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# Carbon Dioxide Exchange in Water-stressed Sorghum[1]

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ABSTRACT

Carbon dioxide exchange was monitored in potted RS 610 sorghum [*Sorghum bicolor* (L.) Moench] in a growth chamber as it dried out from a freshly watered condition to severe water stress. Carbon dioxide uptake in the light decreased to near the compensation point at water potentials near —25 atmospheres. Leaf resistance to gaseous exchange was high at this point. Evolution of $CO_2$ in the light occurred as stress became greater. Dark evolution of $CO_2$ was higher at water potentials near —20 atmospheres than in either the more severely stressed or non-stressed condition. The activity of phosphoenolpyruvic carboxylase remained comparatively high as water stress increased.

*Additional key words:* $CO_2$ exchange, Photosynthesis, Respiration, Leaf resistance, Phosphoenolpyruvic carboxylase.

SORGHUM is a crop known for its ability to persist under heat and moisture stress and often to recover once stresses are alleviated. Mechanisms which permit sorghum plants to survive water stress conditions are not well understood.

Results of studies regarding the influence of water stress on photosynthesis and respiration are varied and inconclusive. Dastur (1925) and Slavik (1963) and (1965) found a direct correlation between photosynthesis and hydration of the plant tissue. Chrelashvili (1941) found in all species which he tested that a certain degree of mild water stress (the degree varying among species) caused $CO_2$ uptake to be at a maximum; after that point it decreased. Veihmeyer (1956) concluded that $CO_2$ uptake was not affected by water stress until permanent wilting percentage was reached. Kramer (1963) questioned Veihmeyer's theories and pointed out that soil water is not equally available to plants over the range of moisture equivalent to wilting percentage.

One complicating factor in studying the effect of water stress on $CO_2$ uptake or evolution is stomatal closure. Vaadia, Raney and Hagen (1961) found that stomatal closure at low light intensities had little effect on rate of $CO_2$ uptake, whereas under high light intensity, such as field conditions, diffusion rate of $CO_2$ was limiting. Similar conclusions were drawn from studies by Nir and Poljakoff-Mayber (1967) and Shimshi (1963). However, Troughton (1969) found that photosynthetic rate reduction in cotton leaves was due solely to stomatal closure until severe stress occurred.

Enzymatic reactions are also greatly affected by water balance according to Crafts et al. (1949). Hydrolytic reactions often increase while synthetic reactions decrease with increasing stress.

In order to compare results of water stress effects on physiologic rates among species, Brix (1962) emphasized the importance of determining plant water stress in terms of the physical status of water. Water potential ($\Psi$), a thermodynamic term proposed by Slatyer and Taylor (1960), is used in this paper to describe water status of the plant tissue.

This investigation was initiated to partially characterize the effect of increasing water stress on photosynthesis and respiration in sorghum and on activity of phosphoenolpyruvic (PEP) carboxylase, the primary carboxylating enzyme (Hatch and Slack, 1966; Slack and Hatch, 1967).

## MATERIALS AND METHODS

*Sorghum bicolor* (L.) Moench, hybrid 'RS 610' was used since it is widely grown and has shown a degree of heat tolerance which is a characteristic often found associated with drought resistance (Sullivan et al. 1968). Two plants per 18-cm pot were grown and tested in a growth chamber at 29 C and 40% relative humidity. A 12-hour light and 12-hour dark cycle was used. Two plants per pot at the 8 to 9 leaf stage, treated as one experimental unit, were enclosed (aerial portion) in a plexiglass chamber (26 liters volume). Light intensity within the plexiglass plant chamber in a horizontal plane at the top leaf level was $37.4 \times 10^{-4}$ microeinsteins $cm^{-2}$ $sec^{-1}$ (400-700 m$\mu$ range).

Air was circulated within the chamber by a fan with a capacity of 190 liters per minute (lpm) and flowed through the chamber at 25 to 40 lpm. Air was sampled every 15 or 30 minutes and analyzed in a Beckman differential infrared gas analyzer (IRGA) according to the method of Hesketh and Moss (1963). Leaf temperatures were measured with thermocouples and ranged from 30 to 34 C.

