**Contrasting Photosynthetic Behavior in Leaves and Twigs of Hymenoclea salsola, a Green-Twigged Warm Desert Shrub**

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# CONTRASTING PHOTOSYNTHETIC BEHAVIOR IN LEAVES AND TWIGS OF HYMENOCLEA SALSOLA, A GREEN-TWIGGED WARM DESERT SHRUB[1]

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ABSTRACT

The photosynthetic behavior of leaves and twigs was compared in *Hymenoclea salsola* T. and G., a subshrub of the Mohave and Sonoran deserts, in which both leaves and green twigs make substantial contributions to whole-plant carbon gain. Light saturated photosynthesis in twigs was 0.62 times that of leaves (36.9 $\mu$mol $m^{-2}$ $s^{-1}$) when plants were well watered. Similar ratios were consistently observed in contrasting the photosynthetic responses of the two organ types to light, temperature, and intercellular $CO_2$, regardless of whether rates were compared under saturating or highly limiting conditions of light or intercellular $CO_2$. These scalar differences in photosynthetic rate between leaves and green twigs under a wide range of conditions were correlated with contrasting anatomical features such as chlorenchyma volume per projected area. Under normal ambient $CO_2$ concentrations (350 $\mu$l $l^{-1}$), twigs on well watered plants operated at lower intercellular $CO_2$ concentrations than the leaves. Possible causes of this difference are discussed with respect to performance under well-watered conditions, organ lifespans, and contrasting anatomical constraints. Twigs require larger investments than do leaves of both carbon and nitrogen per projected area of the respective organs, yet they realize lower photosynthetic rates per intercepted light. Twigs, however, fulfill additional roles besides photosynthesis such as structural support and vascular transport which does not allow them to be as anatomically specialized as leaves for photosynthesis. Twigs also have a longer expected lifespan than leaves with a larger fraction of them surviving the summer drought period. This was correlated with a greater tolerance of twig than leaf photosynthesis to low plant water potentials.

THE OCCURRENCE of functional chloroplasts in the cortical tissue of the stem is quite widespread in Angiosperms, especially among deciduous trees and shrubs (Schaedle, 1975). In most nondesert species, however, it is well established that these weakly developed chlorenchymous tissues serve only to partially offset respiration rates of illuminated twigs and stems (Keller, 1973; Foote and Schaedle, 1978; Parker, 1978; Coe and Mclaughlin, 1980). Such stems have a high resistance to gaseous diffusion through the well-developed periderm, and only endogenously derived $CO_2$ is utilized by the cortical chloroplasts (Brayman and Schaedle, 1982). In contrast, the stems and twigs of many desert shrub species have thick chlorenchyma layers. Associated with these cortical chlorenchymas are specializations in stem anatomy such as delayed formation of periderm and the retention of functional stomata (Cannon, 1908; Gibson, 1983). The stems and twigs of a few of these desert species have been shown to possess positive net photosynthetic rates (utilizing atmospheric $CO_2$) (Adams, Strain, and Ting, 1967; Adams and Strain, 1969; DePuit and Caldwell, 1975; Szarek and Woodhouse, 1978). Based on anatomical structure, sizable fractions of the Mohave and Sonoran desert floras are believed to do so (Cannon, 1908; Gibson, 1983).

While some of these green-stemmed desert shrubs are completely aphyllus, many species have functional leaves as well as green stems/twigs, at least during the favorable growing seasons. While the presence of photosynthetic stems and twigs as a major assimilatory pathway in carbon gain has been established in the tree *Cercidium floridum*, its importance in other species is poorly understood.

*Hymenoclea salsola* is a dominant subshrub of the Mohave and Sonoran deserts of North America. After winter/summer rains, new growth is initiated in which both green leaves and green twigs are produced. Both leaf and twig tissues are thus present during the periods of peak seasonal productivity. As drought stress

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develops, the drought-deciduous leaves wither as do, eventually, many of the twigs. A portion of the twigs remains green until their second spring season at which time they may flower. The phenology differences between leaves and twigs in *H. salsola* and a number of other photosynthetic-twig shrubs are currently being studied under field conditions. The purpose of this study was a comparison of leaf and twig photosynthesis in *H. salsola.* We have measured the photosynthetic and transpirational responses of leaves and twigs of *H. salsola* to light, temperature, intercellular $CO_2$, and predawn plant water potential.

