1984

Guralnick LJ, PA Rorabaugh, Z Hanscom (1984) Influence of photoperiod and leaf age on Crassulacean acid metabolism in Portulacaria afra (L.) Jacq.

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Plant Physiol. (1984) 75, 454–457
0032-0889/84/75/0454/04/$01.00/0

Influence of Photoperiod and Leaf Age on Crassulacean Acid Metabolism in *Portulacaria afra* (L.) Jacq.[1]

Received for publication September 13, 1983 and in revised form January 15, 1984

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ABSTRACT

The possibility that Crassulacean acid metabolism (CAM) is subject to long day photoperiodic control in *Portulacaria afra* (L.) Jacq., a facultative CAM plant, was studied. Periodic measurements of $^{14}CO_2$ uptake, stomatal resistance, and titratable acidity were made on plants exposed to long and short day photoperiods. Results indicates that water-stressed *P. afra* had primarily nocturnal CO_2 uptake, daytime stomatal closure, and a large diurnal acid fluctuation in either photoperiod. Mature leaf tissue from nonstressed plants under long days exhibited a moderate diurnal acid fluctuation and midday stomatal closure. Under short days, there was a reduced diurnal acid fluctuation in mature leaf tissue. Young leaf tissue taken from nonstressed plants did not utilize the CAM pathway under either photoperiod as indicated by daytime CO_2 uptake, lack of diurnal acid fluctuation, and incomplete daytime stomatal closure.

The induction of CAM in *P. afra* appears to be related to the water status of the plant and the age of the leaf tissue. The photosynthetic metabolism of mature leaves may be partly under the control of water stress and of photoperiod, where CAM is favored under long days.

Photoperiodic induction of CAM was first reported for *Kalanchoë blossfeldiana* (3), which under a LD photoperiod exhibited a C_3 mode of photosynthesis. With an increasing number of SD, there was increased nocturnal CO_2 uptake. Subsequent studies of *K. blossfeldiana* showed that malic acid and the enzymes associated with CAM increased in activity after a switch to a SD photoperiod (1, 9). *K. blossfeldiana* undergoes a winter drought in its natural habitat; thus, it is hypothesized that induction of CAM by SD constitutes preparation of a metabolic mechanism to withstand this drought (2). There is still very little information on the influence of photoperiod in other facultative CAM plants.

Portulacaria afra is a faculative CAM species which responds to water stress by switching from C_3 photosynthesis to CAM (5, 11). Irrigated plants of *P. afra* exhibit daytime CO_2 uptake and little diurnal organic acid fluctutation. Nighttime CO_2 uptake occurs in conjunction with a large diurnal fluctuation of organic acids only when the plants are water- or salt-stressed. *P. afra* is endemic to South Africa in an area which is characterized by a distinct summer drought (Richard Cowling, personal communication). Based on preliminary findings, we suggest that CAM is photoperiodically triggered in *P. afra* by LD. We report here a set of experiments testing the hypothesis that CAM may be induced in *P. afra* by LD and short nights.

[1] Supported by the Departments of Biology and Botany and the Systems Ecology Research Group at San Diego State University

MATERIALS AND METHODS

Plants. *Portulacaria afra* (L.) Jacq. clones were propagated from a large potted plant growing in the San Diego State University greenhouse. The shrub (2.5–3 m in height) was watered biweekly and fertilized monthly. Cuttings from the parent plant were rooted in U.C.-2 potting mix and irrigated biweekly with deionized H_2O. After rooting, six nonstressed plants were watered every 3rd d to maintain high tissue water potentials, and six stressed plants were watered every 6th d with one-half the amount of water applied to the nonstressed group. Full-strength Hoagland solution was applied to all plants biweekly. Plants were trimmed to induce new seasonal growth at the beginning of the growing season.

Growth Chamber Experiments. To study the effect of photoperiod, 12 randomly selected plants were transferred to a growth chamber and were allowed to adjust to a LD (15 h light/9 h dark) photoperiod for 2 months. Light was provided by fluorescent (Sylvania F48T12-CW-VHO) and incandescent (100 w) lamps with a photosynthetic photon flux density, measured below the top of the canopy, of 500 μmol m^{-2} s^{-1} (Li-Cor LI-185 quantum meter). After analyses, the same plants were allowed to adjust to a SD (9 h light/15 h dark) photoperiod for 2 months. Daytime temperatures during each photoperiod regime were maintained at 30°C and nighttime temperatures at 15°C. The RH fluctuated between 65% during the day and 75% at night.