Leaf area was determined three times during the experiment, because of the gradual reduction of living leaf tissue induced by water stress. The total leaf area per plant was estimated from leaf length multiplied by the greatest width multiplied by 0.68 (a factor derived from leaf area and length $\times$ width measurements).

Plant water potential was determined by use of a Spanner (1951) type thermocouple psychrometer similar to that described by Box (1965). Water potential determinations were made from 1-cm-diameter samples cut from the widest part of the longest leaf on each plant. Water potential measurements listed were taken at the end of each light or dark $CO_2$ monitoring period and represent the plants near their points of greatest stress for each respective period. Leaf resistance to water loss was measured with the diffusive resistance instrument described by van Bavel et al. (1965).

Carbon dioxide exchange rates were measured simultaneously every ½ hour on two plants during the first 8 hours of a light and dark period. Only 8 hours of each 12-hour period were monitored because the rest of the period was needed for other measurements, standardization and equilibration of the system. Carbon dioxide uptake and evolution rates were averaged for the measurements during the 8 hour period and their standard deviations computed.

Assays for PEP carboxylase activities were made after monitoring $CO_2$ exchange. Green leaf tissue of each plant, minus the midribs, was cut into 1-cm pieces and quickly chilled. During the isolation procedure the plant tissue was kept near 0 C. Ten grams (fresh weight) of tissue was homogenized for 1 minute at half speed in an omnimixer equipped with a razor blade rotor. The extractions were made in 80 ml of solution containing 0.01 *M* NaCl, 0.01 *M* mercaptoethanol and 0.05 *M* glycylglycine at pH 7.6. Sucrose concentration of each extraction solution was determined by the water potential of the plant

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tissue. The homogenate was filtered through glass wool and then centrifuged at 121 × *g* for 2 min to remove debris, at 480 × *g* for 5 min to obtain a chloroplast pellet, and at 14,000 × *g* for 20 min to obtain a mitochondrial pellet. Enzyme activities were determined on the bulk fraction (after debris removal), resuspended mitochondrial and chloroplast fractions, and the combined supernatants from centrifugation. The resuspension solution was identical to the extraction solution except that the NaCl was deleted. The reaction solution minus the enzyme source contained 4 μmoles of PEP, 16 μmoles of $MgSO_4$, 4 μmoles of sodium glutamate, 200 μmoles of glycylglycine, and 12 μmoles of $NaHCO_3$. To 3.75 ml of the reaction mixture were added 0.25 ml of $NaH^{14}CO_3$ solution (approximately 2.5 μc) and ½ ml of the enzyme source. The reaction mixture was maintained at 30 C. One ml of each reaction mixture was acidified after 3, 6, and 9 min by injection into 0.5 ml of 1N HCl. Reaction rates were reasonably linear over ranges of 6 and 9 min. Air was bubbled through each acidified mixture for 3 min in order to remove unfixed $^{14}CO_2$. A 0.2-ml sample was removed from these mixtures and counted in a Packard Tri-Carb scintillation counter. Chlorophyll content of each enzyme source, when determined, was by Arnon's (1949) procedure and protein was determined by the procedure described by Lowry et al. (1951).

## RESULTS AND DISCUSSION

The $CO_2$ uptake rates in Table 1 are grouped arbitrarily into three categories according to degree of stress. Carbon dioxide uptake values did not decrease directly in proportion to the increase in water potential. An average $CO_2$ uptake rate of 8.9 mg $dm^{-2}hr^{-1}$ was observed for the group of turgid plants. Moderately stressed plants (—18.6 ± 0.8 atm) showed no decrease in $CO_2$ uptake rate from the nonstressed values but about a 50% decrease (4.20 mg $dm^{-2}hr^{-1}$) was observed at —20.3 ± 1.5 atm. The severely stressed plants show very low $CO_2$ uptake values averaging 0.40 mg $dm^{-2}hr^{-1}$. The rapid decrease in $CO_2$ uptake occurred around a water potential of —20 atm.

