped thermocouple psychrometer probes (Model PT51-10, Wescor, Inc., Logan, VT), located at the center of each soil container at a depth of 15 cm. Leaf-water potentials were measured by the isopiestic technique, using ther- mocouple psychrometers (3). Measurements were made on leaf discs (7-mm diameter) in a constant temperature water bath at 25.5°C.

**Experimental Water Stress.** Water stress was induced in glasshouse-grown plants by three methods. First, nutrient solution was withheld from 25 plants of each cultivar for a period of 4 days, and control plants were maintained on the normal watering regime. Soil- and leaf-water potential and gas exchange measurements were made daily, between 1200 and 1300 h, on five plants of each cultivar. Because sampling was destructive, individual plants were used only once. Following the 4th day, the remaining plants were rewated and evaluated for recovery 48 h later. In the second method, four plants of each cultivar were severed at the base of the stem on 3 successive days at 1200 h. The severed stems were coated with petroleum jelly and held in the original upright position with a ringstand and clamp. Following detachment, we measured the stomatal conductance to water vapor diffusion of the abaxial leaf surface (a) at 2-min intervals for 30 min, (b) once after 40 min, and (c) once after 120 min. For the third method, 10 plants of each cultivar were treated with one of four solutions of PEG (Carbowax 6000, Union Carbide Chemical Corp., New York, NY). The solution concentrations were 0, 100, 150, and 200 g/kg water, and they corresponded to osmotic potentials of 0, −0.12, −0.24, and −0.43 MPa, respectively (21). The solutions were applied in two 1-liter aliquots, 12 and 6 h prior to measuring water potential at 1200 h.

The variations in all data presented are represented as ± SE.

**RESULTS**

**Stomatal Characteristics.** Adaxial leaf surfaces of both cultivars have fewer stomata and greater guard cell dimensions than do the abaxial surfaces, but there are no significant differences (P = 0.05) between cultivars with respect to either characteristic (Table I). In addition, no significant differences in these characteristics are observed between field-grown and glasshouse-grown plants of either cultivar. These results indicate that the known difference in foliar ozone sensitivity in these cultivars is unrelated to either stomatal frequency or guard cell length, per se.

**Root and Shoot Fresh Weights.** Visual comparison of the root systems suggested that these cultivars differed in root mass. Since a previous report (25) had indicated that differences in root mass may contribute to differential ozone sensitivity in tobacco cultivars, we investigated this apparent difference (Table II). Relative to the weight of the shoot tissue, the weight of the root system of Bonanza (ozone-resistant) is significantly smaller (P = 0.02) than that of Monarch Advance (ozone-sensitive) in both glasshouse- and field-grown plants. Field-grown plants of both cultivars have higher fresh weight ratios than those observed for glasshouse-grown plants. This difference reflects both a more vigorous shoot growth and an incomplete recovery of root material for field-grown plants. We estimate a 70% recovery of total root mass by the present field methods. Our estimate is based on a previous report (12) of the lateral and vertical distribution of root material in corn plants of a similar age.

**Leaf-Water Potentials.** The midday leaf-water potentials of Bonanza are significantly lower (P = 0.01) than those of Monarch Advance in both glasshouse- and field-grown plants (Table II).

**Stomatal Conductance to Gas Exchange.** Typical diurnal changes in stomatal conductance to water vapor diffusion for field-grown plants are presented in Figure 1, along with changes in meteorological conditions. These conductances are based on data obtained from 10 plants of each cultivar on two separate occasions. On each occasion, the meteorological conditions were similar and the peak ozone concentrations were low to moderate (0.1−0.15 μL/L; 1200 to 1300 h). Measurements prior to 0800 h were prevented by the presence of dew on the leaves. Initially, the abaxial surface conductances of both cultivars are high; but they decrease as irradiance increases, increase to high values at midday, and finally decrease in the afternoon with decreasing irradiance (Fig. 1, A and B). Stomatal surface conductances are lower than those observed for the abaxial surfaces, presumably reflecting a lower stomatal frequency on the upper leaf surfaces (Table I). The adaxial surface conductances of both cultivars increase and decrease corresponding to changes in irradiance (Fig. 1C). Total leaf conductances are calculated from data presented in Figure 1 (A, B, and C) assuming parallel conductance for each leaf surface (Fig. 1D). Total conductances at midday are in the range of 0.55 to 0.65 cm/s for both cultivars. Similar values have been recorded for corn in the field under nonstress conditions (8) and in numerous growth room studies (1, 3, 10).

