Photosynthetic Pathway Types of Evergreen Rosette Plants (Liliaceae) of the Chihuahuan Desert

Paul R. Kemp and Pietra E. Gardetto
Department of Biology, New Mexico State University, Las Cruces, New Mexico 88003, USA

Summary. Diurnal patterns of CO₂ exchange and titratable acidity were monitored in six species of evergreen rosette plants growing in controlled environment chambers and under outdoor environmental conditions. These patterns indicated that two of the species, *Yucca baccata* and *Y. torreyi*, were constitutive CAM plants while the other species, *Y. elata*, *Y. campestris*, *Nolina microcarpa*, and *Dasylirion wheeleri*, were C₃ plants. The C₃ species did not exhibit CAM when grown in any of several different temperature, photoperiod, and moisture regimes. Both photosynthetic pathway types appear adapted to desert environments and all species show environmentally induced changes in their photosynthetic responses consistent with desert adaptation. The results of this study do not indicate that changes in the photosynthetic pathway type are an adaptation in any of these species.

Introduction
The deserts of North America contain a number of evergreen rosette species which are related both taxonomically and morphologically. These species, of the genera *Dasylirion*, *Hesperaloe*, *Nolina* and *Yucca*, are generally considered to belong to the Liliaceae (Correll and Johnston 1970) although some authors have placed them in the more specialized Agavaceae which also contains the evergreen rosette genus *Agave* (Hutchinson 1973). The four Liliaceous genera, which are the focus of this paper, are characterized morphologically by having a large woody caudex with numerous elongate, coriaceous leaves in large rosettes at the apex of the caudex or its branches (Correll and Johnston 1970). The morphological similarities of this group coupled with their occurrence in similar arid environments leads to the assumption that they may be similar physiologically. However, little is known about the physiology, and in particular the photosynthetic physiology, of the plants within this group. There have been a number of studies in which investigators made various assumptions about the photosynthetic physiology of plants in this group, usually based on little experimental data. It has frequently been assumed (Bender et al. 1973; Syvertsen et al. 1976; Kluge and Ting 1978; Wallen and Ludwig 1978) that these are CAM plants even though some data (Bender et al. 1973; Wallen and Ludwig 1978) suggest a photosynthetic metabolism more characteristic of C₃ plants. The photosynthetic metabolism of plants in the related genus *Agave* has been fairly extensively studied (Neales et al. 1968; Neales 1973; Eickmeier and Adams 1978; Nobel 1976; Nobel and Hartsock 1978) and has been the basis for some of the assumptions about photosynthesis in other evergreen rosette plants. Szarek and Troughton (1976) have also presented substantial evidence for CAM in *Yucca baccata*. However the extensive literature on CAM photosynthesis indicates that there is great variability in photosynthetic response among even closely related species or plants within a single species. Because the *Liliaceous* evergreen rosette plants are important components of desert vegetation (Little and Campbell 1943; Shreve and Wiggins 1964; Moir 1963; Weber 1953) as well as economically important (Weber 1953) it would seem prudent to understand how these plants are physiologically adapted to their arid environment. To this end the following experiments were undertaken with the objectives of determining (1) the photosynthetic pathway type of representatives of this group, (2) the effects of environmental parameters of moisture, temperature and irradiance on photosynthesis and the constancy of the photosynthetic pathway, and (3) the extent to which the photosynthetic responses foster adaptation of these plants to desert environments.

Materials and Methods

Plants
Young plants (10–30 cm tall) of the following six species were transplanted from their native southern New Mexico locations into 1 or 5 gallon buckets and grown in a greenhouse for four months before being transferred into a controlled environment chamber: *Yucca baccata* (Engelm) Trel. (acaulescent and broadleaved); *Yucca torreyi* Shafer (acaulescent and broadleaved); *Yucca campestris* Mc Kelvey (acaulescent, narrow leaved); *Yucca elata* Engelm. (acaulescent, narrow leaved); *Nolina microcarpa* Wats. (acaulescent, narrow leaved); and *Dasylirion wheeleri* Wats. (shortly caulescent, broad leaved) (nomenclature follows Correll and Johnston 1970). All plants were collected within 30 kilometers of Las Cruces, Doña Ana Co., NM, USA, except for *Yucca campestris* which was collected near Carlsbad, Eddy Co., NM, USA. Plants were grown under two temperature/photoperiod regimes in the controlled environment chamber. First, plants were grown at 12/12 h day/night photoperiod with 22/10 °C d/n temperatures with daily wa-
tering. After 4 weeks gas exchange and titratable acidity were measured on 3 replicates of each species. Water was then withheld for varying times (but no longer than 2 weeks) and gas exchange and titratable acidity were monitored as plants became water stressed. Upon completion of these measurements on water stressed plants, all plants were again watered daily and the photoperiod was changed to 14/10 h with 32/15°C d/n temperatures. Plants were grown under these conditions for four weeks before measuring gas exchange and titratable acidity. Water was then withheld from plants and gas exchange and titratable acidity were monitored as before. In addition to experiments with these small plants grown in environmental chambers, gas exchange was monitored in larger plants growing in a garden near the New Mexico State University campus, Dona Ana Co., NM, USA, and titratable acidity was monitored in plants growing in their natural habitats. The general environment in this region has been described by Little and Campbell (1943) and Buffington and Herbal (1965).

