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Carbon Balance of Sorghum Plants during Osmotic Adjustment to Water Stress[1]

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ABSTRACT

The daily (24-hour) carbon balances of whole sorghum plants (*Sorghum bicolor* L. Moench cv BTX616) were continuously measured throughout 15 days of water stress, followed by rewatering and 4 more days of measurements. The plants were grown under controlled environment conditions typical of warm, humid, sunny days. During the first 12 days, osmotic potentials decreased in parallel with decreased water potentials to maintain pressure potentials near 0.5 kilojoules per kilogram (5 bars). Immediately before rewatering on day 15, the water potential was −3.0 kilojoules per kilogram. Osmotic adjustment at this point was 1.0 kilojoules per kilogram, as measured by the decrease in the water potential at zero turgor from its initial value of −1.4 kilojoules per kilogram.

Gross input of carbon was less but the fraction retained was greater because a smaller fraction was lost through respiration in stressed plants than in unstressed plants. This was attributed to a lower rate of biomass synthesis, and conversely a higher rate of storage of photosynthate, due to inhibition of leaf expansion. The reduction in the cost associated with biomass synthesis more than balanced any metabolic cost of osmotic adjustment. The net daily gain of carbon was always positive in the stressed plants.

There was a large burst of respiration on rewatering, due to renewed synthesis of biomass from stored photosynthate. Over the next 3 days, osmotic adjustment was lost and the daily carbon balance returned to that typical of nonstressed plants. Thus, osmotic adjustment allowed the stressed plants to accumulate biomass carbon throughout the cycle, with little additional metabolic cost. Carbon stored during stress was immediately available for biomass synthesis on rewatering.

Many plants adjust osmotically by accumulating solutes when they are exposed to water stress (7, 14). Osmotic adjustment in sorghum (*Sorghum bicolor* L. Moench.) has been well documented (1, 5, 9–11). The adjustment is especially marked when the stress is applied slowly, either in the field or in controlled environments with the plants growing in large containers.

Osmotic adjustment has a positive effect on the daily carbon balance of a stressed plant, since it allows the plant to photosynthesize down to lower leaf water potentials than would otherwise have been possible. However, there may well be an additional metabolic cost of accumulating the solutes (6). Certainly, carbohydrates that are accumulated during the adjustment are not being used for synthesis of new biomass.

The objective of the experiments reported here was to analyze the daily carbon balances of sorghum plants exposed to a complete cycle of water stress and irrigation. The components of the daily (24-h) carbon balance of the whole plant, consisting of gross carbon input from photosynthesis, carbon loss through respiration, and net carbon gain, were calculated from the CO_2 exchange rate, which was continuously measured throughout a 19-d stress/irrigation cycle. Comparisons were made with plants that were irrigated every 2 d.

Osmotic adjustment was documented by following the water potential and osmotic potential of exposed leaves throughout the cycle, and also by drying leaf samples to determine the water potential at zero turgor and the pressure potential at zero water potential. There are no reports of previous attempts to link the whole plant carbon balance to osmotic adjustment. Since the photosynthetic carbon input per plant is strongly dependent on the leaf area (15), and it is known that the daily increment of leaf area is highly sensitive to water deficit (3), and that leaves senesce under severe stress (2), we also measured the leaf area per plant daily throughout the stress/irrigation cycle.

The daily carbon balances of 24 sorghum plants that had been exposed to various levels of water stress were reported by Wilson *et al.* (15). They showed that the photosynthetic input per plant, as well as both the growth and the maintenance components of the respiratory loss, decreased with decreasing leaf water potential. The yield of the growth processes, which is the ratio of net carbon gain to carbon input to the growth processes after subtracting maintenance losses, was found to be independent of leaf water potential.