Leaf resistances for these low-light growth chamber plants were all relatively high. Average leaf resistances for typical plants were 13.2, 30.6, and > 60 sec $cm^{-1}$ for approximate water potentials of < —13.5 (turgid), —21, and —24 atmospheres, respectively. The fairly sharp drop in $CO_2$ uptake at about —20 atmospheres corresponds with the increased leaf resistance noted at this approximate water potential. Leaf resistance increased more sharply at —24 atmospheres at which time $CO_2$ uptake approached the compensation point. In fact the diffusive resistance meter detected no water loss from the more severely stressed plants.

The response of moderately and severely stressed plants to watering is illustrated in Figure 1. The wide fluctuation in $CO_2$ uptake measured several hours after watering is likely associated with rapid changes in stomatal aperture. The day after watering, $CO_2$ uptake in the moderately stressed plants reached the original maximal level. However, recovery was less than complete in plants stressed severely enough to reduce $CO_2$ uptake to near the compensation level.

Dark $CO_2$ evolution data are given in Table 2. Nonstressed plants showed an average $CO_2$ evolution rate of 0.81 mg $dm^{-2}hr^{-1}$ which increased under moderate stress (approaching —20 atmospheres) to 1.31 mg $dm^{-2}hr^{-1}$. Values for severely stressed plants were greatly reduced from either the nonstressed or moderately stressed figures. The $CO_2$ evolution values for the samples with water potentials of —26.3 ±

**Table 1. $CO_2$ exchange at low light level as affected by water stress. Figures for mg$CO_2$dm$^{-2}$hr$^{-1}$ of uptake were averaged from readings taken every ½ hour during the first 8 hrs of a regular 12 hr dark-12 hr light cycle.**

| Turgid | | Moderate stress | | Severe stress | |
|---|---|---|---|---|---|
| $CO_2$ uptake mg/dm²/hr± st. dev. | Ψ atm ± avg. dev. | $CO_2$ uptake mg/dm²/hr± st. dev. | Ψ atm ± avg. dev. | $CO_2$ uptake mg/dm²/hr± st. dev. | Ψ atm ± avg. dev. |
| 9.87±0.26 | -12.2±0.1 | | | | |
| 8.60† | -12.8±2.2 | 9.14±1.87 | -18.6±0.6 | | |
| 6.20±0.15 | -13.0±2.2 | 4.20±0.72 | -20.3±1.5 | 0.45±0.06 | -25.3±1.5 |
| 9.62±1.44 | ≤13.5* | | | 0.20±0.03 | -28.4±2.5 |
| 9.20±0.63 | ≤13.5* | | | 0.69±0.15 | -34.2±1.9 |
| 9.80±0.27 | -13.5±0.4 | | | 0.27±0.09 | -55.7±0.0 |
| Average | | | | | |
| 8.93±0.28 | -13.1±1.2 | 6.77±1.30 | -19.5±1.2 | 0.40±0.08 | -36.2±1.5 |

* Due to equipment failure, these water potential values were estimated from measurements on similar plants. † Single reading.

**Table 2. Dark $CO_2$ exchange at increasingly severe stages of water stress. Figures for mg $CO_2$ dm$^{-2}$hr$^{-1}$ evolved were averaged from readings taken every ½ hour during the first 8 hrs of darkness of a regular 12 hr dark-12 hr light cycle.**

