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Water Status of Drought-Resistant and Drought-Sensitive Sorghum Treated with Ethephon

J. Zhang* and M. B. Kirkham

Evapotranspiration Laboratory, Waters Annex, Department of Agronomy, Kansas State University, Manhattan, Kansas 66502, USA

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Abstract. The effects of ethephon on stomatal resistance, water potential, osmotic potential, turgor potential, and ethylene production were determined on leaves of a drought-resistant (KS 65) and a drought-sensitive (IA 25) genotype of sorghum [*Sorghum bicolor* (L.) Moench] grown under well-watered or drought-stressed conditions. With both sufficient and limited water supply, ethephon had no effect on the adaxial, abaxial, or total stomatal resistance of either genotype. For both water treatments, the adaxial stomatal resistance of the drought-sensitive genotype was higher than that of the drought-resistant genotype. Ethephon increased the amount of ethylene produced by the plants under both levels of water. For plants with sufficient water, water potentials of both genotypes were lowered by ethephon. Ethephon had no effect on the water potentials under drought or on the osmotic potentials under either water regime. With drought, the turgor potential of the drought-sensitive genotype, but not that of the drought-resistant, was increased by ethephon.

Drought has been shown to increase ethylene production in plants previously studied (Yang and Hoffman 1984). However, little work has been done to investigate the effect of ethylene on plant–water relations (stomatal resistance, water potential, osmotic potential, turgor potential). Even less has been done to determine if drought-resistant plants respond differently to ethylene than drought-sensitive plants. Kirkham (1983) found that foliar treatments with ethephon, an ethylene-releasing compound (Beaudry and Kays 1988), increased the stomatal resistance of both a drought-sensitive and a drought-resistant winter wheat (*Tritrium aestivum* L.) cultivar. Water and osmotic potentials of the drought-resistant plants were lowered by ethephon, but ethephon did not affect the water or osmotic potentials of the drought-sensitive plants. Other than this experiment with wheat, there appears to be no work relating the effect of ethylene on the plant–water relations of genotypes varying in drought resistance. Drought resistance may be regulated by hormones, like ethylene, which act at the gene level (Broglie et al. 1989). Therefore, it is important to determine if genotypes differing in drought resistance also differ in their response to ethylene. The objective of this experiment was to determine if a drought-resistant genotype of sorghum had a different water status than a drought-sensitive genotype of sorghum, when both genotypes were treated with ethephon and grown under well-watered or drought-stressed conditions. In addition, the effect of ethephon on the evolution of ethylene by the two genotypes was determined.

Materials and Methods

Plant Culture

The experiment was conducted during the spring in a greenhouse at Kansas State University, Manhattan, Kansas. The temperature and humidity varied from 25–40°C and 8–70%, respectively. No extra light was provided for the plants.

The two genotypes of sorghum [*Sorghum bicolor* (L.) Moench] used in the study were KS 65 (drought resistant) and IA 25 (drought sensitive) (Gaosegelwe 1988, Kirkham 1988, Majerus 1987). The plants were grown in 24 plastic pots (15 cm diameter; total volume 2700 cm^3; no drainage holes) filled with a commercial greenhouse mixture (Sunshine Mix, Swecker Knipp, Inc., Topeka, Kansas, USA) at pH 5.5. The composition of the mix

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*Present address: Hebei Academy of Agricultural and Forestry Sciences, 24 Airport Road, Shijiazhuang, Hebei, People's Republic of China.

(μg/g) was as follows: NO_3–N, 42; available P, >100; and exchangeable K, 300. Twelve pots were seeded with KS 65 and 12 with IA 25 on April 5, 1988. Plants were thinned to 13 per pot 5 days after emergence.

Ethephon Treatment

On day 18 after planting (April 23), days 19–21, the upper surfaces of leaves in half of the pots (six pots with KS 65; six pots with IA 25) were sprayed with a 100 mg/L solution of 2-chloroethyl phosphonic acid (lot no. 106F-0121; Sigma Chemical Co., St. Louis, Missouri, USA) to the run-off point, 125 ml being applied each day. No attempt was made to prevent run-off into the potting mix. The upper surfaces of the leaves in the other half of the pots were sprayed with distilled water.

Water-Stress Treatment

Eighteen days after planting (April 23), all pots were watered with tap water until the soil throughout the pot was wet. Subsequently, 12 pots were watered daily with 150 ml/pot tap water, whereas the remaining 12 pots received no water until the end of the experiment (May 2, day 27).

