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PHYSIOLOGY OF PHOTOSYNTHESIS IN CHIMALACATE (*Viguiera dentata*) IN THE ZAPOTITLAN DE LAS SALINAS VALLEY OF THE TEHUACAN BIOSPHERE RESERVE IN PUEBLA, MEXICO

[FISIOLOGIA DE LA FOTOSINTESIS DEL CHIMALACATE (*Viguiera dentata*) EN EL VALLE DE ZAPOTITLAN DE LAS SALINAS EN LA RESERVA DE LA BIOSFERA DE TEHUACAN PUEBLA, MEXICO]

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SUMMARY

Growing interest in understanding ecological deterioration phenomena and natural restoration of native landscapes has led us to initiate a research effort on a wild species which may be instrumental in the recovery of severely disturbed ecosystems. Here we report on the basic characterization of growth and gas exchange in the perennial desert shrub known as "chimalacate" (*Viguiera dentata*) in the Tehuacan Biosphere Reserve in Puebla, Mexico. Chimalacate plants grew almost twice as tall, developed twice as many tillers, showed 54% greater dry weight, 40% greater leaf expansion rate and over 5 times as many flowers when exposed to full sun as compared to plants growing in the shade. However, shaded plants developed 48% more leaf area per plant mainly due to more leaves per plant. Optimum leaf temperature for photosynthesis at high light intensity and ambient CO_2 concentration was 34°C. Light and CO_2 compensation points under a controlled environment ranged between 23 and 48 μmol m^{-2} s^{-1} and between 22 and 32 μL L^{-1} CO_2, respectively. The photosynthesis data presented indicates chimalacate is a C3 plant well adapted for growth in a hot dry climate.

Key words: *Viguiera dentate;* desert shrubs; plant growth; photosynthesis; transpiration; temperature.

RESUMEN

El creciente interés en la comprensión de los fenómenos asociados al deterioro ecológico y a la restauración natural de paisajes nativos ha llevado a investigar sobre una especie silvestre que puede ser fundamental en la recuperación de algunos ecosistemas severamente perturbados. El presente estudio reporta los resultados de mediciones *in situ* y en laboratorio para caracterizar el crecimiento y el intercambio gaseoso en el arbusto perenne del desierto conocido como "chimalacate" (*Viguiera dentata)* en la Reserva de la Biósfera de Tehuacán, en Puebla, México. Las plantas de chimalacate que crecieron a cielo abierto alcanzaron casi el doble de la altura, desarrollaron el doble de macollos y más de 5 veces el número de flores que las plantas que crecieron a la sombra de un dosel. Sin embargo, estas últimas desarrollaron 48% más área foliar por planta que a cielo abierto, mientras en esta condición, las plantas mostraron pesos secos 54% mayores y tasas de expansión foliar 40% más altas que las típicas de plantas que crecieron a la sombra. La temperatura óptima para la fotosíntesis a alta intensidad lumínica, a concentración ambiental de CO_2, fue de 34ºC. Los puntos de compensación lumínica y de CO_2 en ambiente controlado oscilaron entre 23 y 48 μmol m^{-2} s^{-1} y entre 22 y 32 μL L^{-1} CO_2, respectivamente. Los datos sobre fotosíntesis indican de manera concluyente que *V. dentata* es una planta C3 bien adaptada para crecer en un clima caliente y seco.

Palabras clave: *Viguiera dentata;* arbusto desértico; crecimiento; fotosíntesis; transpiración; temperatura.

INTRODUCTION

The Tehuacan-Cuicatlan Valley in Puebla, Mexico has been home of human groups for several thousand years and is regarded as the cradle of cultivated corn (Piperno and Pearsall, 1993). Recently, this area was declared a Biosphere Reserve by the Mexican Government and UNESCO in 1998, along with 12 other centers of biodiversity in the “Middle America” region, as it has been identified by the International

Union for the Conservation of Nature (Dávila and Herrera-MacBryde, 1997; Davis *et al*., 1997). A small area of hill country in the southwestern corner of this reserve, known as the Zapotitlan de las Salinas Valley, has been the object of much botanical research by several national and international groups for the past few years. Floristic listings, vegetation types and phytogeographic studies in the area are numerous (Dávila *et al*., 1993; Osorio-Beristain *et al*., 1996; Flores-Hernández *et al*., 1999). In general, these studies have focused on phytodiversity, endemism, plant community structure and plant population dynamics. However, since its conversion into a Biosphere Reserve, the region has also become a model to study ecological deterioration.