Acid Titrations. Six leaves per treatment were collected randomly, weighed, and placed on dry ice. The samples were lyophilized and stored in a desiccator until assayed. The dried samples were ground to a fine powder with a mortar and pestle, and a homogenate was made with 20 ml glass-distilled H_2O. The homogenate was titrated with 0.01 N KOH to a pH 7.0 endpoint. In addition, morning and evening organic acid levels of mature leaves from a large potted shrub growing in the San Diego State University greenhouse were assayed for a year.

Gas Exchange Measurements. Stomatal resistance to water vapor was measured with an autoporometer (Li-Cor, Li-65). The porometer was calibrated at several temperatures with known resistances to obtain regression lines of resistance *versus* time. Values exceeding 90 s cm^{-1} were assumed to indicate stomatal closure (5). The sensor was placed on the abaxial surface of attached leaves.

$^{14}CO_2$ uptake was measured using the system of Oechel and Mustafa (8). A single detached leaf was enclosed in a cuvette and exposed to $^{14}CO_2$ for 45 s in the growth chamber. Exposed plant material (0.168 cm^2 leaf punch) was immediately harvested and dropped into a 1:1 mixture of cold phenethylamine:methanol. After 48 h, the plant sample was washed with 1 ml of ethylene glycol monoethyl ether (cellosolve) and then dried for 24 h at 80°C, weighed, and combusted. Radioactivity of the phenethyl-

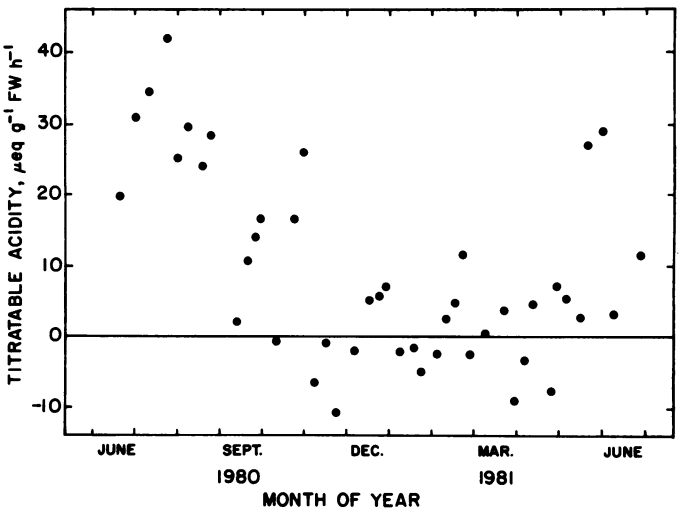

FIG. 1. Accumulation of titratable acidity for *P. afra* from June 1980 to June 1981. Each datum was calculated from a minimum of 12 acidity determinations, six each at sunrise and sunset.

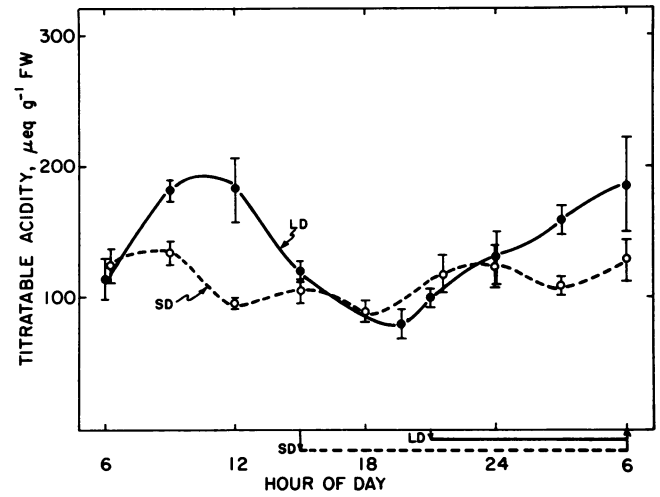

FIG. 2. Diurnal variation in titratable acidity of mature, nonstressed *P. afra* leaves grown under a LD (●——●) or SD (○- - -○) photoperiod. Night period indicated ——(LD) or - - -(SD). Each point represents the mean of six determinations ± SE.

amine:methanol, cellosolve, and combusted plant tissue was measured with liquid scintillation methods, pooled, and used to derive $^{14}CO_2$ rates for one side of leaf tissue (8).