Methods for Measurement of Water Stress

Stomatal resistance on both surfaces of a recently matured leaf of two plants in each pot was measured daily on days 18–26 after planting between 9:00 and 10:00 a.m. using a diffusion porometer (Model LI-700, Li-Cor, Inc., Lincoln, Nebraska, USA). The adaxial and abaxial stomatal resistances were measured separately on adjacent portions of the same leaf. Resistances of the adaxial and abaxial stomata were considered to act in parallel (Kramer 1983), and the total stomatal resistance of the leaf (r_{leaf}) was calculated by $1/r_{leaf} = 1/r_{ad} + 1/r_{ab}$, where r_{ad} and r_{ab} are the resistances of the adaxial and abaxial surfaces, respectively.

After stomatal resistance was measured (and day 27 after planting), a 6.4-mm diameter leaf disc (one/treatment) was placed in a thermocouple-psychrometer chamber (Model 75-3AC; J.R.D. Merrill Specialty Equipment, Logan, Utah, USA) and after 3 h the water potential was obtained using a microvoltmeter (Model HR-33T Dew Point Microvoltmeter, Wescor, Inc., Logan, Utah, USA). The tissue was frozen, thawed, and equilibrated again in the thermocouple-psychrometer chamber to determine the osmotic potential. Turgor potential was calculated as the difference between osmotic and water potential. The matric and gravitational potentials, also components of the water potential, were considered to be negligible (Kirkham 1990).

Determination of Leaf Ethylene Production

Ethylene production was measured 18, 20, 22, and 24 days after planting and expressed on a fresh-weight basis [nl (g fresh weight)$^{-1}$]. Intact leaves (1 g fresh weight) were placed in test tubes (15 ml) and stopped with rubber caps. Ethylene in the gas phase of the enclosed tubes was determined from a 1-ml sample withdrawn with a hypodermic syringe 2 h after the test tubes were capped. Ethylene was assayed using a gas chromatograph (Series 2400, Varian, Walnut Creek, California, USA). The carrier gas was nitrogen at a flow rate of 30 ml/min. The flame gases were air at 240–300 ml/min and hydrogen at 30 ml/min. The injector and detector temperatures were 145–150°C. The 3-m stainless steel column was packed with Porapak R (Sigma Chemical Co.) and maintained at 65–70°C. Ethylene standards from Fisher Scientific (St. Louis, Missouri, USA) ran through this column 2–3 min after injection. Concentrations of ethylene in the samples were determined by measuring peak heights and comparing these with known concentrations of the ethylene standards.

Statistics

There were three replications for each of the eight treatments (with and without ethephon; two cultivars; two watering regimes) arranged in a completely random design. Each value reported for stomatal resistance is the mean and standard deviation of six measurements (two leaves per pot × three replications). Eight water-potential and eight osmotic-potential measurements were made daily, each selected randomly from a different treatment. Values obtained every 2 days for water potential, osmotic potential, and turgor potential were averaged together to give a mean and standard deviation. Values for ethylene production are the means of three replications and standard deviations were determined.

The *t* test (Steel and Torrie 1980) was used to compare means of KS 65 with and without ethephon and means of IA 25 with and without ethephon, and to determine if ethephon had a significant effect on the measurements.

Results

Adaxial Stomatal Resistance

The drought-sensitive genotype (IA 25) (± ethephon) had a higher adaxial stomatal resistance than did the drought-resistant genotype (KS 65) (± ethephon) under water-sufficient conditions (Fig. 1A and B). Ethephon had no effect on adaxial stomatal resistance. Results for water-stressed plants (Fig. 1C and D) were similar to those for well-watered plants, except that resistances increased to higher values (>60 s/cm) as the plants became drought stressed.

Abaxial Stomatal Resistance

The abaxial stomatal resistance of drought-resistant plants was similar to that of drought-sensitive plants (± ethephon) under well-watered conditions (data not shown). Ethephon had no effect on the abaxial stomatal resistance under either well-watered or water-stressed conditions. Maximum abaxial stomatal resistances under water-stressed conditions were about four times those under well-watered conditions (7–9 s/cm vs. 25–35 s/cm, respectively).