In Mexico and other countries, deforestation on a large scale for the incorporation of more land for cultivation has accelerated processes leading to severe soil erosion and has promoted the development of infertile soils (Landa *et al*., 1997; Brookfield, 1999; Ozturk, 1999), particularly in areas which are highly susceptible to excessive run off, as is the case in the Tehuacan area (Cuanalao de la Cerda *et al*., 1989). From a conservation perspective, demographic growth and the concomitant change in land use to facilitate agricultural and urban development were identified as the major threats to the native plant communities (Landa *et al*., 1997). Thus, there is an urgent need to generate the knowledge that may lead to an understanding of the processes involved in deterioration and restoration of such fragile environments. In this context, we have begun the study of the wild perennial shrub *Viguiera dentata* (Asteraceae), “chimalacate” as it is locally known, which can be used as fresh forage to feed goats, or for wood and honey production. Chimalacate is quite an invasive weed in cultivated plots as well as most any other space available. It is an herbaceous perennial which grows erect reaching up to 3 m height. It develops many hispid purple colored tillers on which opposite or alternate leaves develop whose foliar blades are oval to rhomboidal in shape, 3.5 to 12 cm long and 1 to 8 cm wide. Numerous bright yellow flowers grouped in panicles develop on 15 cm long peduncles; they grow elliptic in shape, and large heads set up to 35 fruits of the type known as achene, each 3.5 to 4 mm long and provided with 2 to 3 mm long awns on the vilan (Rzedowski and Rzedowski, 2001). This species shows remarkable phenotypic plasticity, considerable drought and heat resistance and abundant seed production in the fall and winter; protein and oil content in the seeds average 25% each, and 1000 seed weight averages 1.4 g (Jones and Earle, 1966). These characteristics have led ecologists to use similar species as deterioration “indicators” in other areas of the world (Breckenridge *et al*., 1995; Rapport *et al*., 1998). For instance, the photosynthetic responses of plants to current climate change will determine their survival and performance and consequently their competitive ability, which may lead to long-term changes in biomass accumulation at a plant or community level (González-Rodríguez *et al*., 2005; Prieto *et al*., 2009). Such characterization may underestimate the relevance of these species as initiators of a succession process that may eventually lead to natural restoration of native plant communities.

This study focuses on describing the growth response associated with variation in exposure to sunlight and the physiological characterization of photosynthesis for *V. dentata,* as part of the diagnostic work which involves the description of the ecologically and anthropocentrically important plant species in the study site**.**

MATERIAL AND METHODS

Study site

The Zapotitlan de las Salinas Valley is the southernmost portion of the larger semiarid Tehuacan-Cuicatlan Valley. The area (2,193 km^2) is located on the limits between the states of Puebla and Oaxaca in the southern part of Central Mexico (between 17°39´ and 18°53´N and between 96°55´ and 97°44´W). The small Zapotitlan Valley located within the Cerro Viejo and Cerro Zotoltepec mountain ranges at elevations between 900 and 2,100 masl. Average annual rainfall is 600 mm, distributed from May through November, but most rainfall occurs between July and September. However, maximum rainfall over a 24 hr period can be as high as 140 mm (Cuanalao de la Cerda *et al*., 1989). Mean annual temperature is 17.7°C, but large fluctuations characterize the diurnal and seasonal patterns, with minimum temperatures in winter often falling below freezing and maximum temperatures in the summer reaching 42°C in the shade. The dry conditions of the climate are due to the rain shadow effect on the valley by the Sierra Madre Oriental that runs along the coast of the Gulf of Mexico and the gradual depletion of underground water (Villaseñor, 1990). Soils in the valley are eutric regosols (FAO/UNESCO, 1990) and the landscape has a history of dramatic geological change on a large scale (Brunet, 1967). The study site is a densely populated chimalacate community (3 plants m^{-2}) that has been undisturbed for the past 7 years. Prior to the development of this chimalacate community, the area was cultivated with corn or beans on a regular basis at least every other year.

Growth characterization

Two sites within the study area were selected to compare growth parameters of *V. dentata*. In site A, plants grew in the open, under unobstructed sunlight (approximately 1,800 µmol m^{-2} s^{-1}) during the length of the natural photoperiod. In site B, plants grew under the shade of an old *Prosopis laevigata* tree next to the chimalacate field, which effectively blocked about 40 to 60% of sunlight (approximately 700 to 1,000 µmol m^{-2} s^{-1}) during most of the day over the growing season.

Vegetative growth in chimalacate goes on continuously; however, the bulk of new dry matter production occurs over the months of the short rainy season, from July through November; the plant flowers profusely in the fall, from October through December and sets seed from November through January. From then on and until July, very little growth occurs as the dry period sets in rapidly and temperatures reach limiting values as early as February. Thus, in order to compare the extent of growth under the described contrasting light regimes in the field over the most relevant period within a full growing season, plants as similar as possible within the study area were selected and marked in early July of 2007, when the rainy season began. About seven months later, in early February 2008 after seed had matured, growth parameters were recorded on the plants previously marked. Measurements included plant height, number of tillers, total leaf area (estimated by means of a LI-3000A portable leaf area meter, LI-COR, Lincoln, NE) and above ground fresh and dry weight. Additionally, leaf primordia from plants at the two selected sites were marked to measure leaf expansion rate by recording leaf area on a daily basis until the leaf reached full size. Leaf water potential (MPa) was estimated in the field from midday measurements of xylem tension made with a Scholander pressure bomb (SoilMoisture Equipment Corp., Santa Barbara, CA). Growth measurements were made on 5 plants per site. Xylem tension measurements were replicated on each plant by sampling 3 to 5 young tillers cut 15 cm down from the apex, with at least 3 fully extended leaves.

