Plant Physiol. (1988) 88, 375-378
0032-0889/88/88/0375/04/$01.00/0

# Water Deficit and Associated Changes in Some Photosynthetic Parameters in Leaves of 'Valencia' Orange (*Citrus sinensis* [L.] Osbeck)

Received for publication November 3, 1987 and in revised form May 2, 1988

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## ABSTRACT

**Photosynthetic $CO_2$ assimilation, transpiration, ribulose-1,5-bisphosphate carboxylase (RuBPCase), and soluble protein were reduced in leaves of water-deficit (stress) 'Valencia' orange (*Citrus sinensis* [L.] Osbeck). Maximum photosynthetic $CO_2$ assimilation and transpiration, which occurred before midday for both control and stressed plants, was 58 and 40%, respectively, for the stress (−2.0 megapascals leaf water potential) as compared to the control (−0.6 megapascals leaf water potential). As water deficit became more severe in the afternoon, with water potential of −3.1 megapascals for the stressed leaves vs. −1.1 megapascals for control leaves, stressed-leaf transpiration declined and photosynthetic $CO_2$ assimilation rapidly dropped to zero. Water deficit decreased both activation and total activity of RuBPCase. Activation of the enzyme was about 62% (of fully activated enzyme *in vitro*) for the stress, compared to 80% for the control. Water deficit reduced RuBPCase initial activity by 40% and $HCO_3^-/Mg^{2+}$-saturated activity by 22%. However, RuBPCase for both stressed and control leaves were similar in $K_{cat}$ (25 moles $CO_2$ per mole enzyme per second) and $K_m$ for $CO_2$ (18.9 micromolar). Concentrations of RuBPCase and soluble protein of stressed leaves averaged 80 and 85%, respectively, of control leaves. Thus, reductions in activation and concentration of RuBPCase in Valencia orange leaves contributed to reductions in enzyme activities during water-deficit periods. Declines in leaf photosynthesis, soluble protein, and RuBPCase activation and concentration due to water deficit were, however, recoverable at 5 days after rewatering.**

Water deficit affects citrus vegetative growth as well as fruit yield and quality (8, 9). Information is limited, however, on the effects of water deficit on the physiological and biochemical processes of photosynthesis in citrus. The understanding of water deficit on these processes will help to identify critical problem areas in citriculture for further study (12). Such information is also important in developing citrus water management strategies and in identifying and/or developing new citrus cultivars highly efficient in water use.

Reduction of leaf A[1] due to water deficit have been reported in a variety of plant species (6, 7, 15, 17), but the nonstomatal mechanisms contributing to these reductions are not well understood. Particularly for RuBPCase, the enzyme being responsible for fixing atmospheric $CO_2$ into the PCR cycle of $C_3$ plants, water deficit causes a decrease in enzyme activity (2, 3, 6, 10, 13, 17). However, the effect of water deficit on the enzyme concentration during and after the stress period, if there was any, is not known.

In this study, the effects of water deficit on a number of photosynthetic parameters were determined in leaves of 'Valencia' orange. We found that water deficit reduced the photosynthetic $CO_2$ assimilation rate, the carboxylation reaction, and the soluble protein content in leaves of citrus trees. Data from this study indicate that the reduction in RuBPCase activity of Valencia orange leaves as a result of water deficit was partly the result of a reduction in concentration of the RuBPCase protein and partly a result of reduced $CO_2$-$Mg^{2+}$ activation of the enzyme.

## MATERIALS AND METHODS

**Plant Material.** Trees of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) grafted on rough lemon (*C. jambhiri* Lush) rootstocks were grown in 6-L containers in washed sand and maintained outdoors. Trees were watered daily and fertilized biweekly with a 1.6% solution of 15-7-7 (N-P-K) liquid fertilizer. On March 3, 1986, when trees were 33 months old, water deficit (stress) was imposed on 20 trees by withholding daily watering. A second or control group of 20 trees continued to receive water. Visible water deficit (leaf wilting) started to develop at midday on the 6th day and was evident in all stressed trees by the 9th day. On the 10th day, March 12, 1986, leaf A, g, E, and water potential were monitored from 0745 to 1825 EST on top, fully expanded sun leaves. Additional leaf samplings were taken at 1430 and 1700 EST for determinations of RuBPCase, Chl, and soluble protein. All stressed trees were rewatered to field capacity at 1830 EST. Photosynthetic measurements and leaf samplings were performed again from 1000 to 1200 EST of the next day and on March 17, 1986, for observations of recovery.