Data do not indicate differences which can be related directly to ozone sensitivity. However, a midmorning depression in abaxial leaf surface conductance can be observed 1 h earlier in Monarch (1030 h) than it can in Monarch Advance (1130 h) (Fig. 1, A and B). Similar observations were made on numerous occasions in 1977 and 1978 (data not presented). We assume that this depression in conductance reflects a decrease in water potential resulting from the lag time between water absorption from the soil and transpiratory water loss from the leaves (19). Thus, the earlier observed depression in conductance for Monarch is consistent with a higher resistance to water movement in this cultivar’s water-conducting tissues.

**Table I. The Stomatal Frequency and Guard Cell Length of the Sweet Corn Hybrids, Bonanza and Monarch Advance**

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<tr>
<th>Stomatal Frequency</th>
<th>Guard Cell Length</th>
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<tbody>
<tr>
<td></td>
<td>Glasshouse-grown</td>
</tr>
<tr>
<td>Adaxial surface</td>
<td>Abaxial surface</td>
</tr>
<tr>
<td>1/μm²</td>
<td>51.0 ± 1.2</td>
</tr>
<tr>
<td>Bonanza</td>
<td>56.2 ± 1.2</td>
</tr>
<tr>
<td>Monarch Advance</td>
<td>51.0 ± 1.0</td>
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Each value is the mean ± SE of ten microscope fields (200X) per leaf for five leaves. Samples were taken on the sixth leaf numbered from the base of 30-day-old plants.
The diurnal changes in stomatal conductance to water vapor diffusion for glasshouse-grown plants follow patterns similar to those observed in the field, but the conductance values of both leaf surfaces are greater in both cultivars. Midday conductances ranged from 0.71 to 0.77 cm/s (abaxial) and 0.53 to 0.66 cm/s (axial) and correspond to a range in total conductance of 1.24 to 1.43 cm/s for both cultivars. The data do not indicate differences which can be related to ozone sensitivity.

Midday transpiration rates (g H$_2$O/dm$^2$-h) and CO$_2$ uptake (mg CO$_2$/dm$^2$-h) are presented in Table II. Although Bonanza apparently has higher transpiration and CO$_2$ uptake rates under both growth conditions, the differences are not significant (P = 0.05).

**Response to Water Stress.** Following the cessation of watering, both the soil-water potential in the root zone and the midday leaf-water potential decline in each cultivar (Fig. 2, A and B). During the first 3 days of desiccation, Bonanza has higher soil-water potentials and lower leaf-water potentials than those observed for Monarch Advance. This trend is reversed on the 4th day. A plot of leaf-water potential versus soil-water potential (Fig. 3; generated from data presented in Fig. 2, A and B) indicates that, in the soil-water potential range of −0.15 to −0.40 MPa, Bonanza has consistently lower leaf-water potentials than does Monarch Advance. In addition, these data indicate that, under mild stress, the decline in midday leaf-water potential for a given decline in soil-water potential is greater in Bonanza than it is in Monarch Advance (slope of lines in Fig. 3).

The abaxial leaf surface conductance to water vapor diffusion declines with a decrease in leaf-water potential (Fig. 4). Changes in conductance indicate two distinct regimes in leaf-water relations following the imposition of water stress: (a) a linear decline in conductance with declining leaf-water potentials at potentials above −0.60 (Monarch Advance) to −0.80 (Bonanza) MPa (Fig. 4A); and (b) a dramatic drop in conductance at a critical leaf-water potential of approximately −0.65 MPa for Monarch Advance and −0.85 MPa for Bonanza (Fig. 4B). From a leaf-water potential of −0.35 to −0.60 MPa for Monarch Advance and −0.35 to −0.80 MPa for Bonanza, the conductance decreases to approximately 50% of the observed maximum (a decrease of 0.70 cm/s from a maximum of 1.4 cm/s). However, at the lower leaf-water potential, a further decline in leaf-water potential of 0.05 MPa in each case resulted in conductances one order of magnitude smaller.