Total Stomatal Resistance

Under both water treatments, the drought-resistant

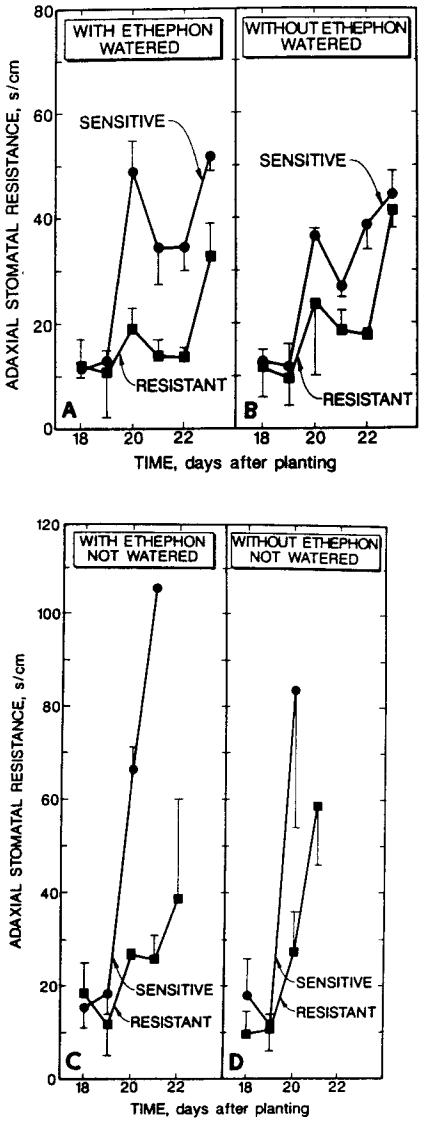

Fig. 1. Effect of ethephon on the adaxial stomatal resistance of a drought-resistant (KS 65) and a drought-sensitive (IA 25) genotype of sorghum. **(A, B)** Watered plants; **(C, D)** water-stressed plants. Under water-stressed conditions, values are missing because plants died. The means ± SD from three plants are shown. Only half of the SD bar is drawn for easier viewing of the figure. Symbols with no bar represent only one value without the mean.

and drought-sensitive genotypes had similar stomatal resistances (data not shown). Ethephon did not affect total stomatal resistance. The higher adaxial stomatal resistance of the drought-sensitive genotype did not result in a higher total stomatal resistance, because the adaxial stomatal resistance for all plants was greater than the abaxial resistances, which were in parallel ($1/r_{ad} + 1/r_{ab}$). The smaller resistance (the abaxial one, in the case of sorghum) dominates the total stomatal resistance.

Water and Osmotic Potentials

Plants of both genotypes treated with ethephon had a lower water potential than nonethephon treated plants under watered conditions (difference significant at the 0.01 and 0.05 level for KS 65 and IA 25, respectively) (Fig. 2, see dark symbols). Under drought, the water potential of the plants treated with ethephon was not significantly different from those plants without ethephon (Fig. 2C and D). Under both water treatments, the osmotic potential of ethephon-treated plants did not differ significantly from that of controls (Fig. 2, see light symbols).

Under well-watered conditions, water and osmotic potentials of the two genotypes were similar (Fig. 2A and B). However, water-deficient IA 25 had lower water and osmotic potentials than KS 65 (Fig. 2C and D).

Turgor Potential

Under well-watered conditions, the turgor potential of the two genotypes was similar both with and without ethephon (Fig. 3A and B). However, under drought, the turgor potential of IA 25 plus ethephon was greater than that of IA 25 minus ethephon (difference significant at the 0.05 level) (Fig. 3C and D). Ethephon did not affect the turgor potential of drought-stressed KS 65. The turgor potential of the two genotypes (−ethephon) was similar under water-stressed conditions (Fig. 3D).

Ethylene Production

Under well-watered conditions, the drought-sensitive and drought-resistant genotypes released similar amounts of ethylene, with or without ethephon treatments (Fig. 4A and B). However, more ethylene was produced by both genotypes when treated with ethephon than when not treated (difference significant at the 0.01 level for both genotypes).

Under water-stressed conditions, the drought-sensitive and drought-resistant genotypes also gave off similar amounts of ethylene, both when treated and not treated with ethephon (Fig. 4C and D). Again, both genotypes emitted more ethylene when treated with ethephon (days 18–21) than when not treated.