In situ gas exchange

Gas exchange measurements were made *in situ* with a portable system (LI-6400, LI-COR Lincoln, NE) configured as an open system, at midday on at least 10 fully expanded upper leaves on 5 plants chosen at random at sites A and B. Source of CO_2 was ambient air passed through a 20 L bottle, which effectively provided an air stream that did not fluctuate significantly around 360 µL L^{-1} of CO_2 flowing through the leaf chamber during the incubation period. Individual leaves were clamped into a remote leaf chamber mounted on a tripod to avoid artifacts due to excessive manipulation. The leaf was allowed to equilibrate until a steady state photosynthesis rate was achieved, which usually did not exceed 4 to 5 min. The leaf was further incubated for 30 to 45 s before photosynthesis rate was recorded along with leaf and air temperature, leaf vapor pressure deficit (Vpdl) and air vapor pressure deficit (Vpda), stomatal conductance (g), transpiration rate, ambient (Ca) and intercellular (Ci) CO_2 concentration, Ci/Ca ratio, and photosynthetically active radiation (PAR) directly from the automated LI-6400 console.

Temperature dependence of photosynthesis; light and CO_2 compensation points

Seeds were collected in January of 2008, germinated in 20 L pots filled with soil from the location. The pots were placed at the two experimental sites among the randomly selected plants used to compare growth characteristics between plants exposed to full sunlight and shade grown plants. Each pot contained 1 plant and a total of 5 pots were placed at each site. These potted plants were grown for one entire growing season under field conditions. In June 2009, these plants were alternatively brought into the lab and used to determine basic photosynthetic metabolism under controlled conditions and for determination of light (CP_l) and CO_2 ($CPco_2$) compensation points under a controlled environment made to vary according to a pre-established pattern.

On each day when measurements were conducted, one potted plant was brought from the field into the lab (usually in less than 60 min) at about 9:00 am. The pot was watered to field capacity and the plant was allowed to acclimate to ambient conditions in the lab (430 µmol m^{-2} s^{-1}, 23 ± 2°C) until a steady rate of photosynthesis could be measured, usually within 60 min. Next, a fully extended upper leaf was clamped into the leaf chamber and the measuring conditions were set. A further equilibration interval of 5 or 6 min was conducted, which was followed by a measuring period of less than 1 min. This scheme was repeated at least 4 times to do each, CP_l and $CPco_2$ evaluation. Under the conditions described, variation among leaves and plants over the many days on which characterizations were made was negligible.

First, an experiment was designed to document the photosynthetic response of chimalacate leaves to air temperature under conditions which simulated a typical summer day in the Zapotitlan Valley, i.e., high light intensity (1,800 µmol m^{-2} s^{-1}), 340 to 380 µL L^{-1} CO_2 and a high evaporative demand (30% relative humidity and Vpda at 3.2 kPa), but nonlimiting water availability, which was provided to stimulate the unrestricted expression of photosynthetic potential under the conditions described.

Secondly, CP_l and CP_{CO2} were estimated from regression analysis on separate experiments in which

CO_2 concentration or light intensity in the leaf chamber were gradually increased from zero or decreased towards zero while all other variables were held constant (57% relative humidity, Vpda at 1.7 kPa, 34°C air temperature and 1,800 ± 200 μmol m^{-2} s^{-1} or 380 ± 24 μL L^{-1} CO_2 for CP_{CO2} and CP_l, respectively). The stepwise increase or decrease of light intensity or CO_2 concentration in the leaf chamber required a period of at least 3 h to ensure gradual and complete acclimation to each level of intensity/concentration. Usually, an interval of 20 min proved adequate at each level; thus, when decreasing, light intensity was turned down at an average rate of 10 μmol min^{-1} from 1,800 μmol m^{-2} s^{-1} to complete darkness over an interval of 3 h. In practice, the stepwise decrease/increase in light intensity followed a reproducible nine-step pattern in which intensity was reduced/increased by 200 μmol m^{-2} s^{-1} approximately every 17 min and 3 min equilibration followed, prior to recording measurements to ensure steady state assimilation rates. Similarly, the stepwise decrease/increase in CO_2 concentration followed a 9 step pattern in which concentration was reduced or increased by 50 μL L^{-1} CO_2 approximately every 17 min with 3 min equilibration time following, prior to recording measurements. Larger magnitude changes were less convenient, as longer equilibration times were required. For instance, when 400 μmol m^{-2} s^{-1} changes in light intensity were used, equilibration times were longer than 25 min and a steady state rate of photosynthesis was difficult to achieve.