**Photosynthesis Measurements.** A, g, and E of single attached leaves were measured outdoors under natural solar irradiance, using the LI-6000 portable photosynthesis system (LICOR[2], Lincoln, NE) as previously reported (19). The return flow rate of air circulating within the closed system was 16 $cm^3\ s^{-1}$, and five 30-s observations were used for each measurement.

[1] Abbreviations: A, photosynthetic $CO_2$ assimilation; CABP, carboxyarabinatol bisphosphate; E, transpiration; EST, eastern standard time; g, stomatal conductance; PCR, photosynthetic carbon reduction; RuBPCase, ribulose-1,5-bisphosphate carboxylase.

[2] Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

**Measurements of Leaf Water Potential.** Water potentials of single leaves were determined at each sampling time immediately after leaves were detached from trees by the pressure chamber technique (11).

**Extraction and Assay of RuBPCase.** Leaf-sampling procedures and storage conditions for RuBPCase studies have been described in a previous report (18). The extractions and assays of nonactivated (initial) and $HCO_3^-/Mg^{2+}$-activated (total) RuBPCase were performed in a manner similar to that described previously (17). Kinetic determinations were made on leaf samples of control and stressed trees harvested at 1430 EST on March 12, 1986. Chl content in the leaf extracts was measured according to Arnon (1). Soluble protein in the supernatant was precipitated with 5% (w/v) TCA, and the concentration determined as described by Bradford (4).

**Quantitation of RuBPCase.** RuBPCase concentrations in leaf tissues from both control and stressed treatments were determined using a modification of the radioimmune technique of Collatz *et al.* (5). About 250 mg of liquid $N_2$-frozen leaf powder were ground in 5 mL of 50 mM Hepes-NaOH buffer containing 5 mM DTT, 0.1 mM EDTA, 10 mM $MgCl_2$, 10 mM $NaHCO_3$, and 2% (w/v) PVP-40 at pH 7.5, and centrifuged for 5 min at 5000*g*. A 30 $\mu$L aliquot of the supernatant was then added to 100 $\mu$L of buffer (100 mM bicine, 20 mM $MgCl_2$, 1 mM EDTA at pH 7.8) having 4 nmol [2-$^{14}$C]CABP and 100 $\mu$L of antiserum to purified tobacco RuBPCase raised from rabbits. After incubating for 2 h at 37° C, the precipitate was collected on a Millipore cellulose acetate filter (0.5 $\mu$m pore size), washed with 5 mL of a 0.85% (w/v) NaCl solution containing 10 mM $MgCl_2$, and the bound $^{14}$C was determined by liquid scintillation counting.

## RESULTS

Photosynthetic $CO_2$ assimilation rates of attached leaves from control and water-deficit Valencia orange trees were determined throughout the day of March 12, and from 1000 to 1200 EST on March 13 and 17, 1986 (Fig. 1A). In both control and stressed treatments, A increased in the morning and reached its saturation level when solar PAR approached 800 $\mu$mol $m^{-2}$ $s^{-1}$ at about 0900 EST (Fig. 1A). Maximum A averaged 7.8 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$ for the control, compared to 4.5 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$ for the stressed trees. These maximum levels remained relatively stable from 0930 to 1130 EST, as solar PAR fluctuated between 800 and 1700 $\mu$mol $m^{-2}$ $s^{-1}$ because of variable cumulus cloud cover in the morning of March 12. In the well-watered control, there was a midday depression in A which started at about 1200 EST. By 1330 EST, A of the control was about 3 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$. However, A of the control partially recovered thereafter, with value of about 5 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$ at 1530 EST, and gradually declined with the continued drop of solar PAR during late afternoon. Like in the control treatment, decline in A of the stressed trees started at about midday, but decreased to near zero by 1400 EST. There was no apparent recovery of A of the stressed trees for the remainder of the afternoon (Fig. 1A).