Discussion

The results for adaxial stomatal resistance are at variance with those of Kirkham (1983), who found

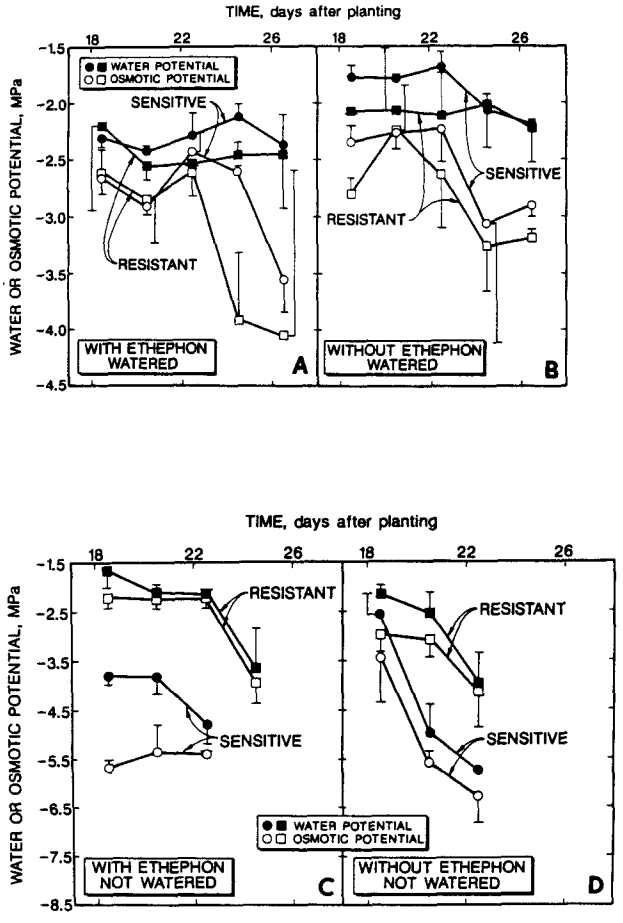

Fig. 2. Effect of ethephon on the water and osmotic potentials of a drought-resistant (KS 65) and a drought-sensitive (IA 25) genotype of sorghum. (A, B) Watered plants; (C, D) water-stressed plants. For vertical bars, see legend of Fig. 1.

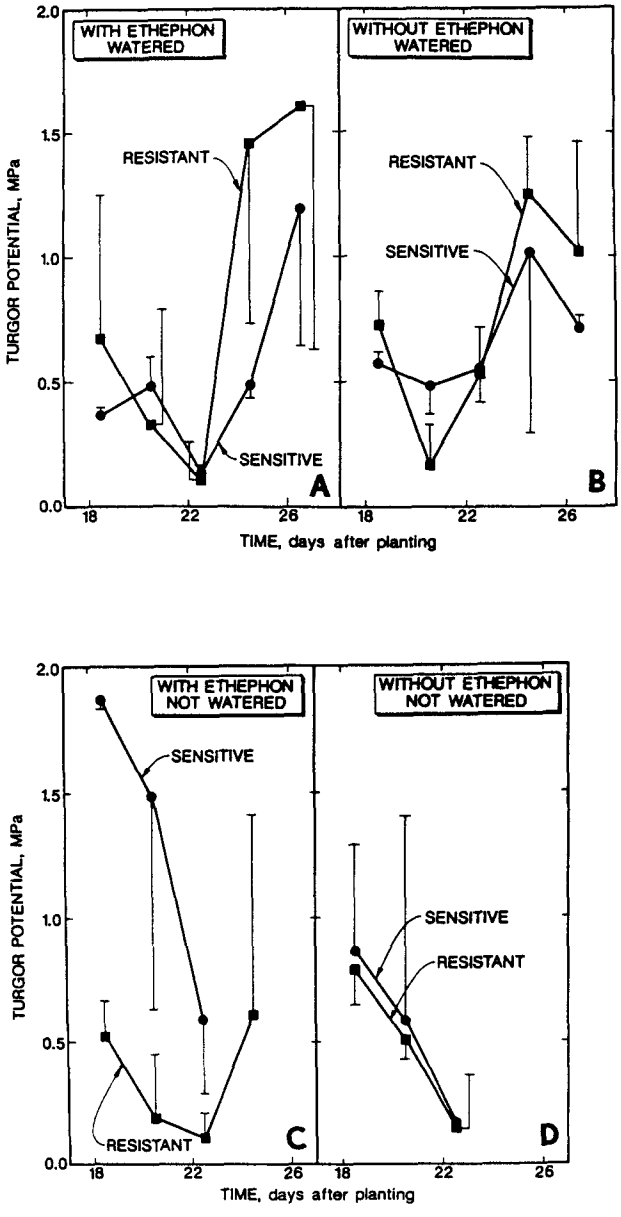

Fig. 3. Effect of ethephon on the turgor potential of a drought-resistant (KS 65) and a drought-sensitive (IA 25) genotype of sorghum. (A, B) Watered plants; (C, D) water-stressed plants. For vertical bars, see legend of Fig. 1.