PEP carboxylase *in vitro* activity

In order to determine conclusively if chimalacate is a C3 or a C4 species, phosphoenol-piruvate (PEP) carboxylase *in vitro* activity was assayed (Arnosis and Barneix, 1989). Briefly, mature, fully expanded leaves were sampled at random from plants growing in the field under various environmental conditions, placed in liquid nitrogen and stored at -70°C until assayed. Five independent leaf tissue samples (0.5 g) were homogenized under liquid nitrogen in 2.5 mL of extraction buffer consisting of 100 mM Hepes-KOH (pH 7.3), 50 mM $MgCl_2$, 2.5 mM EDTA, 2.5 mM DTT, 1 mM PMSF and 1 mM 2-β-mercaptoethanol. The extract was filtered through cheesecloth and centrifuged at 13,000 rpm for 3 min. The assay mixture contained 100 mM Hepes-KOH (pH 7.3), 0.2 mM NADH, 10 mM $MgCl_2$, 5 mM PEP, 10 mM $NaHCO_3$, 4 μg mL^{-1} MDH and 0.2 mg mL^{-1} of extract. The reaction was started by addition of the extract and was run for 10 min at 30 °C. PEP carboxylase activity was reported as absorbance readings (A_{340} nm; UV/Vis Lambda 12 Spectrometer, Perkin Elmer, Norwalk, CT) caused by oxidation of NADH. Partially purified maize-PEP carboxylase, a fresh maize extract, a blank and a potato leaf extract were included in each run as checks. The assay was replicated five times for mean comparison. Additionally, light microscopy on leaf tissue was conducted to determine the presence of a vascular bundle sheath, i.e., Kranz anatomy characteristic of C4 plants.

RESULTS AND DISCUSSION

Growth characterization

Figure 1 offers some views of chimalacate plants growing under the environmental conditions of the Tehuacan-Cuicatlan Valley in Puebla, Mexico.

Table 1 summarizes the data on the growth of chimalacate plants over a seven month period, from the beginning of the rainy season until the termination of flower senescence. Plants in the open under full sun grew almost twice as tall, developed twice as many tillers, produced over 5 times as many flowers, and showed 54% greater dry matter weight and 40% greater leaf expansion rates than shaded plants. However, shaded plants developed 48% more leaf area per plant than plants grown in full sun. A number of native grasses in southern Arizona showed similar growth differences when growing in full sun or association with a diversity of overstory plant canopies (Livingston *et al*., 1997). These marked phenotypic differences in growth within a relatively small area are as striking as the phenotypic differences observed among chimalacate plants growing in quite diverse habitats and climatic conditions. Whether the differences are due to true genotypic variability or phenotypic plasticity is currently unknown.

Leaf dry weight was a small fraction (5 and 10% for sun and shade plants, respectively) of total dry weight per plant, while fast elongating and highly branched tillers accounted for up to 95% (sun plants) of the newly allocated dry matter. Competition for light, a process often associated with the extent of stem elongation, did not result in shaded plants growing taller than plants in full sun as might be expected. Additionally, more tillers and flower heads were developed in the sun than in the shaded plants. In contrast, a large difference in leaf area favored understory plants. In summary, these data indicate that aerial biomass accumulation by chimalacate was reduced in absolute terms under a canopy, basically due to the effect of shade on tiller formation and tiller elongation, while the light regime in the shade was not inductive of profuse flowering as is normally the case for this species growing in the sun, where one single plant may produce over 65,000 seeds, as estimated from seed number on sample flower heads, average number of flower heads per tiller and average tiller number per plant. Seed quality was not evaluated in this study.

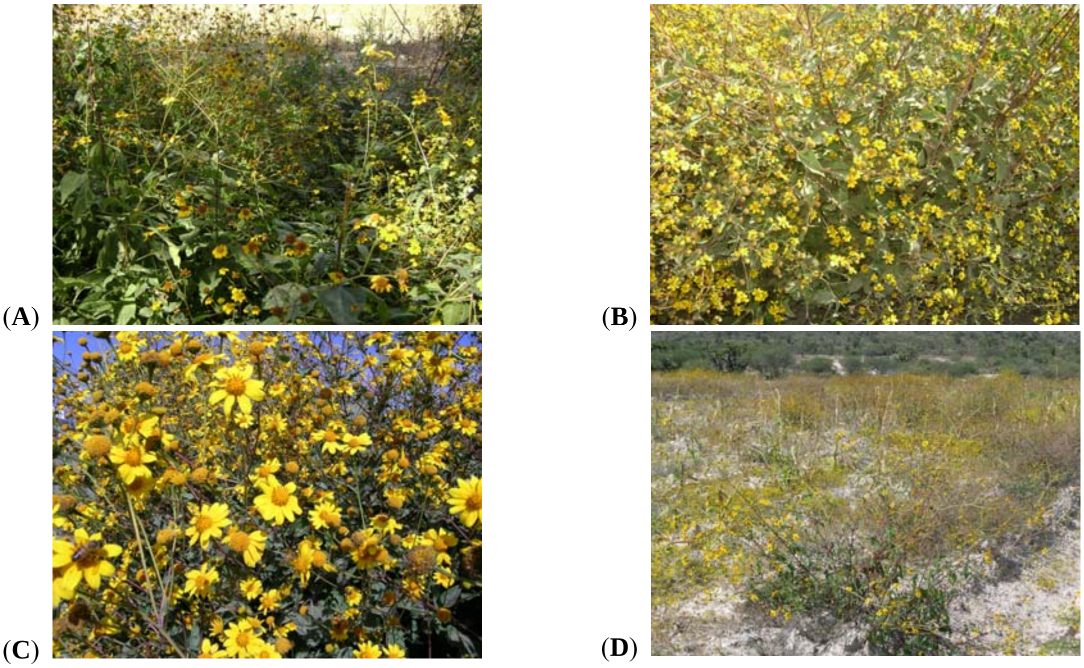

Figure 1. *Viguiera dentata* plants in the Tehuacan-Cuicatlan Valley, Puebla, Mexico. Panels A and B show the typical plants growing under a *Prosopis laevigata* canopy and in the sun, respectively, as the plants used in this study; C, profusion of flowers in a sun plant, note bee on lower right corner; D, a cultivated plot invaded by *V. dentata*.