We assumed that our plants would respond in a similar fashion, so that there was no need to separate the growth and maintenance components by changing the light level (12, 15). This enabled us to maintain constant and optimal (aerial) environmental conditions throughout the cycle, and to avoid sacrificing plants for determination of the maintenance requirement per unit biomass. All data could be taken on the same plant, with no interruption of the cycle.

MATERIALS AND METHODS

Prestress growth conditions were similar to those reported previously (12). Seeds of sorghum (*Sorghum bicolor* L. Moench., cv BTX 616) were soaked in distilled H_2O, germinated for 2 d, and planted in pots containing 2.5 L of fritted clay growth medium (Absorb-N-Dry). Twenty-one d after germination, the plants were repotted into 8 L pots. Growth conditions were: air temperature 30°C, dewpoint temperature 10 to 15°C, CO_2 concentration 330 to 420 $\mu l\ l^{-1}$, windspeed about 1 m s^{-1}, PPFD[2] 1000 $\mu mol\ s^{-1}\ m^{-2}$, daylength 12 h. Full strength nutrient solution was applied to excess daily. Tillers and dead leaves were removed, and leaves were numbered as they emerged.

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[2] Abbreviations: PPFD, photosynthetic photon flux density; CER, CO_2 exchange rate.

Leaf areas were calculated as length × maximum width × 0.68. The multiplying factor was based on areas of detached leaves from a sample plant, as measured by a LiCor model 3100 leaf area meter. Leaf length was measured as the distance from the tip to the point of attachment to the blade on fully expanded leaves, and from the tip to the deepest visible part of the whorl on expanding leaves. Senescent leaf area was subtracted from the calculated leaf area to give green leaf area.

Twenty-five d after germination, when the 8th leaf was fully expanded and the 12th leaf was emerging from the whorl, irrigation was stopped and the test plants were moved to a whole-plant assimilation chamber for determination of the daily carbon balance. The air temperature in the chamber was 30°C, the dewpoint temperature was 23°C, and the PPFD at the top of the plant was 1600 μmol s^{-1} m^{-2} for 12 h. As in previous experiments (12, 15), integrated CO_2 exchange rates (CER, per plant) were used to calculate values of the three daily (24-h) carbon balance parameters:

ΔS = gross input of substrate carbon from photosynthesis (gC $plant^{-1}$ d^{-1})
= integrated CER in 12 h of light − integrated CER in 12 h of darkness (where dark CER is negative)

ΔW = net 24-h gain of carbon by the plant (gC $plant^{-1}$ d^{-1})
= integrated CER in 12 h of light + integrated CER in 12 h of darkness

and

ΔR = 24-h loss of carbon due to respiration (2 × night loss)
= $\Delta S - \Delta W$

Oven (85°C) dry weight of the whole plant was determined at the end of the experiment, and multiplied by 0.38 to obtain the biomass carbon (W) at that time (12). Values of W for each previous day were back-calculated by subtracting values of ΔW from the final value of W.

To determine the water status of the plants during the stress cycle, a port in the assimilation chamber was opened briefly and samples of fully exposed leaves were taken for psychrometric measurement of water and osmotic potentials (16). Two 6-mm discs from different leaves were punched with a paper punch and placed within seconds in Wescor C-52 sample chambers. The sample chambers were kept in a temperature-controlled laboratory and were shielded from drafts with a polystyrene box. After 2 h of equilibration, the water potentials of the discs were measured with a Wescor model PR-55 psychrometric microvoltmeter. Microvolt output was corrected from the room temperature to 25°C using the equation provided with the instrument. A cooling current of 8 mamp and a delay time of 5 s were used. Cooling times varied from 5 s at the highest potentials to 10 s at the lowest potentials. Sample chambers were individually calibrated against NaCl standards.