that both drought-resistant and drought-sensitive wheat plants treated with ethephon had a higher adaxial stomatal resistance than control plants. (Adaxial stomatal resistance of drought-sensitive wheat was similar to that of drought-resistant wheat.) Wheat leaves may be more sensitive to ethephon, since more stomata occur on the adaxial surface than on the abaxial surface (Teare et al. 1971). However, sorghum has more stomata on the abaxial surface than on the adaxial surface (Liang et al. 1975). Future studies are needed to determine if ethephon treatment on the abaxial surface of sorghum leaves would increase abaxial stomatal resistance. When using ethephon, perhaps the leaf surface with the most stomata should be treated to insure that the spray has an effect (e.g., increased stomatal resistance). Differential effects of ethephon on adaxial and abaxial resistance may be due to poor distribution or penetration of the material because of the lack of surfactant or adjuvant. With adequate uptake and distribution, resistances might be similarly affected. Nevertheless, the results suggested that the higher adaxial stomatal resistance of the drought-sensitive sorghum, compared to the drought-resistant sorghum, was not caused by ethylene.

Ethephon increased the amount of ethylene produced by the plants. Others also have shown that ethephon stimulates ethylene production (Robinson

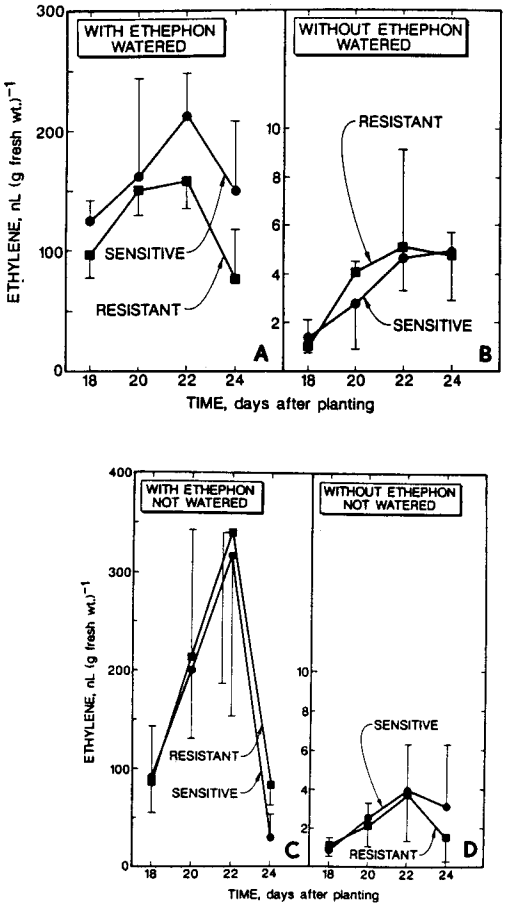

Fig. 4. Effect of ethephon on the production of ethylene by a drought-resistant (KS 65) and a drought-sensitive (IA 25) genotype of sorghum. **(A, B)** Watered plants; **(C, D)** water-stressed plants. For vertical bars, see legend of Fig. 1.

1983, Yamamoto and Kozlowski 1987). Ethephon decomposes into ethylene (Audley et al. 1976) so when ethephon was applied to plants between days 18–21 after planting, ethylene production increased (Fig. 4). However, when the sprays were discontinued, ethylene evolution declined as has been observed by others (Van Andel and Verkerke 1978).

Kirkham (1983) found under both watered and water-stressed conditions that water and osmotic potentials of a drought-resistant wheat were lower with ethephon than without ethephon. Ethephon did not affect the water or osmotic potentials of drought-sensitive wheat. In the current study, ethephon lowered the water potential of both genotypes, but only under well-watered conditions. The results of the present experiment and those of Kirkham (1983) show that drought-resistant and drought-sensitive genotypes of wheat and sorghum respond differently to ethephon.