Table 1. Growth characteristics of chimalacate (*V. dentata*) plants exposed to full sun or shaded environmental conditions with the evaluation conducted after flower senescence, approximately seven months after initiation of the study period.

	Environmental Growth Conditions	
Variable	Sun	Shade
Plant height (m)	1.84±0.49	0.97±0.32
No. of tillers per plant	30.70±10	14.40±6
Leaf area (m^2 per plant)	0.27±0.05	0.40±0.03
Fresh weight (g per plant)	2,905.00±347	1,960.00±503
Number of flowers per plant	2,072.00±649	388.00±200
Leaf dry weight (g per plant)	102.00±26	135.00±43
Dry weight (g per plant)	2,016.00±258	1,329.00±301
Leaf expansion rate (cm^2 day^{-1})	2.10±0.29	1.50±0.15

Values are means of 5 independent plants, followed by confidence intervals (P=0.95).

It is well established that desert plants tend to have small leaves compared with closely related species living in less extreme environments (Smith and Nobel, 1977; Miller and Poole, 1979; Gill and Mashall, 1986). This adaptation acts by increasing convective heat dissipation which cools the leaf, which in turn helps to reduce the water vapor concentration gradient between leaf and air, the driving force of transpirational water loss (Smith, 1978). The net result of these events is a significant increase in water use efficiency (Smith and Nobel, 1977; Smith, 1978). Although the area of individual leaves was not evaluated, visual observations of leaf size indicated that this species looks very much like many broadleaf mesophytes, and that shaded chimalacate plants had greater total leaf area per plant due to more leaves present, but not significantly larger leaves than those on sun plants. In fact, using instantaneous measurements of assimilation and transpiration rates we did not find any diffference in water use efficiency (WUE) between sun and shade chimalacate leaves. WUE values ranged between 5.71 and 10.13 g CO_2 Kg^{-1} H_2O, irrespective of plant location and apparently depending more on the individual plant water status.

***In situ* gas exchange**

Table 2 shows midday gas exchange data for sun and shade grown chimalacate plants on a typical summer day in the Zapotitlan de las Salinas Valley in June 2009. Shaded plants, with less than one third of the light incident of sun plants, showed significantly lower photosynthesis and transpiration rates, although ambient CO_2 and Vpda were similar in the two environments. Under these conditions, a one degree difference in air temperature translated into a 6% difference in ambient relative humidity and almost twice the value of g. Sun plants showed twice the transpirational loss as compared to shade plants, with a -0.33 MPa difference in leaf water potential commonly observed between sun and shade plants around solar noon. Similarly, Zhang *et al*. (1995) reported a greater photosynthetic rate in sun leaves than in shaded leaves for the C3 desert species *Encelia farinosa*, an effect that was largely accounted for by the exponential increase in g as leaf water potential increased from -3.5 MPa to -0.5 MPa. Indeed, it is generally accepted that the contribution of g to limitations in photosynthesis becomes more important as the soil becomes dry and water stress develops in the plant. Plants regulate transpiration via both short-term changes in crown-scale stomatal conductance in response to variation in light, vapor pressure deficit and leaf water potential, and long-term changes in canopy leaf area and the structure of the roots and shoots that supply the canopy with water (Bréda *et al*., 2006; McDowell *et al*., 2008).

Temperature dependence of photosynthesis; light and CO_2 compensation points

Plants which are native to hot environments generally exhibit higher photosynthetic rates at high temperatures and optimum photosynthetic rates occur at higher temperatures in comparison with plants which are native to cool environments. We do not know for a fact what sort of climate chimalacate is native to, but this shrub is widely distributed in the semiarid regions of Central Mexico. As shown in Figure 2, gross assimilation activity in chimalacate leaves increased little from 25 to 31°C, but surged rapidly from 19.5 μmoles CO_2 m^{-2} s^{-1} at 31°C to a maximum rate of 29.4 μmoles CO_2 m^{-2} s^{-1} as leaf temperature increased to an optimum of 34°C (37°C in the air), from which point a gradual decline brought the rate back down to 24.3 μmoles CO_2 m^{-2} s^{-1} at a leaf temperature of 38°C (43°C in the air). Similar temperature optima for assimilation have been recorded for several C3 desert species which are well adapted to hot and dry climates, including *Larrea divaricata, Heliotropum curassivicum, Nerium oleander* and several C3 shrubs from Western Australia (Berry and Björkman, 1980). Using the temperature optimum, response curves for photosynthesis rate by a chimalacate mature leaf to increasing PAR or CO_2 concentration were obtained (Figure 3). Results indicated that 50% maximum apparent assimilation rate occurred at a PAR flux of 350 μmol m^{-2} s^{-1} -at ambient CO_2 (Figure 3A) and at about 200 μL L^{-1} CO_2 -at a PAR flux of 1500 μmol m^{-2} s^{-1} (Figure 3B) in the leaf chamber.