Leaf E patterns of both control and water-deficit trees are shown in Fig. 1B. Maximum E, which occurred near midday for both control and stressed treatments, averaged 1 and 2.4 mmol $m^{-2}$ $s^{-1}$ for water-deficit and well-watered control leaves, respectively (Fig. 1B). Stomatal conductance patterns of both control and stressed leaves followed those of their corresponding A (data not presented). In the morning of March 12, g of both treatments increased with increased solar PAR and reached the maximum level at midmorning, about 0.1 cm $s^{-1}$ for stressed leaves and 0.25 cm $s^{-1}$ for control leaves. In the control, midday depressions of A were followed by similar depressions of E and g, with partial recoveries at about 1530 EST. In the stressed leaves, however, E and g declined with the continued drop of A, as water deficit became more severe in the afternoon. There were no apparent recoveries thereafter.

Figure 2 shows leaf water potential of control and water-deficit Valencia orange trees, determined from 0800 to 1700 EST on March 12, at 1200 EST on March 13, and at 1100 EST on

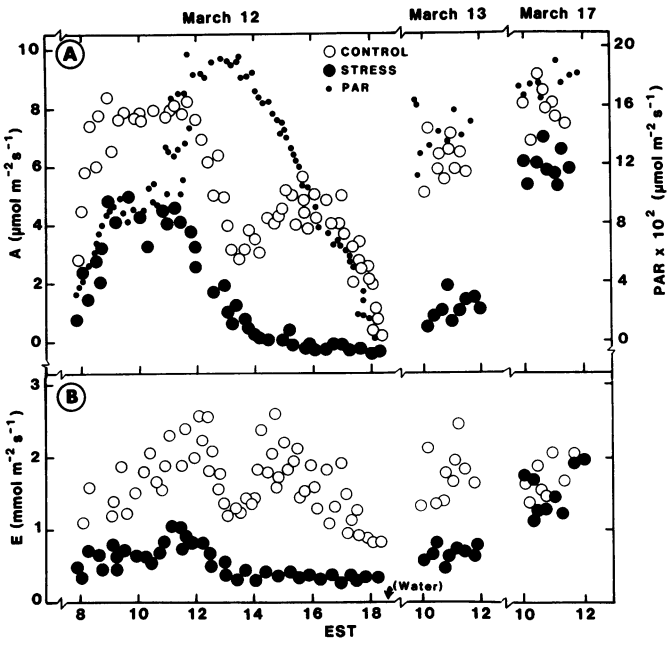


FIG. 1. Photosynthetic $CO_2$ assimilation (A, Fig. 1A) and transpiration (E, Fig. 1B) of single attached leaves of control and water-deficit trees of Valencia orange measured from 0745 to 1825 EST on March 12, and from 1000 to 1200 EST on March 13 and 17, 1986, with the LI-COR LI-6000 portable photosynthesis system. Water was added to all stressed trees (arrow) at 1830 EST on March 12, 1986. Solar PAR data are plotted in Figure 1A for reference.