| Turgid | | Moderate stress | | Severe stress | |
|---|---|---|---|---|---|
| $CO_2$ evolved mg/dm²/hr± st. dev. | Ψ atm ± avg. dev. | $CO_2$ evolved mg/dm²/hr± st. dev. | Ψ atm ± avg. dev. | $CO_2$ evolved mg/dm²/hr± st. dev. | Ψ atm ± avg. dev. |
| 0.92±0.02 | 10.1±0.4 | | | 0.35±0.05 | 20.3±1.5 |
| 0.79±0.11 | 12.2±0.1 | | | | |
| 1.08±0.24 | 12.8±2.2 | 1.30±0.07 | 18.6±0.08 | 0.42±0.11† | 26.3±1.5 |
| 1.04±0.06 | 13.0±2.2 | 1.41±0.09 | 19.6±4.1 | 0.42±0.04† | 28.4±2.5 |
| 0.47±0.05 | ≤13.5* | 12.1±0.20 | 20.3±5.1 | 0.28±0.02 | 34.2±1.9 |
| 0.63±0.08 | ≤13.5* | | | 0.13±0.03 | 47.9±5.0 |
| 0.81±0.09 | 13.5±0.4 | | | 0.14±0.04 | 55.7±0.0 |
| Average | | | | | |
| 0.81±0.09 | 12.7±1.1 | 1.31±0.12 | 19.5±3.1 | 0.29±0.05 | 35.5±2.1 |

* Due to equipment failure, these water potential values were estimated from measurement on similar plants. † These figures were obtained by averaging 1/2 hour readings over the first 4 hours following darkness and are slight overestimates. See text for explanation.

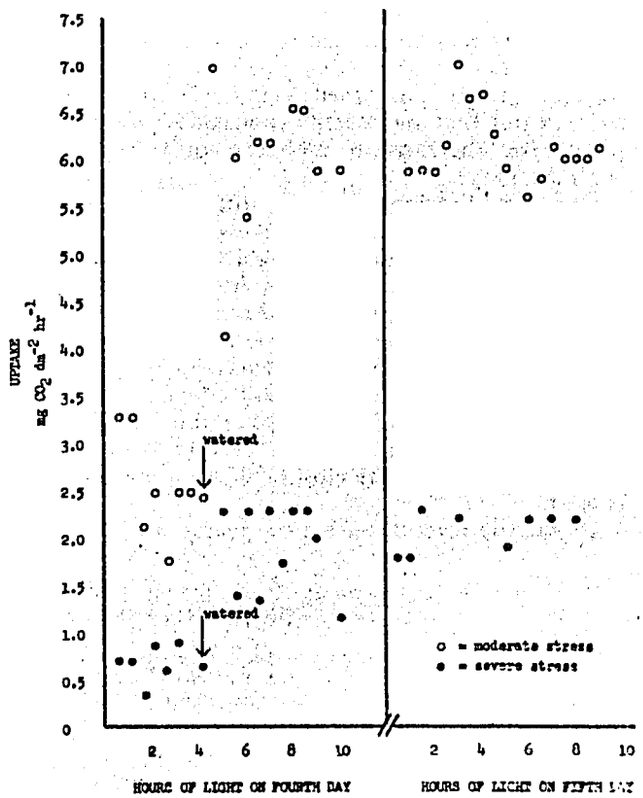


**Fig. 1. $CO_2$ uptake rates of moderately stressed and severely stressed plants immediately following and 1 day after watering to soil saturation.**

**Table 3. Comparative activities of phosphoenolpyruvate carboxylase in sorghum at different degrees of water stress. Cell fractions used as enzyme sources were obtained by centrifugation as described in the test. Activity is expressed as the average dpms fixed from samples taken after 3, 6 and 9 minutes of reaction per mg protein, per micromole substrate, per minute of reaction ± mean deviations. Plants A and B were assayed simultaneously, as were plants C and D and plants E and F.**