Table 2. Midday gas exchange physiological variables in chimalacate (*V. dentata*) plants under ambient conditions on a typical summer day in the Zapotitlan de las Salinas Valley.

	Environmental Growth Conditions	
Variable	Sun	Shade
PAR	1800.00±185	500.00±140
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	26.30±4.8	14.60±5.8
Transpiration (mmol H_2O m^{-2} s^{-1})	6.55±2.2	3.46±0.67
Ambient CO_2 (μmol mol air^{-1})	375.00±60	419.00±45
Intercellular CO_2 (μmol mol air^{-1})	193.00±38	211.00±23
Relative Humidity (%)	36.00±3.1	42.00±3.4
T_a (°C)	30.30±0.14	29.30±0.15
T_l (°C)	30.50±0.17	28.60±0.11
Ci/Ca	0.52±0.02	0.49±0.03
Vpda (kPa)	2.11±0.19	2.11±0.21
Vpdl (kPa)	2.14±0.03	1.93±0.09
g (μmol H_2O m^{-2} s^{-1})	0.27±0.06	0.15±0.03
Midday Leaf Water Potential (MPa)	-1.18±0.12	-0.85±0.15

Values are means of at least 10 independent measurements made on as many leaves on each of two sets of plants located one in the sun and one in the shade, except for xylem tension measurements, which were replicated on each of 5 plants per site, by sampling 3 to 5 young tillers cut 15 cm down from the apex, with at least 3 fully extended leaves. Values are followed by confidence intervals (P=0.95).

Maximum fixation rate attained was 28 µmol CO_2 m^{-2} s^{-1} at saturating levels of CO_2 or PAR (above 480 µL L^{-1} CO_2 or 1,500 µmol m^{-2} s^{-1}, respectively). It is important to bear in mind that the temperature dependence of photosynthesis in chimalacate reported here may be of limited significance in the field, as it is well known that, even for a single leaf, temperature dependence of photosynthesis is strongly influenced by other factors, among which, light intensity and intercellular CO_2 partial pressure have especially pronounced effects (Berry and Björkman, 1980).

The high level of PAR required for light saturation of the photosynthetic response led to closer scrutiny of CO_2 fixation to determine whether the plant may actually be a C4 species. Light (CP_l) and CO_2 (CP_{CO2}) compensation points were estimated by regression from separate experiments in which CO_2 concentration or light intensity in the sample chamber were gradually increased from zero or decreased towards zero, while all other variables were held constant (57% relative humidity, Vpda at 1.7 kPa, 34°C air temperature and 1,800 µmol m^{-2} s^{-1} or 360-380 µL L^{-1} CO_2 for CP_{CO2} and CP_l, respectively). CP_{CO2} and CP_l were invariably greater when CO_2 concentration or light intensity were increased from zero, than when gradually decreased zero, which underlines the importance of light and CO_2-mediated processes involved in the activation of the photosynthetic machinery. CP_l was estimated at 48 µmol m^{-2} s^{-1} as PAR was increased and 23 µmol m^{-2} s^{-1} when PAR was decreased (Figure 4). Similarly, CP_{CO2} was estimated at 32 µL L^{-1} when CO_2 concentration was increased and 22 µL L^{-1} when CO_2 concentration was decreased (Figure 5). Both, CP_l and CP_{CO2} estimates in either condition were two to four times greater than typical values reported for C4 species (Imbamba and Teiszen, 2006; Sikolia *et al*., 2009).

PEP carboxylase activity

Extracts from either shade or sun chimalacate leaves did not show PEP carboxylase activity as compared to the maize samples (Table 3) indicating chimalacate is a C3 species. Additionally, the lack of Kranz anatomy, as observed by means of light microscopy on transverse sections of sun or shade leaves (data not shown), the observed optimum temperature for photosynthesis, the CP_l and CP_{CO2} values and the gas exchange data provide conclusive evidence of the C3 nature of chimalacate.