These results indicate a progressive stomatal closure with declining leaf-water potential up to a critical potential, at which the stomata closed tightly. In conjunction with the previously described relationships between soil- and leaf-water potentials, these data indicate that, at the same soil-water potential (within the potential range of −0.15 to −0.40 MPa), both cultivars have similar leaf conductances but significantly different leaf-water potentials.

A comparison of the reduction in leaf-water potential following the cessation of watering (Fig. 3) and following PEG application (Fig. 5) indicates a similar relationship between leaf-water potential and soil-water potential for both treatments. Applying a 150 g/kg water solution of PEG (osmotic potential of −0.24 MPa) results in a leaf-water potential of −0.81 MPa for Bonanza and −0.68 MPa for Monarch Advance. A comparable water stress induced by a decrease in substrate moisture to −0.24 MPa results in a leaf-water potential of −0.86 MPa for Bonanza and −0.65 MPa for Monarch Advance. These results indicate that PEG application is suitable for rapidly inducing water stress to desirable levels. Furthermore, the resultant declines in leaf-water potential following PEG application are comparable to those resulting from cessation of watering.

A significant difference in the rate of decline in abaxial leaf surface conductance to water vapor diffusion following root detachment is also observed between cultivars (Fig. 6). Although
The diurnal changes in stomatal conductance to water vapor diffusion and in meteorological conditions. A, Abaxial leaf surface conductance for Bonanza (ozone resistant). Data taken with a diffusion porometer (■) and an isotope porometer (△). B, Abaxial leaf surface conductance for Monarch Advance (ozone sensitive). Data taken with a diffusion porometer (■) and an isotope porometer (△). C, Adaxial leaf surface conductance for Bonanza (■) and Monarch Advance (○). Data taken with a diffusion porometer. D, Total leaf conductance for Bonanza (■) and Monarch Advance (○). E, Meteorological conditions: photosynthetically active radiation (○); air temperature (△); and RH (■). The conductance values represent the mean ± se of data taken on the 6th leaf, numbered from the base of the plant, of plants grown in the field for 30 days. The peak ambient ozone concentration was low to moderate (0.1–0.15 μL/L) on each sampling date and occurred between 1200 and 1300 h (data provided by the Statewide Air Pollution Research Center, Riverside, CA).

Fig. 2. Leaf-water potential (A) and soil-water potential (B) of sweet corn hybrids following the cessation of watering. Each value represents the mean ± se of data for five glasshouse plants of Bonanza (■) and Monarch Advance (○) at 30 days of age. Values for control plants which were watered daily were not significantly different from the values for day 1. The test plants were rewated following day 4.

Fig. 3. The relationship between soil-water potential and leaf-water potential for two sweet corn hybrids. Each value represents the mean ± se of data taken for five glasshouse plants of Bonanza (■) and Monarch Advance (○) at 30 days of age.

Initial conductances are not significantly different, the conductance of Bonanza declines to approximately 40% of its original value within 10 min of detachment, whereas the conductance of Monarch Advance remains at nearly 90% of its original value. While this differential response may be attributed to a variety of causes, these results suggest a greater tendency, in the leaves of Bonanza, for stomatal closure with increasing water stress (cf. Fig. 4).
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**DISCUSSION**

Foliar ozone sensitivity in the two sweet corn cultivars examined is unrelated to stomatal frequency per unit leaf area and/or guard cell length, per se (Table I). In addition, inherent gas exchange characteristics do not appear to determine foliar ozone sensitivity (Fig. 1). In this respect, the results differ from those reported for other species, where intraspecies differences in ozone sensitivity were associated with stomatal characteristics and/or stomatal conductance to gas exchange (15). However, the results do indicate a possible relationship between differential response to water stress and foliar ozone sensitivity.

Stomatal closure associated with declining leaf-water potential was observed in both cultivars (Fig. 4A). These results are comparable to the relative decline in stomatal conductance reported by Boyer (2) for the same range in leaf-water potential. In the present study, the stomata of both cultivars were also observed to close tightly at a critical leaf-water potential (Fig. 4B). Rapid stomatal closure with increasing water stress has been observed by a number of workers (1, 10, 23) to occur over a narrow range of leaf-water potential in corn. The critical leaf-water potential reported by these workers was within the range of -0.90 to -1.0 MPa, a value slightly lower than that observed in the present study. However, it has been demonstrated clearly that the leaf-water potential at which the stomata close is not unique for a given species but depends on the stage of development (13), growth conditions (18), and the stress history (4).