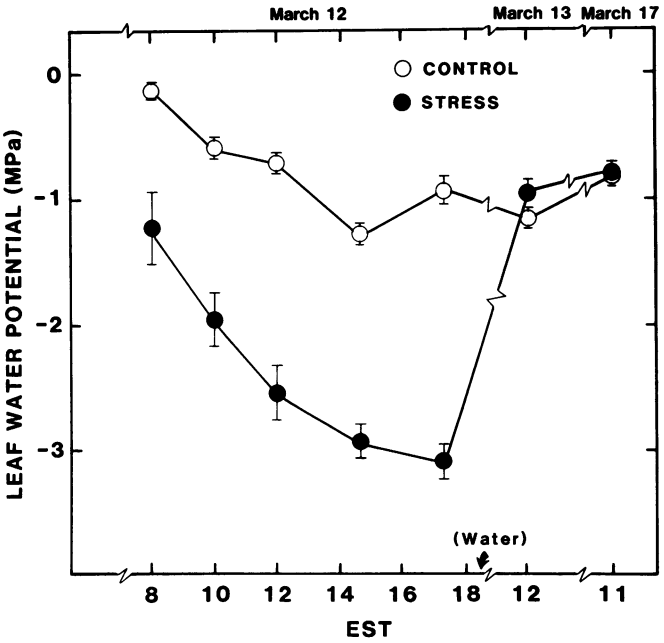


FIG. 2. Water potential of single leaves of control and water-deficit trees of Valencia orange determined from 0800 to 1700 EST on March 12, at 1200 EST on March 13, and at 1100 EST on March 17, 1986. Arrow indicates the addition of water for the stressed trees at 1830 EST on March 12, 1986. Data points are averages of five determinations ± SD.

March 17, 1986. During the day of March 12, leaf water potential of the control treatment decreased gradually from −0.14 MPa at 0800 EST to −1.3 MPa at 1430 EST, and partially recovered to −0.94 MPa at 1730 EST. Leaf water potential of water-deficit trees, which was ninefold more negative than that of control trees at 0800 EST, declined continuously throughout the day to a low value of −3.1 MPa at 1730 EST. Leaf water potentials of both control and rewatered stress-treatment trees were similar, however, when measured at noon of the next day, March 13, because water was added to field capacity to all stressed trees at sunset of March 12. Leaf A and E of water-deficit trees, determined from 1000 to 1200 EST on March 13, were only 19 and 37%, respectively, of well-watered control leaves (Fig. 1A, B). Full recovery of A was not attained even by March 17.

Table I shows RuBPCase, soluble protein, and Chl in leaves of control and water-deficit trees during March 12, 13, and 17, 1986. During the afternoon of March 12, initial and total activities of RuBPCase averaged 363 and 453 $\mu$mol $mg^{-1}$ Chl $h^{-1}$, respectively, for the control, and 218 and 352 $\mu$mol $mg^{-1}$ Chl $h^{-1}$, respectively, for the water deficit, which were about 60 and 78%, respectively, of the control (Table I). Full recoveries in enzyme activation and activities, however, were attained by March 17, as water was added back to all stressed trees at 1830 EST of March 12.

The $K_{cat}$ values, which were computed based on the initial (nonactivated) activity of RuBPCase, averaged 25% less during the afternoon of March 12 and 14% less on March 13 for the water-deficit treatment. However, there was no difference in $K_{cat}$ between the water deficit and control when this turnover number was calculated based on the total, $HCO_3^-/Mg^{2+}$-saturated activities of the enzyme (Table I).

Concentrations of RuBPCase and soluble protein of water-deficit leaves averaged 78 and 85%, respectively, of control leaves, for the afternoon of March 12 (Table I). Total Chl, however, was not affected by water deficit throughout the experimental period.

Double reciprocal plots of RuBPCase activity as a function of $H^{14}CO_3^-$ concentration in extracts of control and stressed Valencia orange leaves, harvested at 1430 EST on March 12 when water deficit was most severe, did not reveal any change in $K_m$ of the enzyme (Fig. 3). The apparent $K_m$ ($HCO_3^-$) and calculated $K_m$ ($CO_2$) values were 2.8 mM and 18.9 $\mu$M, respectively, for both control and stressed treatment. The $V_{max}$ values of the enzyme from stressed leaves were lower than those of control leaves.

## DISCUSSION

The results from this study indicate that water deficit decreased the activation of RuBPCase in Valencia orange, resulting in an average reduction of 18% in activation of the enzyme in stressed leaves (−3.1 MPa leaf water potential), as compared to control leaves (−1.1 MPa leaf water potential), during the afternoon on March 12. As water was added back to all stressed trees at sunset of that day, the inhibition in enzyme activation was partially released the next day, and, on March 17, 5 d after rewatering as leaf water potential was about the same (−0.85 MPa) for both treatments, enzyme activation value of the prestressed was comparable to the control treatment. Our data also suggested that water deficit appreciably inhibited RuBPCase activity in Valencia orange leaves. Nonactivated or initial RuBPCase activity, which has been considered most likely as the *in vivo* potential activity of the enzyme, was inhibited about 40% in stressed leaves during

Table I. *RuBPCase, Soluble Protein, and Chl in Leaves of Control and Water Deficit Valencia Orange Trees*