RESULTS

CAM is usually characterized by a minimum of 100 μeq acid fluctuation from morning to evening (7). Thus, a rate of 12.5 μeq h^{-1} (for an 8-h d) would be an indicator of CAM activity. The change in titratable acidity for *P. afra* decreased from over 40 in late July to below 10 μeq h^{-1} in December, and remained low throughout much of the following spring (Fig. 1). On the basis of these results, we attempted to determine if the change in carbon metabolism was related to photoperiod.

Mature, Nonstressed Leaves. Irrigated plants which were grown under a LD (15 h light/9 h dark) photoperiod for 2 months exhibited a moderate 100 μeq g^{-1} FW[2] fluctuation of organic acids (Fig. 2). Minimum stomatal resistance values on the order of 2 to 10 s cm^{-1} were noted at the end of the dark period and beginning of the light period (Fig. 3). Complete stomatal closure was observed through the middle of the light period. Stomatal resistance decreased during the latter part of the light period. Maximum $^{14}CO_2$ uptake in nonstressed *P. afra* occurred at the beginning of the light period and at the end of the dark period (Fig. 4). Low levels of $^{14}CO_2$ uptake were seen during the remainder of the dark period and again at the end of the light period.

After growth for 30 d under a 9-h light/15-h dark photoperiod, there was a reduced organic acid fluctuation (~50 μeq g^{-1} FW) from that observed under LD (Fig. 2). Stomatal resistance was minimal at the beginning of the light period and at the end of the dark period (Fig. 3). In addition, stomatal resistance increased throughout most of the light period. The stomatal opening observed during the dark period was not accompanied by appreciable $^{14}CO_2$ uptake (Fig. 4). The major peaks of $^{14}CO_2$ uptake in the SD plants occurred during the early portion of the light period and at the very end of the 15-h dark period.

Mature, Stressed Leaves. Titratable acidity of water-stressed *P. afra* grown under a LD photoperiod underwent a large 250 μeq g^{-1} FW fluctuation (Fig. 5). Stomatal resistance was high

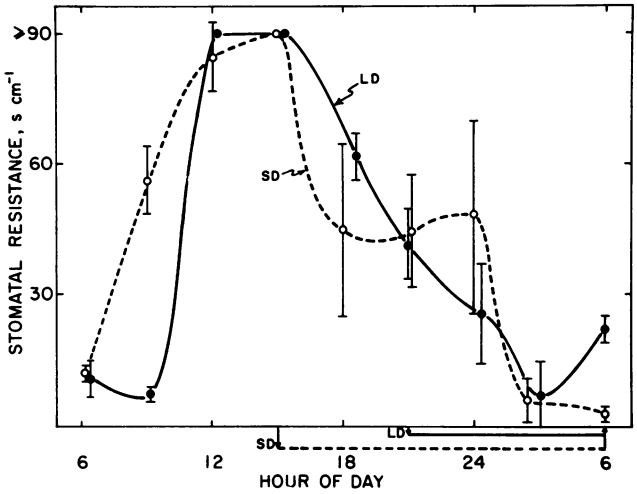

FIG. 3. Diurnal variation in stomatal resistance of mature, nonstressed *P. afra* leaves grown under a LD (●——●) or SD (○- - -○) photoperiod. Each point represents the mean of three determinations ± SE.

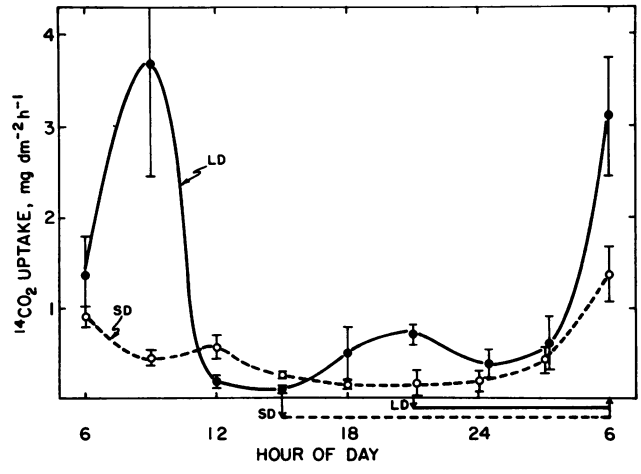

FIG. 4. Time course of $^{14}CO_2$ uptake of mature, nonstressed *P. afra* leaves grown under a LD (●——●) or SD (○- - -○) photoperiod. Each point represents the mean of six determinations ± SE.