**Titrable Acidity**

Weighed samples of approximately 1 g of fresh leaf tissue, were macerated at high speed for 3 min in a small volume blender with 30 ml of distilled water. The water extract was collected and the residue was remacerated with 30 ml of distilled water twice more. The final volume of extract was brought to 100 ml and immediately titrated with 0.01 N NaOH to an end point of pH 7.0. Data are expressed as micro equivalents of acid per g fresh wt of tissue.

**Gas Exchange**

Carbon dioxide and water vapor exchange rates were measured using an open gas exchange system (Neales 1973; Nobel 1976). One to four (depending on size) of the most recently matured leaves were placed into a water-jacketed acrylic leaf cuvette. Carbon dioxide fluxes were monitored with a Beckman 215B differential IRGA and water vapor fluxes were monitored with an EG & G model 880 dew point hygrometer. Cuvette temperature and leaf temperature were measured with a shaded thermocouple and one taped to the bottom of the leaf, respectively. Gas exchange patterns were monitored on 3 plants of each species in the garden experiments and for the well-watered plants grown in the environmental chambers. However, the slightly different timing of CO₂ uptake patterns on a diurnal basis resulted in great variability in mean data particularly during those times when plants were switching from CO₂ evolution to CO₂ uptake. Thus, diurnal responses of gas exchange are shown only for a representative example which more clearly expresses the CO₂ exchange patterns during the course of the day.

**Water Potential, Chlorophyll Content, and Mesophyll Succulence**

The water potential of leaf tissue was assumed to be close to that of the leaf xylem pressure potential which was measured with a PMS xylem pressure chamber. Leaf chlorophyll content was measured by grinding 0.25 g fresh wt. of tissue (from the middle of the leaf) in 25 ml 96% ethanol plus 0.01 g MgCO₃. The extract was centrifuged (1,000 x g for 3 min) and the absorbance of the supernatent read on a Gilford model 2,600 spectrophotometer. Chlorophyll was determined as mg per g fresh wt. using the constants of Wintermans and DeMots (1965). Tissue dry weight was determined by drying leaf samples to a constant weight at 80°C (this required about 48 hrs). From the chlorophyll content and leaf dry and fresh weights the mesophyll succulence (Sₗ) of the leaf tissue was calculated as the ratio of tissue water content to chlorophyll content (Kluge and Ting 1978).

**Results**

The diurnal course of CO₂ exchange exhibited by plants grown in controlled environment chambers indicated that there were two distinct groups of plants; those that assimilated CO₂ in the dark under most environments tested, and those that did not assimilate CO₂ in the dark under any environment tested. These two groups can also be identified on the basis of changes in tissue acidity content (Table 1). D. wheeleri, N. microcarpa, Y. campesiris, and Y. elata did not assimilate CO₂ in the dark and did not have a diurnal change in tissue acidity of greater than 25 µeq g⁻¹. Y. baccata and Y. torreyi plants which were well watered exhibited CO₂ uptake in the dark and showed diurnal changes in tissue acidity of greater than 40 µeq g⁻¹. The diurnal course of CO₂ and water vapor exchange are shown in Figs. 1 and 2 for Y. torreyi and Figs. 3 and 4 for Y. baccata. Photosynthesis in Y. baccata and Y. torreyi was affected only slightly by changes in growth temperature and photoperiod. Both species, when well watered, exhibited uptake of CO₂ in the light as well as in the dark under both growth environments. Growth under the longer photoperiod and warmer temperature regime appeared to enhance the amount of CO₂ fixed in the dark. This was most pronounced in Y. baccata where the total dark CO₂ fixation at 32/15°C was about twice that at 22/10°C. When water was withheld from Y. baccata and Y. torreyi, plant water potentials declined within 1 week and this incipient water stress greatly reduced CO₂ fixation in the light and the dark (Fig. 5). Withholding water for two weeks produced moderately low leaf water potentials (Table 1) and resulted in the elimination of virtually all CO₂ uptake and evolution in both species in both cool and warm temperature regimes. However, there was still moderate stomatal opening in the daytime for most plants.

Transpiration rates in most of these droughted plants reached a maximum of near 30 mg H₂O m⁻² s⁻¹ early in the light period but declined to less than 10 mg H₂O m⁻² s⁻¹ in the dark.

Diurnal courses of gas exchange are not shown for the other species grown in controlled environment chambers, since CO₂ fixation was never a function of time of day but rather was typical of C₃ photosynthesis and normal dark respiration. For these latter species neither cool temperatures, shorter photoperiods, nor water stress induced the CAM responses of dark CO₂ assimilation or acid accumulation. These three environmental stimuli are the most important factors regulating dark CO₂ fixation in those species with the potential for CAM photosynthesis (Osmond 1978; Kluge and Ting 1978). Photosynthesis measured at a quantum flux density of 1,800 µE m⁻² s⁻¹ (400-700 nm) is shown as a function of leaf temperature for these non-CAM plants grown under cool and warm temperatures and at several levels of water stress (Fig. 6). Both growth temperature and water stress affected the pho...