After measurement of the leaf water potential, the discs were removed from the chambers, rapidly sealed in Teflon tape and aluminum foil, and frozen for 4 min on a block of dry ice. The sealed discs were then thawed for 3 min, unwrapped, and returned to the chambers. Osmotic potentials were measured after 45 min of equilibration. Pressure potentials were estimated by subtracting osmotic potentials from water potentials. All potentials in this paper are expressed as the potential energy content per unit mass of water, in kJ·kg^{-1} (4) (1 kJ·kg^{-1} = 1 MPa = 10 bars, assuming the density of water is 1.0 Mg·m^{-3}).

Samples were taken daily, either 1 h before the end of the day ('daytime' samples), or 1 h before the end of the night ('predawn' samples). To conserve plant material for the carbon balance measurements, daytime and predawn samples were taken in separate experiments under identical conditions. In other experiments specifically designed to quantify the degree of osmotic adjustment, four to eight leaf discs that had been punched from fully exposed leaves in the daytime were allowed to dry for various lengths of time (up to 5 min) before placing them in the sample chambers. The water potential at zero turgor and the pressure potential at zero water potential were estimated by linear regression analysis of water potential *versus* pressure potential for these discs. In cases where the pressure potential was already zero, some of the discs were rehydrated by placing a drop of water on them and blotting the surface dry before placing them in the chamber.

Soil water potentials were measured with six calibrated Wescor PCT-55 psychrometers placed randomly in the growth medium. For these measurements, the psychrometer microvoltmeter was set to a cooling current of 8 mamp, a cooling time of 10 s, and a delay time of 7 s.

The carbon balance experiment was repeated 20 times with different combinations of size of plant, size of pot, and length of stress cycle. A similar pattern was observed in every experiment. Data presented are averages from two experiments with similar plant sizes and cycle lengths. Water status data were also replicated twice, with the same plant sizes and cycle lengths as in the carbon balance experiments. Control experiments were run with well watered plants (irrigated every other day), and these experiments were also replicated twice.

RESULTS AND DISCUSSION

Water Status. Changes in the water status of the soil and of exposed leaves of a plant throughout the stress/irrigation cycle are illustrated in Figure 1. Daytime soil and leaf water potentials decreased gradually during the first 10 d after irrigation, then more rapidly, reaching minimum values of near −3.0 kJ·kg^{-1} on the 15th d. The plant was irrigated 5 h after the lights went off on day 15. Leaf water potentials then rose rapidly, reaching the prestress level after 4 d. Predawn water potentials (Fig. 2) were only slightly higher than daytime values throughout the cycle, due to the relatively humid conditions in the assimilation chamber (a saturation vapor pressure deficit of 1.4 kPa at 30°C).

The daytime leaf osmotic potential (Fig. 1) decreased in parallel with the leaf water potential for the first 12 d, the leaf pressure potential remaining in the range 0.4 to 0.6 kJ·kg^{-1} with only a slight downward trend. Pressure potentials became slightly negative on days 13 through 15 (the osmotic potentials were not

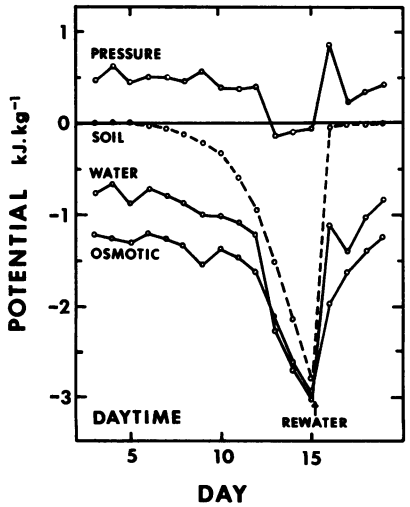

FIG. 1. Daytime water status of sorghum plants during a water stress cycle under controlled environment conditions. Plants were irrigated on day 0 and day 15. Water and osmotic potentials of discs punched from fully exposed attached leaves were measured psychrometrically, and pressure potentials were estimated by difference. Soil water potentials were measured psychrometrically (*in situ*).