Values were determined at 1430 and 1700 EST on March 12, at 1200 EST on March 13, and at 1100 EST on March 17, 1986. Water was added back to all stress trees at 1830 EST on March 12, 1986.

| Components | March 12 | | March 13 | March 17 |
|---|---|---|---|---|
| | 1430 EST | 1700 EST | 1200 EST | 1100 EST |
| 1. RuBPCase | | | | |
| *Activation*[a] (%) | | | | |
| Control | 82.2 | 78.3 | 86.4 | 79.2 |
| Stress | 61.5 | 62.6 | 73.4 | 80.0 |
| *Treatment effect*[b] (%) | | | | |
| Initial[c] | 59.3 | 61.1 | 77.2 | 99.0 |
| Total[d] | 79.3 | 76.3 | 90.8 | 97.9 |
| $K_{cat}$[e] (mol $CO_2$ $mol^{-1}$ enzyme $s^{-1}$) | | | | |
| Control (Initial[c]) | 19.5 | 19.8 | 22.4 | 19.1 |
| Stress (Initial[c]) | 14.5 | 15.0 | 18.8 | 21.0 |
| Control (Total[d]) | 23.8 | 25.2 | 25.9 | 24.9 |
| Stress (Total[d]) | 23.6 | 24.0 | 25.6 | 25.3 |
| *Concentrations* (±SD) (mg $g^{-1}$ leaf dry wt) | | | | |
| Control | 17.1 ± 0.6 | 16.5 ± 0.4 | 16.0 ± 0.7 | 17.7 ± 0.5 |
| Stress | 13.2 ± 0.6 | 13.0 ± 0.5 | 14.0 ± 0.3 | 16.5 ± 0.6 |
| 2. Soluble protein (±SD) (mg $g^{-1}$ leaf dry wt) | | | | |
| Control | 66.8 ± 1.8 | 62.1 ± 2.5 | 60.4 ± 2.1 | 64.4 ± 3.2 |
| Stress | 55.9 ± 2.2 | 54.4 ± 1.5 | 53.8 ± 2.0 | 68.4 ± 3.1 |
| 3. Chl (±SD) (mg $g^{-1}$ leaf dry wt) | | | | |
| Control | 5.9 ± 0.2 | 6.0 ± 0.2 | 5.8 ± 0.3 | 5.9 ± 0.4 |
| Stress | 5.7 ± 0.2 | 5.9 ± 0.2 | 5.5 ± 0.3 | 6.4 ± 0.5 |

[a] [Initial activity/total activity] × 100. Enzyme activities calculated on a Chl basis. [b] [Enzyme activity of stress/enzyme activity of control] × 100. Enzyme activities calculated on a Chl basis. [c] Based on the initial activity of the enzyme. [d] Based on the total activity of the enzyme. [e] Assuming mol wt = 550,000.

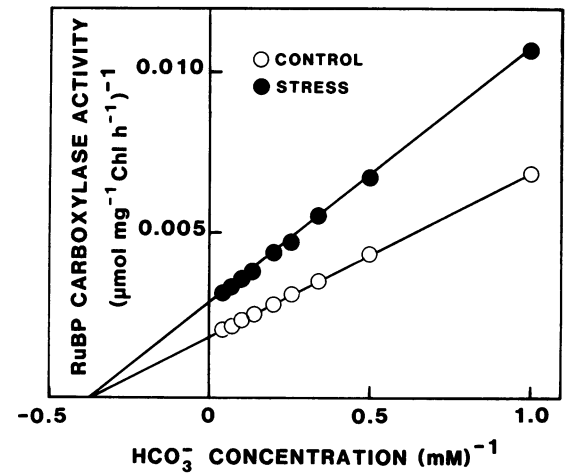


FIG. 3. Double reciprocal plots of $HCO_3^-/Mg^{2+}$-activated RuBPCase activity as a function of $HCO_3^-$ concentration in leaf extracts of control and water-deficit Valencia orange trees. The leaves were sampled at 1430 EST on March 12, 1986.

the afternoon of March 12. The total or fully activated, $HCO_3^-/Mg^{2+}$-saturated RuBPCase activity of stressed leaves was also inhibited about 22% during that water-deficit period. Like in activation state, inhibitions on initial and total activity of the enzyme were mostly released at 5 days after rewatering (Table I).