METHODS—For laboratory work, young individuals of *Hymenoclea salsola* were collected from field sites near Oatman, Arizona, in the spring of 1984. Field-collected material was transferred to tube pots (PVC pipe) 0.155 m in diameter and 0.75 m tall with a soil volume of 12 liters. Plants were then grown at the University of Utah in a naturally-lit greenhouse supplemented with HID lamps (an alternating bank of Na-vapor and Metal Halide lamps) to create a daily photon flux total of 40–50 mol $m^{-2}$ (400–700 nm). Gas exchange measurements were made during winter months, at which time daily greenhouse temperature ranges were approximately 33/20 C (day/night) and midday relative humidity was approximately 10%.

The potting soil was porous to simulate the soils of desert washes and consisted of vermiculite, perlite, peat moss, redwood compost, autoclaved soil, silica sand, and dolomitic sand in ratios of 2:2:1:2:2:3:3. N-P-K was administered from Peter's General Purpose 20-20-20 fertilizer mixture dissolved in the water supply at a concentration of 200 ppm per nutrient. Available nitrogen was as 5.61% nitrate, 3.96% ammonia, and 10.93% urea. Plants treated to decreased soil water availability were watered daily with smaller amounts of water from the same water supply as well-watered plants. Predawn leaf water potentials were measured with a Scholander pressure chamber.

For measurements of internal anatomy, one centimeter long sections of leaf and twig organs were fixed in 2% paraformaldehyde with a 0.1 M Na cacodylate buffer (pH = 6.9), dehydrated in ethanol, embedded in Spurr's resin (Spurr, 1969), sectioned at 2 $\mu$m, and stained with toluidine blue. Twig sections were taken from current season shoots 0.1 m below actively growing apices, and leaf sections were taken from the center of a leaf adjacent to the twig section. To evaluate the percent of the surface area occupied by different tissue types, the degree measure of the respective radial sectors was summed (both organs were cylindrical in shape). Measurements taken in this fashion relate tissue organization to total surface area and the intrinsic structure of the organ; measurements taken with respect to projected area relate anatomical features to expected light interception. Tissue volumes were assumed to be proportional to cross-sectional area, and projected area was proportional to the diameter of the cylindrical organs. Stomatal density was measured by taking a surface impression with clear nailpolish and examining it under the light microscope. Stomatal slit length was measured on electron micrographs for which specimens were fixed, dehydrated in ethanol, and dried by the critical point method from $CO_2$ (Anderson, 1951).

Gas exchange measurements were conducted on an open gas-exchange system (Ehleringer, 1983). Due to the small size of leaves and short internode lengths, neither leaf nor twig tissues could be measured in isolation from each other without manipulations of the plant material. The gas exchange rates of leaves were calculated using two different methodologies. For the measurement of leaf and twig gas exchange activity in response to changes in irradiance and temperature, measurements were taken first on a whole shoot and then subsequently on the twig alone after leaf removal. Leaf photosynthesis rates and conductance were calculated using an area weighted relationship between total shoot flux and the partial fluxes of the two component tissues:

$$\text{Flux}_{\text{shoot}} * \text{Area}_{\text{shoot}} = \text{Flux}_{\text{twig}} * \text{Area}_{\text{twig}} + \text{Flux}_{\text{leaf}} * \text{Area}_{\text{leaf}}$$

which, on rearrangement, gives

$$\text{Flux}_{\text{leaf}} = (\text{Flux}_{\text{shoot}} * \text{Area}_{\text{shoot}} - \text{Flux}_{\text{twig}} * \text{Area}_{\text{twig}})/\text{Area}_{\text{leaf}}$$

To measure the dependence of net photosynthesis on the intercellular $CO_2$ concentration ($c_i$), a different approach was required. This is because twigs, after once being exposed to extreme ambient $CO_2$ values (the independent treatment variable), did not immediately exhibit repeatable stomatal responses to the same ambient conditions during subsequent measurements (although they did have repeatable photosynthetic rates at comparable $c_i$ values). Because of this, whole shoot photosynthetic rates as a function of "whole shoot $c_i$" were both area weighted and, if $c_i$ differed between leaf and twig tissues, $c_i$ weighted averages of the component tissues. Since this introduced too many unknown parameters to solve for the leaf photosynthetic dependence on $c_i$ ($A[c_i]$),

another technique was employed. Whole shoots were placed in the cuvette with twigs that had been coated with vaseline. When leafless twigs (leaves surgically removed) which had been coated with vaseline were placed in the cuvette, and ambient conditions were changed in an analogous manner to that employed during measurement of an A[$c_i$] response curve, no fluxes of water or $CO_2$ could be detected between the vaseline-coated twig and the ambient cuvette atmosphere. It was therefore assumed that measurements on shoots whose stems were coated with vaseline constituted direct measurements of the gas exchange behavior of the leaves. Dental impression material (Healthco low-viscosity polyvinyl silicone) was found to be equally effective as vaseline in this regard and was considerably less messy. Thus, it was the preferred twig-coating method.