[2] Abbreviation: FW, fresh weight.

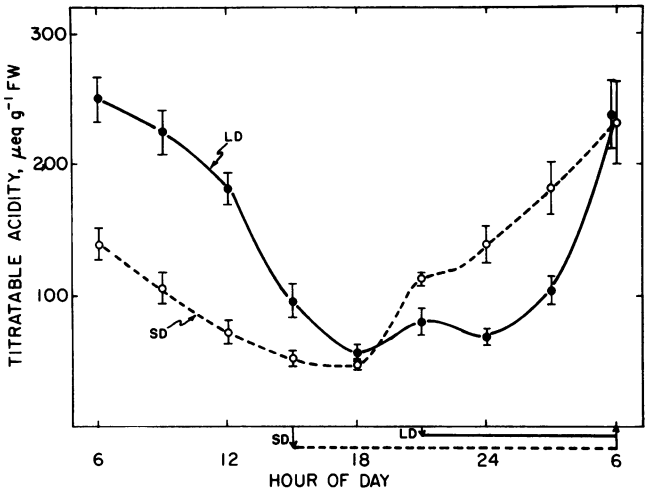

FIG. 5. Diurnal variation in titratable acidity of mature, water-stressed *P. afra* leaves grown under a LD (●——●) or SD (O---O) photoperiod. Each period represents the mean of six determinations ± SE.

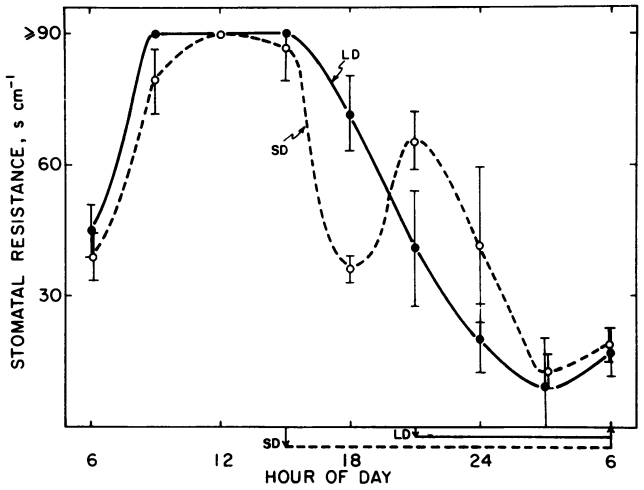

FIG. 6. Diurnal variation in stomatal resistance of mature, water-stressed *P. afra* leaves grown under a LD (●——●) or SD (O---O) photoperiod. Each point represents the mean of three determinations ± SE.

during the light period, with a slight decrease at the beginning and the end of the light period (Fig. 6). Stomatal resistance gradually decreased during the dark period with minimum values occurring near the end of the dark period. $^{14}CO_2$ uptake occurred at the beginning of the light period, was low during the middle of the day, and began to rise during the latter part of the day (Fig. 7). Low rates of $^{14}CO_2$ uptake were observed throughout the dark period.

After growth for 30 d under a SD photoperiod, the acidity results were only slightly different from those of plants grown under a LD photoperiod (Fig. 5). Stomatal resistance patterns were only slightly different under the SD photoperiod relative to that observed under LD (Fig. 6). Low levels of $^{14}CO_2$ uptake occurred during the light period and increased to moderate levels throughout the dark period (Fig. 7).