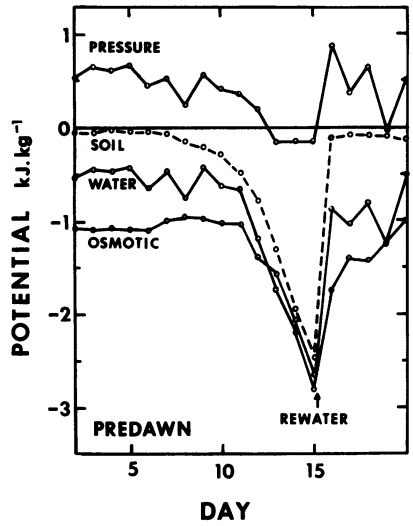

FIG. 2. Predawn water status of sorghum plants during a water stress cycle under controlled environment conditions. (See legend to Fig. 1 for details).

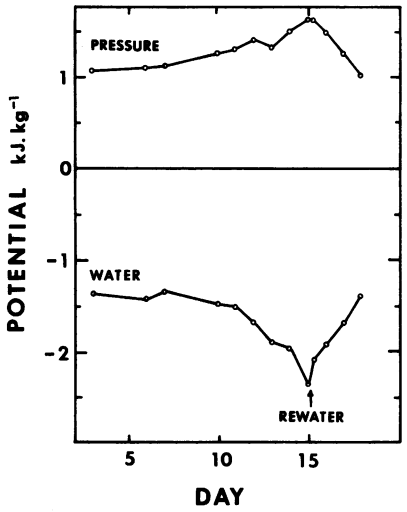

FIG. 3. Osmotic adjustment in sorghum plants during a water cycle similar to that shown in Figure 1. Several leaf discs were dried for various lengths of time and the water and osmotic potentials were measured psychrometrically. The water potential at zero pressure potential and the pressure potential at zero water potential were estimated by linear regression analysis of the psychrometric data and plotted in the figure as 'water' and 'pressure' potential, respectively.

adjusted for dilution by apoplastic water). Predawn osmotic potentials (Fig. 2) were slightly higher than daytime values, while predawn pressure potentials were equal to daytime values.

The pressure potential on the 1st d after rewatering was considerably higher than on day 3, due to a much lower osmotic potential (Figs. 1 and 2). Evidently, at least some of the osmotica accumulated during the stress period were retained until this measurement (which was made 18 h after rewatering). By the following day, pressure potential had returned to prestress levels.

The results of the leaf disc drying/hydrating experiments are shown in Figure 3. The water potential at zero turgor decreased from -1.4 kJ·kg^{-1} on day 3 to -2.4 kJ·kg^{-1} immediately before rewatering on day 15. Since at zero turgor the water potential is equal to the osmotic potential, this difference represents an osmotic adjustment of 1.0 kJ·kg^{-1}. Five h after rewatering, the water potential at zero turgor was -2.1 kJ·kg^{-1}, and 20 h after it was -1.9 kJ·kg^{-1}; thus, 0.5 kJ·kg^{-1} (or about half) of the osmotic adjustment had been lost by this time. The value continued to increase over several days to the original level. There was an adjustment of 0.6 kJ·kg^{-1} in the pressure potential at zero water potential between day 3 and day 15.

The data from all of these experiments (especially Fig. 3) are consistent in showing that osmotic adjustment occurred throughout the drying part of the cycle, continuing after positive turgor in the lamina was lost on day 13. Although concentration of solutes due to dehydration may account for some of the trends shown in Figures 1 and 2, it cannot explain the decrease in the water potential at zero turgor, or the increase in the pressure potential at zero water potential, shown in Figure 3, because these values were obtained at constant levels of hydration, and therefore represent active accumulation of solutes by the plants. Much of the adjustment was lost within 20 h of rewatering, although an appreciable amount was retained for 2 d. Previous measurements, based on the drying of detached leaves after rewatering the whole plant (9–11), may have underestimated the amount of osmotic adjustment.