| Enzyme source | Plant* | Avg dpm mg⁻¹ μmoles⁻¹ min⁻¹ | Plant* | Avg dpm mg⁻¹ μmoles⁻¹ min⁻¹ | Plant* | Avg dpm mg⁻¹ μmoles⁻¹ min⁻¹ |
|---|---|---|---|---|---|---|
| Bulk | A | $3.2 \times 10^3 \pm 750$ | C | $2.2 \times 10^3 \pm 50$ | E | $6.5 \times 10^3 \pm 570$ |
| | B | $3.2 \times 10^3 \pm 360$ | D | $1.9 \times 10^3 \pm 170$ | F | $5.3 \times 10^3 \pm 530$ |
| Chloroplasts | A | $4.2 \times 10^3 \pm 14$ | C | $1.4 \times 10^3 \pm 19$ | E | $5.7 \times 10^3 \pm 40$ |
| | B | $1.1 \times 10^3 \pm 4$ | D | $1.2 \times 10^3 \pm 10$ | F | $5.1 \times 10^3 \pm 43$ |
| Mitochondria | A | $2.3 \times 10^3 \pm 33$ | C | $1.3 \times 10^3 \pm 4$ | E | $1.3 \times 10^3 \pm 67$ |
| | B | $4.7 \times 10^3 \pm 52$ | D | $1.1 \times 1.2 \pm 3$ | F | $6.5 \times 10^3 \pm 83$ |
| Supernatant | A | $3.4 \times 10^3 \pm 1,070$ | C | $1.5 \times 10^3 \pm 100$ | E | $5.9 \times 10^3 \pm 430$ |
| | B | $5.6 \times 10^3 \pm 500$ | D | $1.9 \times 10^3 \pm 100$ | F | $9.1 \times 10^3 \pm 600$ |

* $\Psi_A$ = −13.5 atm; $\Psi_B$ = −13.5 atm; $\Psi_C$ = −20.3 ± 5.1 atm; $\Psi_D$ = −19.6 ± 4.1 atm; $\Psi_E$ = −25.4 ± 2.5 atm; $\Psi_F$ = −28.3 ± 1.5 atm

1.5 and −28.4 ± 2.5 atm are slightly higher in comparison to the other values in this table. This is because these represent averages over a 4-hour instead of an 8-hour period. The overestimate (in comparison to a full dark period average) is caused by a peak in $CO_2$ evolution which occurred in both turgid and stressed plants by the third hour of darkness after which the rate declined and leveled off. The elevation of the peak was less for severely stressed plants than moderately or nonstressed plants.

Increased stomatal resistance may be a factor in the rapid decline in $CO_2$ evolution although Brix (1962) found that high $CO_2$ evolution continued even with high stomatal resistance. Measured stomatal resistances for nonstressed plants in darkness ranged from 16 to 35 sec $cm^{-1}$. No outward diffusion of water vapor from the stressed plants could be detected. Increases in $CO_2$ evolution with stress have been reported by Schneider and Childers (1941) and Kaul (1966), who also reported that following the increase, a significant decrease in $CO_2$ evolution occurred as stress increased. Possibly this relates to substrate availability. Reports of decreases only in $CO_2$ evolution with stress (Brix, 1962; Boyer, 1965) may relate to peculiarities of the different plants used for testing or to the degree of stress inflicted.

*Enzyme Assays.* Increased leaf resistance in the −20 to −25 atmospheres range obviously contributed to a decline in whole plant $CO_2$ uptake in the light. A second factor, the activity of the carboxylating enzyme, appeared to have less effect as water stress increased. Enzyme activities from several sources (Table 3) were generally lower in extracts from plants at about −20 atmospheres than from plants in either the turgid or severely stressed (−25 atmospheres) conditions. Possibly higher activities from severely stressed plants may relate to greater cell breakage and enzyme exposure under those conditions. Slack et al. (1969) feel the enzyme is likely associated with the bounding membrane of mesophyll chloroplasts in maize. Bucke and Long (1971) showed that variation in enzyme activity can be an artifact associated with differential cell breakage due to grindings that inflict different amounts of cellular damage.

The main point is that while enzyme activities from plants at different stress levels do vary, they do not fluctuate from some high level to near zero net activity with increasing water stress as does $CO_2$ uptake in intact plant tissue. Therefore leaf resistance appears to be more limiting to $CO_2$ uptake with increasing water stress than does enzyme activity. Phosphoenolpyruvic carboxylase has a relatively high temperature optimum (Treharne and Cooper, 1968) and the enzyme in sorghum appears to be quite stable as has been demonstrated in other plants by Brandon (1967).

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