Young, Nonstressed Leaves. Fluctuations in titratable acidity of young, nonstressed *P. afra* leaves were markedly different from those seen in mature leaves (Fig. 8). Acid levels remained quite high throughout the day and night, and the fluctuation observed was minor. Stomatal resistances were moderately low

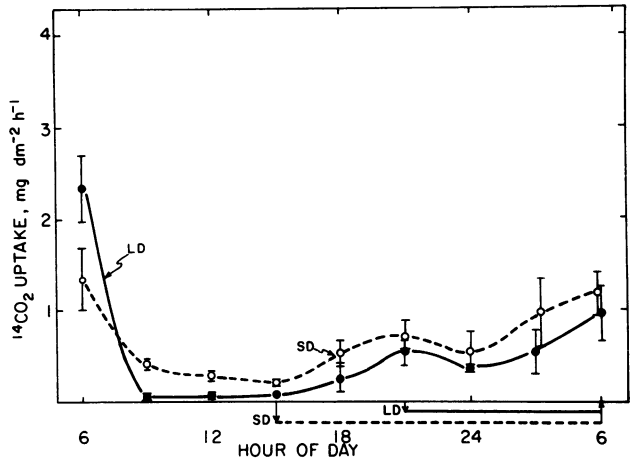

FIG. 7. Time course of $^{14}CO_2$ uptake of mature, water-stressed *P. afra* leaves grown under a LD (●——●) or SD (O---O) photoperiod. Each point represents the mean of six deteminations ± SE.

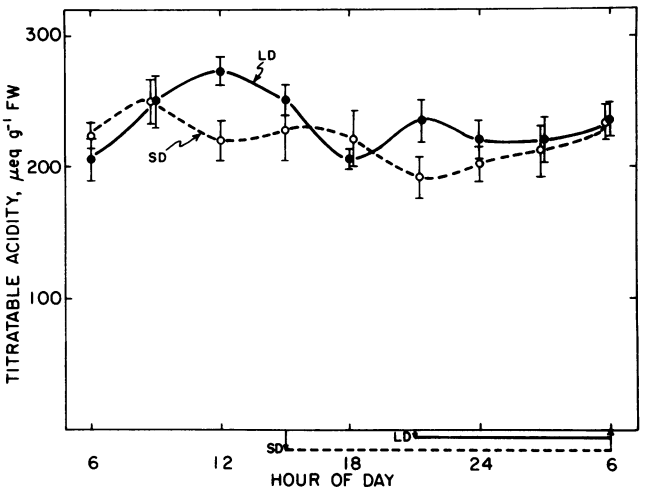

FIG. 8. Diurnal variation in titratable acidity of young *P. afra* leaves grown under a lD (●——●) or SD (O---O) photoperiod. Each point represents the mean of six determinations ± SE.

throughout the 24-h cycle, with minimal levels observed at the beginning of the light period and near the end of the dark period (Fig. 9). Low rates of $^{14}CO_2$ uptake occurred throughout the 24-h cycle, with peaks at the beginning of the light period, near the end of the light period, and near the end of the dark period (Fig. 10). Titratable acidity fluctuation and stomatal resistances in young nonstressed leaves, after 66 d of growth under a SD photoperiod, were similar to those of plants grown under a LD photoperiod (Figs. 8 and 9). After 66 SD, daytime $^{14}CO_2$ uptake was still greater than nighttime uptake, though low levels of uptake occurred throughout the dark period (Fig. 10).

DISCUSSION

Nonstressed *P. afra* showed low organic acid fluctuation when compared to stressed plants (Figs. 2 and 5). These results are similar to those reported earlier for *P. afra* (11). Nonstressed plants underwent a moderate acid fluctuation during LD photoperiods, indicating some CAM activity. With an increasing number of SD, there is a reduction in the magnitude of the acid fluctuation. Nocturnal stomatal opening occurs during both photoperiod regimes, but there is a slight reduction of nocturnal $^{14}CO_2$ uptake during SD, an indication of reduced CAM activity.