Plants that were watered on alternate days maintained leaf water potentials of -0.6 to -0.8 kJ·kg^{-1}, osmotic potentials of -1.2 to -1.4 kJ·kg^{-1}, and pressure potentials of 0.5 to 0.7 kJ·kg^{-1} (data not shown).

Leaf Area. Leaf expansion was very sensitive to water stress (Figs. 4 and 7). The daily increment in leaf area (ΔLA) began to decrease on day 6, when the daytime leaf water potential was -0.8 kJ·kg^{-1}, even though the pressure potential remained in the range 0.4 to 0.6 kJ·kg^{-1} up until day 12 (Fig. 1), at which time ΔLA was zero. Apparently, leaf expansion was not closely related to the pressure potential in the exposed lamina. However, this may not reflect the potentials in the meristem or unemerged portions of the lamina (13), which were not measured.

During the 3 d of zero turgor, ΔLA was negative, due to senescence of leaves. It returned to prestress values 2 d after irrigation.

Carbon Balance Parameters. Changes in the parameters of the daily carbon balance of the plant throughout the stress/irrigation cycle are illustrated in Figure 5. Data for well watered plants are

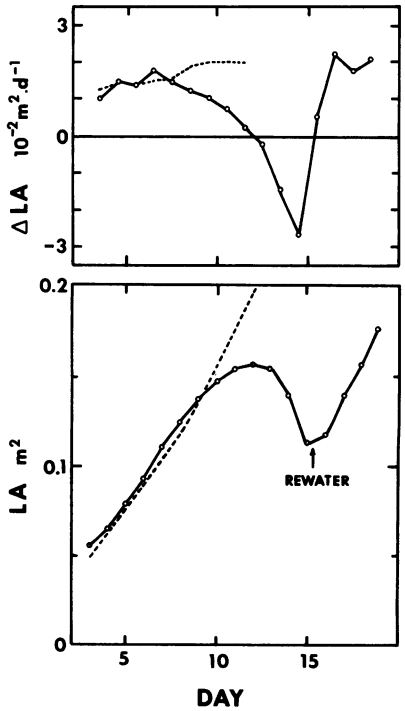

FIG. 4. Leaf area per plant (LA) and daily increment in leaf area (ΔLA) of sorghum plants that were either exposed to the water stress cycle shown in Figure 1 (——), or watered on alternate days (– – –).

shown for comparison. During the first few days of the cycle, the gross input of carbon (ΔS) increased due to the increase in leaf area, while the net gain (ΔW) increased linearly with ΔS (12). Between day 6 and day 12 of the cycle, the rate of increase of ΔS for the stressed plant slowed due to inhibition of leaf expansion (Fig. 4). At the same time, there was a trend toward a higher value of ΔW for a given ΔS, in comparison with a well watered plant. This was due to a decrease in the respiratory loss ΔR for a given ΔS. Wilson *et al.* (15) showed previously that both the growth and the maintenance components of ΔR decrease with increasing water deficit during the drying part of the cycle. We did not attempt to separate the two components in these experiments, because we wished to maintain constant environmental conditions throughout the cycle. Between day 12 and day 15, ΔS decreased rapidly in the stressed plant, presumably due to stomatal closure, as well as to a decrease in green leaf area due to senescence (Fig. 4). ΔW decreased proportionately with ΔS.

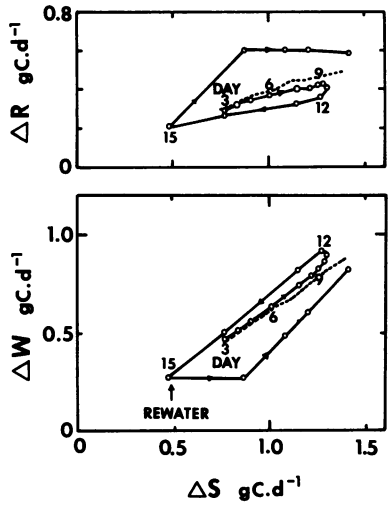

FIG. 5. Whole plant carbon balance parameters of sorghum plants that were either exposed to the water stress cycle of Figure 1 (——), or watered on alternate days (– – –). ΔS, gross input of substrate carbon from photosynthesis; ΔW, net 24-h gain of carbon by the plant; ΔR, 24-h loss of carbon due to respiration. Carbon balance parameters were determined by integration of CO_2 exchange rates.