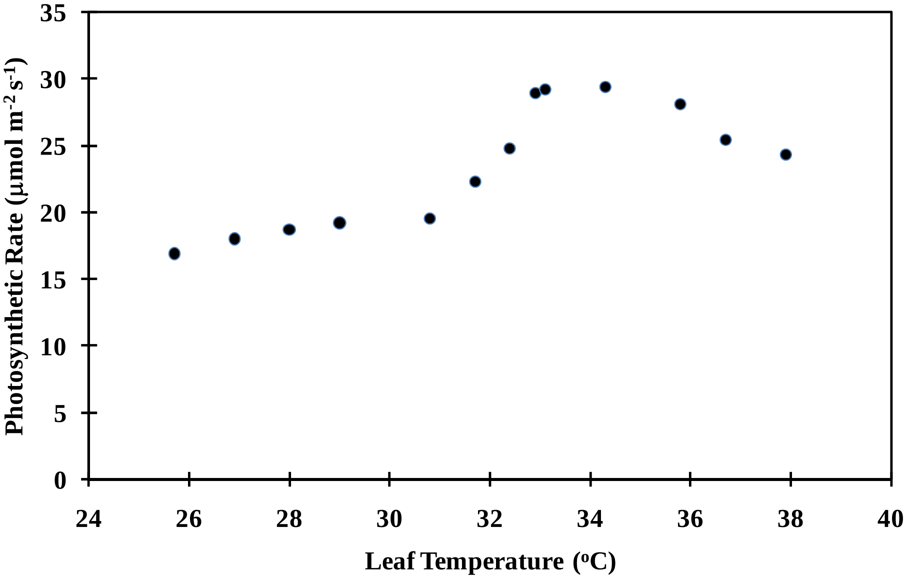

Figure 2. Effect of temperature on rate of photosynthesis by chimalacate (*V. dentata*) mature leaves. Potted plants were grown from seed and kept in the field among intact sun or shade plants. Leaves were incubated in a leaf chamber and allowed to equilibrate at the desired temperature for 20 min prior to photosynthesis measurement (See Materials and Methods).

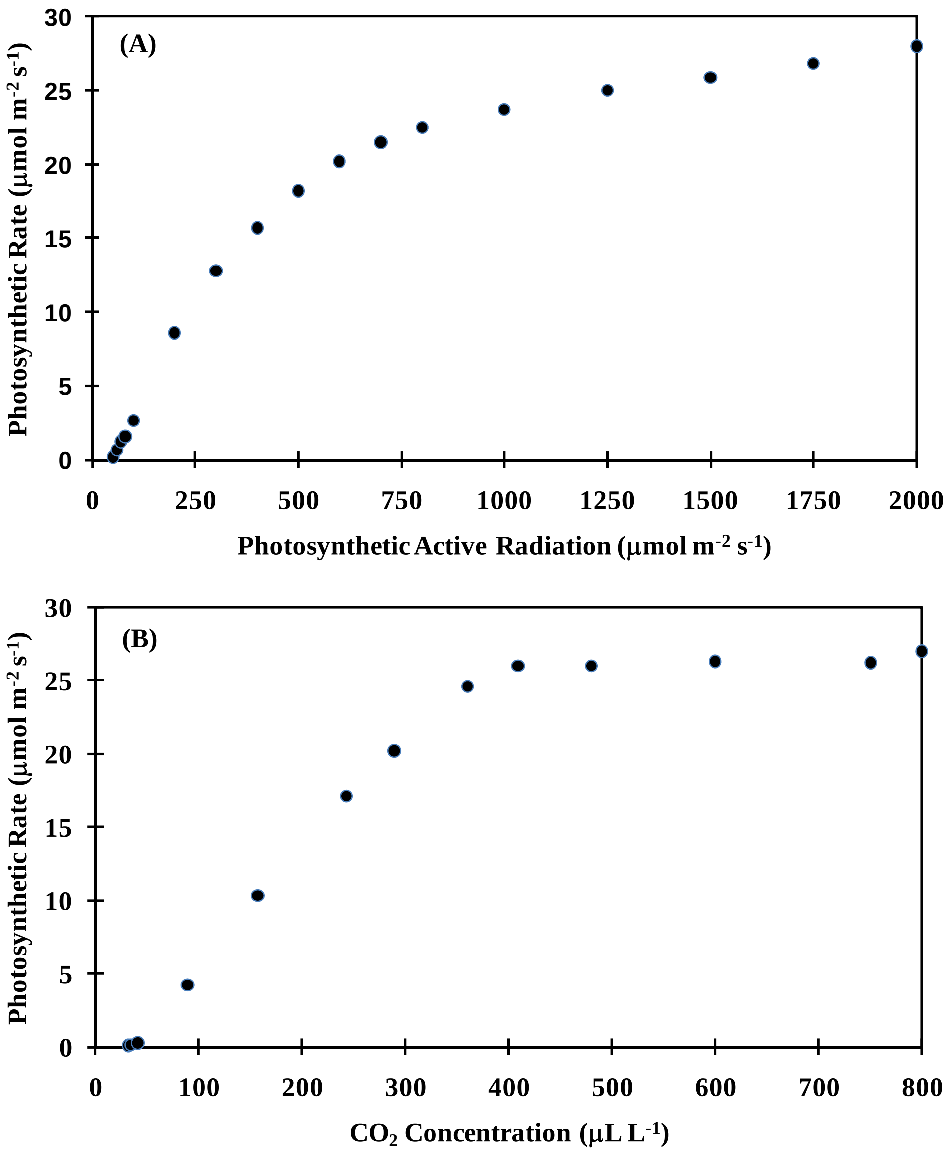

Figure 3. Effect of photon flux density (**A**) and CO_2 concentration (**B**) on photosynthetic rate by chimalacate (*V. dentata*) mature leaves. Potted plants were grown from seed and kept in the field among intact plants. Leaves were incubated in a leaf chamber and allowed to equilibrate at the desired temperature for 20 min prior to photosynthesis measurement (See Materials and Methods).

Table 3. *In vitro* PEP carboxylase activity in leaf extracts from field grown chimalacate (*V. dentata*) plants exposed to contrasting light regimes.