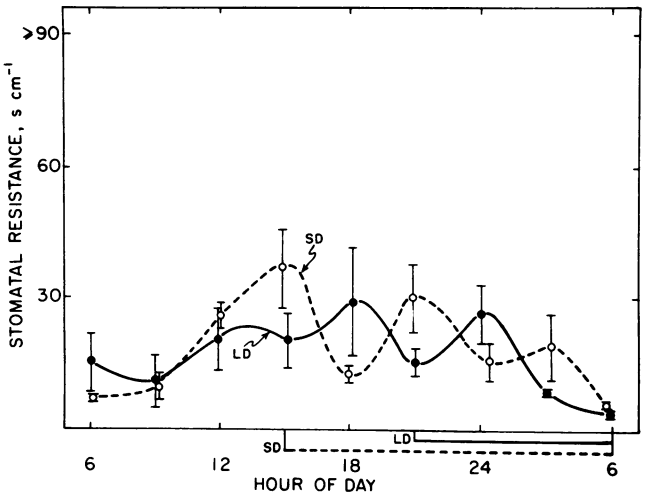

FIG. 9. Diurnal variation in stomatal reisstance of young *P. afra* leaves grown under a LD (●——●) or SD (O- - -O) photoperiod. Each point represents the mean of three determinations ± SE.

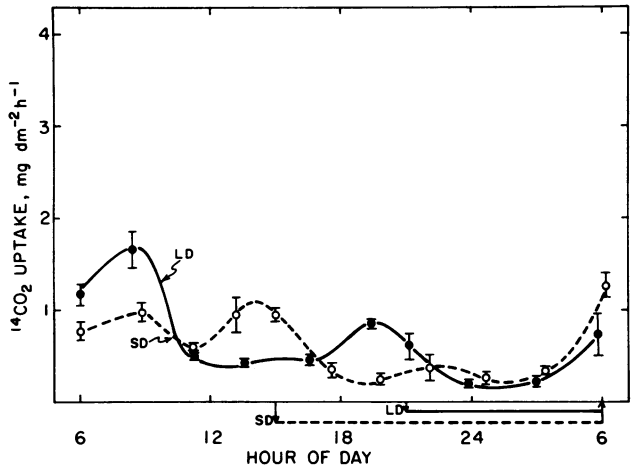

FIG. 10. Time course of $^{14}CO_2$ uptake of young *P. afra* leaves grown under a LD (●——●) or SD (O- - -O) photoperiod. Each point represents the mean of six determinations ± SE.

Differences between LD and SD plants are even less pronounced in young leaves. The magnitude of the acid fluctuation was not sufficiently large to consider the leaves to have switched to CAM. The gas exchange data collected from young plants are indicative of C_3 mode of photosynthesis, with $^{14}CO_2$ uptake occurring primarily during the day. One notable difference between young and mature leaves is that stomatal closure in young leaves is not as complete as in mature leaves. Similar results were reported by Jones (6) whose results indicated that gas exchange patterns in young *Bryophyllum fedtschenkoi* leaves differed from older leaves. This pattern of low daytime stomatal resistance has also been observed in young *Opuntia basilaris* stems (4). It thus appears that young tissues in general, when not stressed, exhibit lower stomatal resistance than do mature tissues.

Data reported here support the contention that baseline acid levels are related to leaf age. Young leaves maintain high acid levels when not fluctuating, whereas older leaves maintain relatively lower acid levels when not fluctuating. *P. afra* maintains high organic acid levels even when the C_3 photosynthetic pathway is operating (10). Other facultative CAM plants do not share this feature (7). We assume that acid fluctuations commence in young leaves when daytime stomatal closure occurs. The hypothesis that daytime stomatal closure is a first response during CAM induction is supported by Ting (10).

Stressed plants had a gas exchange and acid fluctuation pattern typical of CAM regardless of the photoperiod under which they were grown. Plants which were not water-stressed and were grown under a LD/short-night photoperiod had some characteristics of CAM, including some nocturnal CO_2 uptake and a moderate fluctuation of organic acids. Young leaf tissue did not display any characteristics of CAM in either photoperiod. Our data indicate that water stress overrides any effect of photoperiod in *P. afra*, while in well-watered plants the induction of CAM is a maturation process where CAM is favored under LD. In summary, it appears that CAM in *P. afra* is induced by a combination of factors which include water stress, photoperiod, and leaf aging. Thus, the original hypothesis which suggests that CAM is induced in *P. afra* by a LD/short night photoperiod was only partially supported by our data.

Acknowledgment—We would like to extend our thanks to Dr. Irwin P. Ting for his help during the initial stages of the research and to Drs. Ken Johnson, Jochen Kummerow, and Michael Donoghue for their advice and input on the manuscript. Jim Malusa helped by doing numerous acid titrations, and Jan Lippert did the graphics.

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