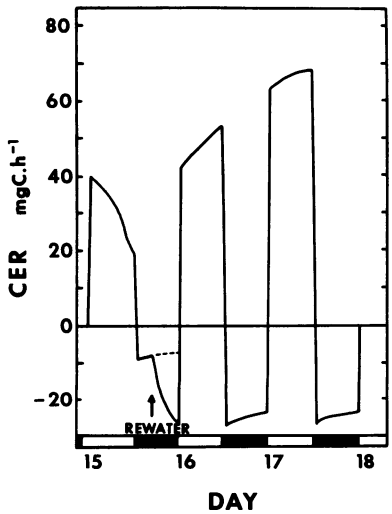

FIG. 6. CO_2 exchange rates (CER, converted to carbon basis) of sorghum plants immediately before and after rewatering. Light and dark periods are shown at the bottom of the figure. (– – –), extrapolated CER used to estimate the carbon balance parameters for day 15 (see Fig. 5).

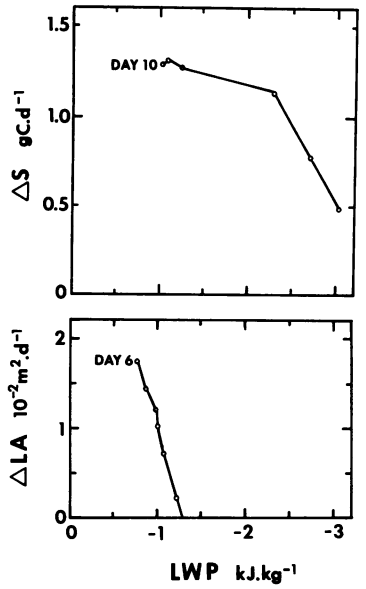

FIG. 7. Relationship between leaf water potential (*LWP*) and daily increment in leaf area (ΔLA), and daily substrate input from photosynthesis (ΔS), for sorghum plants exposed to the water stress cycle in Figure 1.

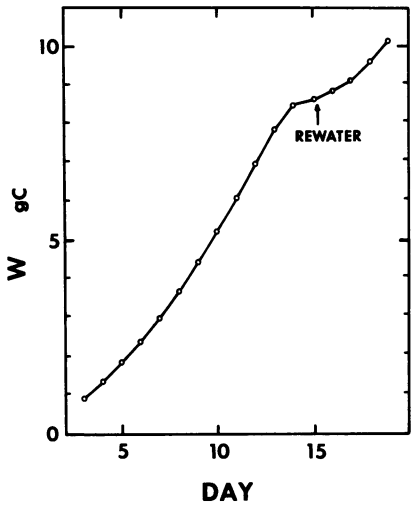

FIG. 8. Biomass carbon per plant (*W*) plotted against time, for sorghum plants exposed to the water stress cycle shown in Figure 1.

There was a large and immediate increase in ΔS upon rewatering, indicating reopening of the stomates. This increase was accompanied by a large increase in ΔR, so that ΔW did not increase at all between day 15 and day 16. Details of the increase in the hourly respiratory loss rate are shown in Figure 6. We attribute this increase in loss rate to rapid conversion of stored photosynthate into new biomass, which would be accompanied by a net carbon loss of about 30% (assuming a 70% conversion efficiency) (12). The leaf area data (Fig. 4) show that there was indeed a rapid synthesis of new leaves after rewatering. On subsequent days, ΔS and ΔW increased together, until the original trend line was re-established. ΔR for a given ΔS reached a value comparable with that found for a well watered plant 9 d earlier.