At leaf-water potentials higher than the one critical for stomatal closure, a given decline in the leaf-water potential resulted in a slightly greater decrease, for Monarch Advance over Bonanza, in stomatal conductance to water vapor diffusion (Fig. 4A). Furthermore (see Fig. 4B), the critical leaf-water potential occurred for Monarch Advance at a value 0.20 MPa higher than that observed for Bonanza. Taken alone, these observations might be considered evidence for a greater degree of water stress tolerance in Bonanza, *i.e.* a higher stomatal conductance at a lower leaf-water potential. However, a comparison of the relationship between soil- and leaf-water potential (Fig. 3) demonstrates that, over a wide range in soil-water potential, the leaf-water potential of Bonanza is 0.20 MPa lower than that observed for Monarch Advance. Therefore,

**Fig. 5.** The effect of treatment with solutions of PEG-6000 on leaf water potential for sweet corn hybrids, Bonanza (•) and Monarch Advance (○). Corresponding osmotic potentials are 0, -0.12, -0.24, and -0.41 MPa (21). Each value represents the mean ± SE of data for 10 glasshouse plants at 30 days of age.

**Fig. 6.** The decline in leaf conductance to water vapor diffusion following the root detachment for sweet corn hybrids, Bonanza (•) and Monarch Advance (○). Conductance values at time 0 for both cultivars were not significantly different and were in the range of 0.70 to 0.75 cm/s. Each value represents the mean ± SE of data for 12 glasshouse plants at 30 days of age.
any apparent tolerance suggested by higher stomatal conductance at lower leaf-water potentials is negated by lower comparable leaf-water potentials at similar soil-water potentials.

The lower leaf-water potentials observed for Bonanza at similar soil-water potentials indicate that the two cultivars differ in their capacities to maintain water supply to the leaves. The difference may reflect a higher resistance to water movement in the roots and/or shoots of Bonanza. Assuming that the smaller comparable root fresh weights for Bonanza (see “Results”) imply a smaller root surface area, a higher root resistance to water uptake might be anticipated due to a greater transpirational water flux per unit surface area of root. It has been suggested (26) that a greater transpirational water flux per unit surface area of root results in perirhizal water-potential gradients which decrease the capacity for water extraction from the soil. In addition, the more rapid decline in stomatal conductance to water vapor diffusion following the root detachment (Fig. 6) may indicate that a higher resistance to water movement also occurs in the water-conducting tissues of the stems and/or leaves of Bonanza.

Dube et al. (10, 11) have reported cultivar differences in resistance to water movement in corn. These workers described two inbreds that exhibited different phenotypic responses to water stress. One was observed to wilt under field conditions, whereas, the other exhibited ‘at least some’ heat and drought resistance under field conditions. Dube et al. (10) observed that the change in leaf-water potential, as a function of time and decreasing soil-water potential, differed markedly. Following the cessation of watering, the leaf-water potential of the wilting inbred decreased more rapidly. This difference was subsequently related to a higher resistance of water movement in the water-conducting tissue of the wilting inbred (11). Our results may represent a similar, but less extreme, difference in resistance to water movement.

Our observations on shoot-to-root fresh weight ratios (see “Results”) are similar to those reported by Ting and Dugger (25) for tobacco cultivars, Bel-B and Bel-W3; a smaller comparable root mass was observed for the ozone-resistant cultivar, Bel-B. More recently, evidence using reciprocal grafts (22) has refuted the importance of root system characteristics as partial determinants of foliar ozone sensitivity in these tobacco cultivars. However, differential injury expression in sweet corn, in contrast to tobacco, is much more contingent upon environmental conditions which would be expected to induce a water stress (7). Therefore, our observations suggest that root and water delivery system characteristics play a major contributory role in the determination of foliar ozone sensitivity in this species. Our preliminary data (not presented) indicate that Bonanza may be predisposed to stomatal closure when exposed to ozone under conditions of water stress. This response would be expected to afford a degree of protection via a higher impedance to the further entry of pollutant (20). Other unpublished results indicate that these cultivars also differ in the mesophyll cell surface area exposed to the intercellular spaces which could likewise affect the uptake of pollutant and further alter cultivar sensitivity to ozone.

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**LITERATURE CITED**