Even though water and osmotic potentials of the sensitive genotype (IA 25) under drought stress were not significantly affected by ethephon, when the two measurements were taken together to obtain turgor potential, the difference was significant. The water potential is the sum of four components (Kirkham 1990):

$$\psi = \psi_s + \psi_p + \psi_m + \psi_g \quad (1)$$

where ψ is the water potential, ψ_s is the osmotic (solute)-potential component, ψ_p is the pressure (turgor)-potential component, ψ_m is the matric component, and ψ_g is the component due to gravity. The matric potential and the gravitational potential are usually neglected and Eq. (1) reduces to the classical equation of plant physiology, as follows:

$$DPD = OP - TP \quad (2)$$

where the diffusion pressure deficit (DPD) is a measure of the water potential, OP is the osmotic pressure, and TP is the turgor pressure. The water potential can remain constant, even though the components of water potential change, which apparently occurred in this experiment. As stated in Materials and Methods, when turgor potential was calculated, matric and gravitational potentials were disregarded. In the current study, gravitational potential would not be important because the plants were too short for gravity to have a measurable effect. (If a plant is 1020 cm tall, the potential from top to bottom varies by 1 bar = 0.1 MPa.) The matric component of the water potential is due to capillary or adsorption forces, such as those in the cell wall (Kirkham 1990). It may be that the matric potential was not negligible. Ethylene affects cellulose microfibril orientation in the cell wall (Eisinger 1983), and cell walls are an important factor in drought resistance. Drought-resistant plants have been shown to have thick cell walls (Kirkham 1990). The cell wall thickness of the two genotypes in this experiment was not measured. However, the results suggested that wall thickness might differ, which could create differences in matric potential between the two genotypes. Further work is needed to determine the effect of ethephon (ethylene) on matric potential of plants.

References

Audley BG, Archer BL, Carruthers IB (1976) Metabolism of ethephon (2-chloroethylphosphonic acid) and related compounds in *Hevea brasiliensis*. Arch Environ Contamin Toxicol 4:183–200

Beaudry RM, Kays SJ (1988) Application of ethylene-releasing compounds in agriculture. In: Neumann PM (ed) Plant

growth and leaf-applied chemicals. CRC Press, Boca Raton, pp 127–155

Broglie KE, Biddle P, Cressman R, Broglie R (1989) Functional analysis of DNA sequences responsible for ethylene regulation of a bean chitinase gene in transgenic tobacco. Plant Cell 1:599–607

Eisinger W (1983) Regulation of pea internode expansion by ethylene. Annu Rev Plant Physiol 34:225–240

Gaosegelwe PL (1988) Evaluation of different sorghum genotypes for drought resistance. MS Thesis, Manhattan, Kansas

Kirkham MB (1983) Effect of ethephon on the water status of a drought-resistant and a drought-sensitive cultivar of winter wheat. Z Pflanzenphysiol 112:103–112

Kirkham MB (1988) Hydraulic resistance of two sorghums varying in drought resistance. Plant Soil 105:19–24

Kirkham MB (1990) Plant responses to water deficits. In: Stewart BA, Nielsen DR (eds) Irrigation of agricultural Crops. Amer Soc Agron, Crop Sci Soc Amer, Soil Sci Soc Amer, Madison, Wisconsin, pp 323–342

Kramer PJ (1983) Water relations of plants. Academic Press, New York

Liang GH, Dayton AD, Chu CC, Casady AJ (1975) Heritability of stomatal density and distribution on leaves of grain sorghum. Crop Sci 15:567–570

Majerus WJ (1987) Comparison of screening techniques for drought tolerance in grain sorghum. MS Thesis, Manhattan, Kansas

Robinson M (1983) Influence of abscisic acid and ethylene on assimilate distribution in *Gladiolus grandiflorus*. Ann Bot 51:779–785

Steel RGD, Torrie JH (1980) Principles and procedures of statistics, 2nd edn. McGraw-Hill, New York, pp 102–104

Teare ID, Peterson CJ, Law AG (1971) Size and frequency of leaf stomata in cultivars of *Triticum aestivum* and other *Triticum* species. Crop Sci 11:496–498

Van Andel OM, Verkerke DR (1978) Simulation and inhibition by ethephon of stem and leaf growth of some Gramineae at different stages of development. J Exp Bot 29:639–651

Yamamoto F, Kozlowski TT (1987) Effects of flooding, tilting of stems, and ethrel application on growth, stem anatomy and ethylene production of *Pinus densiflora* seedlings. J Exp Bot 38:293–310

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35:155–189