During the drying part of the cycle, the rate of increase of leaf area was affected before the photosynthetic input was reduced (3). Thus, the rate of change of leaf area (ΔLA) started to decrease on day 6, at a (daytime) leaf water potential of -0.8 kJ·kg^{-1} (Fig. 7), while ΔS started to decrease only on day 12, at a leaf

water potential of -1.2 kJ·kg^{-1}, and remained high down to -2.3 kJ·kg^{-1}. Beyond this point, leaves senesced and ΔS rapidly decreased. However, the net daily gain ΔW remained positive at a leaf water potential of -3.0 kJ·kg^{-1}, due to the concomitant decrease in ΔR (Fig. 5). It should be noted that these plants were growing under conditions that were optimal apart from the water stress (a high light level for 12 h/d and a constant temperature of 30°C).

The progression of biomass carbon (W) throughout the cycle is shown in Figure 8. The rapid increase during the first 8 d was due to expansion of the leaf area. The rate of increase slowed as the leaf expansion rate decreased due to water stress, then decreased to a low value as stomates closed and leaves senesced. At no time did the plant lose weight. Rapid increase in biomass carbon resumed a few days after rewatering.

CONCLUSIONS

The data show that during the first 12 d of water stress, the sorghum plants adjusted their osmotic potentials so that the pressure potential remained in the range 0.4 to 0.6 kJ·kg^{-1}. During this time, the photosynthetic input per day continued to increase, while the respiratory loss also increased but at a lower rate than was observed with well-watered plants. We attribute this difference to storage of substrate in the stressed plants, as well as to a decreasing maintenance requirement (15). Certainly there was no sign of an additional metabolic cost of accumulating the osmotica, which would have led to a higher than expected respiratory loss and a lower net daily gain than in the well watered controls.

The plants continued to photosynthesize at a high rate beyond the point where leaf area expansion began to be inhibited, on day 6. During this time there would have been large amounts of photosynthate available to contribute to the osmotic adjustment. The metabolic cost of using photosynthate for osmotic adjustment would probably be less than the cost of synthesizing new biomass from the same photosynthate, which would have occurred had the water potentials been high enough to allow leaf expansion. This would explain why the slope of the ΔW *versus* ΔS plot was greater in the stressed plants than in the well watered plants, while at the same time the daily increment in ΔS was smaller. That is, gross input of carbon was less in stressed plants but a greater fraction of the carbon was retained and a smaller fraction lost through respiration.

During the later stages of stress (days 13 through 15), photosynthetic input decreased rapidly due, presumably, to a combination of stomatal closure, loss of chloroplast activity, and leaf senescence. Leaf pressure potential fell to zero and leaf expansion ceased at a leaf water potential of -1.3 kJ·kg^{-1}, but photosynthate accumulation and osmotic adjustment continued down to the lowest leaf water potential tested, -3.0 kJ·kg^{-1}. Based on reports of osmotically adjusted plants maintaining higher leaf conductance and photosynthetic rates (9) and having greater ability to resist tissue death at lower water potentials than non-adjusted plants (8), we suggest that osmotic adjustment also enhanced the ability of sorghum plants in our experiments to continue to gain carbon at low water potentials. The positive carbon gain when leaf turgor was zero indicates that stomata remained at least partially open, and hence guard cells remained at least partially turgid, even though the bulk of the leaf had lost turgor.

The large burst of respiration on rewatering is consistent with rapid conversion of the stored photosynthate to new biomass, as well as a return of the maintenance coefficient to the original value. The observed flush of new leaf growth confirms this interpretation.

The overall pattern is one of a steady increase in biomass throughout the cycle, with a brief interruption during the days of severe stress. Without osmotic adjustment, it is doubtful that the sorghum plants would have continued to add biomass throughout this experiment.

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