Calculations of the catalytic capacity, $K_{cat}$, of the enzyme at the initial activity levels also reveal some decrease in $K_{cat}$ as a result of water deficit (Table I). There was, however, no difference in $K_{cat}$ between control and stressed leaves when this turnover number was computed based on the maximum $HCO_3^-/Mg^{2+}$-saturated enzyme activities. The $K_{cat}$ value of RuBPCase from Valencia orange leaves, which averaged 25 mol $CO_2$ $mol^{-1}$ enzyme $s^{-1}$, is typical for $C_3$ photosynthetic categories as reported by Seemann *et al* (14).

Reductions in RuBPCase protein concentration by water deficit averaged about 22% for the afternoon of March 12 (Table I). Thus the inhibition of RuBPCase activity in Valencia orange leaves, as a result of water deficit, may be explained by nearly equivalent losses in both activation state and amount of the enzyme. Furthermore, since up to 25% of the total soluble leaf protein of Valencia orange was allocated to RuBPCase, decreases in concentrations of the enzyme by water deficit reduced the total soluble fraction of the leaves. Upon rewatering and 5 d after, however, concentrations of RuBPCase and soluble protein in prestressed leaves were about the same as in control leaves. This indicated that in our water-deficit experiment with fully developed Valencia orange leaves, as water was added back to the stressed trees at 1830 EST on March 12, the loss of RuBPCase protein as a result of water deficit was reversible, which was evidenced by nearly identical amounts of the enzyme protein in both control and prestressed leaves on March 17 (Table I).

In $C_3$ plants, the fully activated, $HCO_3^-/Mg^{2+}$-saturated RuBPCase activities are four to five times higher than the photosynthetic $CO_2$ assimilation rates observed at normal atmospheric $CO_2$ (16). Determinations of the water status in Valencia orange leaves during the day of March 12 gave an average leaf fresh weight of 2.65 and 2.23 g per $dm^2$ leaf area, and an average leaf dry weight/fresh weight ratio of 0.38 and 0.44 for well-watered and water-deficit treatment, respectively. This resulted in about 1 g leaf dry weight per $dm^2$ leaf area for both treatments. A comparison of the photosynthetic $CO_2$ assimilation rates to RuBPCase activities was made for Valencia orange, using the Chl data presented in Table I. Total activity of RuBPCase in the afternoon of March 12 averaged 453 μmol $CO_2$ $mg^{-1}$ Chl $h^{-1}$ for the control and 352 μmol $CO_2$ $mg^{-1}$ Chl $h^{-1}$ for the water deficit, which were equivalent to about 75 and 57 μmol $CO_2$ $m^{-2}$ $s^{-1}$, respectively. This indicates that activities of the enzyme from leaves of both treatments, extracted and measured in the afternoon of March 12 when water deficit was most severe, were still more than adequate to support even the highest $CO_2$ assimilation rates of attached leaves as observed in the morning of March 12 (Fig. 1). During the afternoon of that day, however, photosynthetic $CO_2$ assimilation rates were depressed, either partially as for the control or completely as for the water-deficit treatment. These decreases in A were mostly due to partial opening (control leaves) or full closing (water-deficit leaves) of the stomata.

From our studies with Valencia orange, there was no indication that severe water-deficit effects on RuBPCase were due to changes in the $K_m$ of the enzyme (Fig. 3). The depression in initial activity (*i.e. in situ* activity) of RuBPCase by water deficit was, therefore, a result of the cumulative effects of decreases in both activation state and concentration of the enzyme.

*Acknowledgments*—We thank Dr. Jeffrey R. Seemann at the Biochemistry Department of the University of Nevada in Reno, Nevada, for providing information on preparation of [$^{14}$C]carboxyarabinatol bisphosphate, and Dr. Michael G. Bausher and Ms. Dorothy Payne at the U.S. Department of Agriculture Horticultural Research Laboratory in Orlando, Florida, for help in preparation of the RuBPCase antiserum.

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