Sample	A_{340}	A_{340}^{*}	% Variation
Blank	0.825±0.022	0.835±0.013	+1.2
Partially purified maize PEPase	0.803±0.007	0.427±0.011	-46.8
Maize	0.905±0.021	0.350±0.028	-61.3
Chimalacate (unshaded)	0.814±0.020	0.818±0.025	+0.5
Chimalacate (shaded)	0.842±0.030	0.868±0.034	+3.1
Potato	0.900±0.015	0.884±0.009	-1.7

*The reaction was allowed to proceed for 10 min at 30°C. Readings remained stable for at least 3 h after reaction was stopped. Values are means of 5 independent samples followed by confidence intervals (P=0.95).

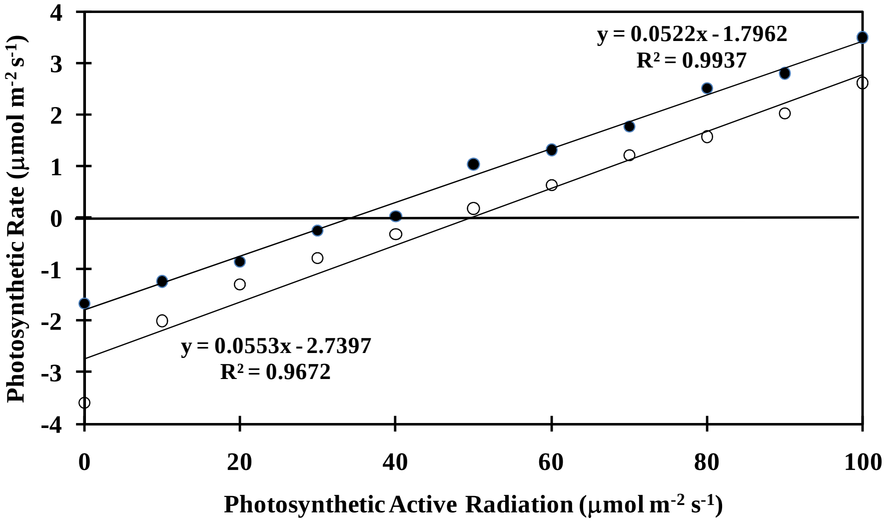

Figure 4. Light compensation point for chimalacate (*V. dentata*) photosynthetic activity. PAR was either increased from zero or decreased towards zero at regular flux and time intervals to allow for leaf equilibration within the chamber prior to recording photosynthetic rates (See Materials and Methods). Open and closed symbols correspond to regression data for decreasing and increasing PAR, respectively.

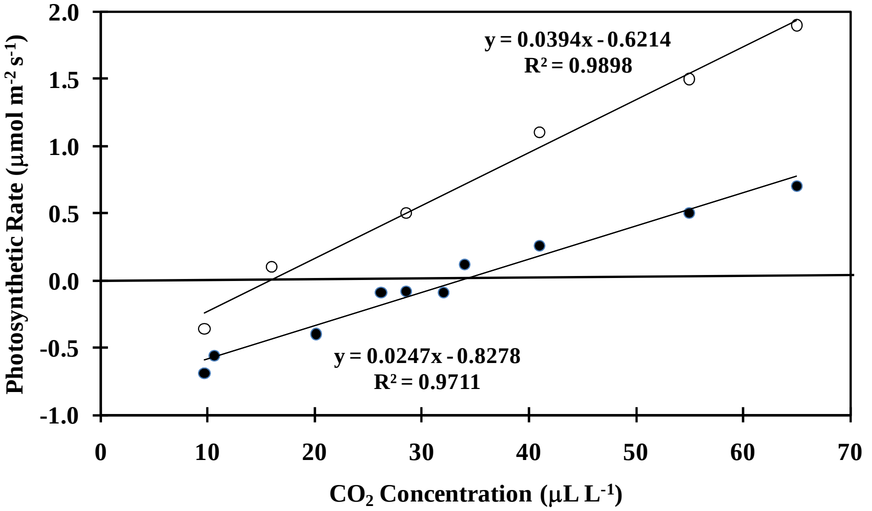

Figure 5. CO_2 compensation point for chimalacate (*V. dentata*) photosynthetic activity. CO_2 concentration was either increased from zero or decreased towards zero at regular concentrations and time intervals to allow for leaf equilibration within the chamber prior to recording photosynthetic rates (See Materials and Methods). Open and closed symbols correspond to regression data for increasing and decreasing CO_2 concentration, respectively.

In order to investigate the ecological significance of this species in terms of restoration of the native plant communities in the Zapotitlan de las Salinas Valley, follow up research should focus on the microenvironmental conditions which prevail under a chimalacate canopy, as opposed to those prevailing on bare soil.

CONCLUSION

In situ gas exchange measurements and laboratory experiments to determine: photosynthesis dependence on light and CO_2, light compensation point, CO_2 compensation point and phosphoenol piruvate carboxylase activity, have made it clear that chimalacate (*V. dentata*) is a C3 plant. As environmental conditions allow, chimalacate may grow more or less rapidly and vigorously, invading even severely limiting environments in the dessert. Our observations and measurements provide a foundation for further study of this perennial shrub which is of much importance both ecologically and anthropologically, as it plays a pivotal role on the natural reconstruction of vegetation while at the same time, it is also an important component of economic activity in the region of the large Tehuacan-Cuicatlan Valley.

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