In order to measure the gas exchange rates of green twigs, it was necessary to first remove the leaves with a razor. Two experiments were performed to check for confounding fluxes of wound respiration and/or water loss from the leaf scars. In the first experiment, dark respiration was monitored for 24 hr after removal of leaves and compared to controls on which the leaves were not removed. Respiration rates slowly declined over the 24-hr period in both experimental and control groups, and no evidence of a large wound respiration was observed. Conductance to water vapor was very low in the dark and was much lower for leafless twigs than for whole shoots despite the presence of fresh leaf scars. In the second experiment, the photosynthetic rate at light saturation was compared between twigs which had their leaves removed three days prior to measurement and twigs on which the leaves were removed immediately before measurements. No significant differences were found between these two treatments in phostosynthetic rate, conductance to water vapor diffusion, or $c_i$. Based on these preliminary measurements, it was concluded that neither wound respiration nor water loss from fresh leaf scars represented a large enough flux to have an appreciable effect on gas exchange calculations for twig tissues. These potential fluxes are consequently ignored in gas exchange calculations, and the importance of possible error in this assumption is discussed where pertinent.

The gas-exchange procedures for measurement of photosynthetic dependence on temperature and $c_i$ were as described by Comstock and Ehleringer (1984). Photosynthetic light-dependence curves were measured by exposing tissue to an irradiance of slightly over 2.0 mmol $m^{-2}$ $s^{-1}$ and allowing a stable photosynthetic rate to be achieved. Irradiance levels were then decreased in a stepwise fashion using neutral-density filters to complete darkness. Ambient $CO_2$ levels during light and temperature response curves were 350 $\mu$l $l^{-1}$, irradiance levels were 2.0 mmol photon $m^{-2}$ $s^{-1}$ (400–700 nm) during temperature and A[$c_i$] curves, atmospheric oxygen was 21%, and the leaf-to-air water vapor gradient ($\Delta w$) was 20 mbar $bar^{-1}$ for all measurements.

$A_{max}$ refers to light saturated (2.0 mmol photon 400–700 nm $m^{-2}$ $s^{-1}$) net photosynthetic rate measured at a tissue temperature of 30 C, $\Delta w$ = 22 mbar $bar^{-1}$, and ambient $CO_2$ = 350 $\mu$l $l^{-1}$. Incident quantum yield was measured at the same ambient conditions as $A_{max}$ over an incident quantum flux range of 0.05–0.15 mmol $m^{-2}$ $s^{-1}$. $A_o$ is the net photosynthetic rate with ambient $CO_2$ varied as necessary to produce a $c_i$ of 350 $\mu$l $l^{-1}$ and with other ambient conditions equivalent to those during the measurement of $A_{max}$. The initial slope of the dependence of net photosynthesis on $c_i$ was calculated by linear regression over the range of $c_i$ between 50–80 $\mu$l $l^{-1}$.

All gas exchange calculations were made as described by von Caemmerer and Farquhar (1981). When calculating $\Delta w$, the partial pressure of water inside the leaf airspace was assumed to be in equilibrium with the leaf water potential (Nobel, 1974). All gas exchange measurements were conducted at the University of Utah at an elevation of 1,460 m with an average atmospheric pressure of approximately 860 mbar.

Amino nitrogen was analyzed on both twig and leaf tissues from each gas exchange experiment using Kjeldahl analyses on an autoanalyzer (Technicon, Tarrytown, NY).

RESULTS—Both leaves and twigs of *Hymenoclea salsola* were cylindrical organs (Fig. 1). While organ diameters vary with growth conditions and age, green twig diameters rarely exceeded 2 mm under any conditions and leaf diameters were always smaller than those of the twigs to which they were attached (Table 1). A substantial fraction of the volume of both organ types was occupied by chlorenchyma tissues, but it was a significantly smaller fraction in twigs than in leaves. Chlorenchyma volume

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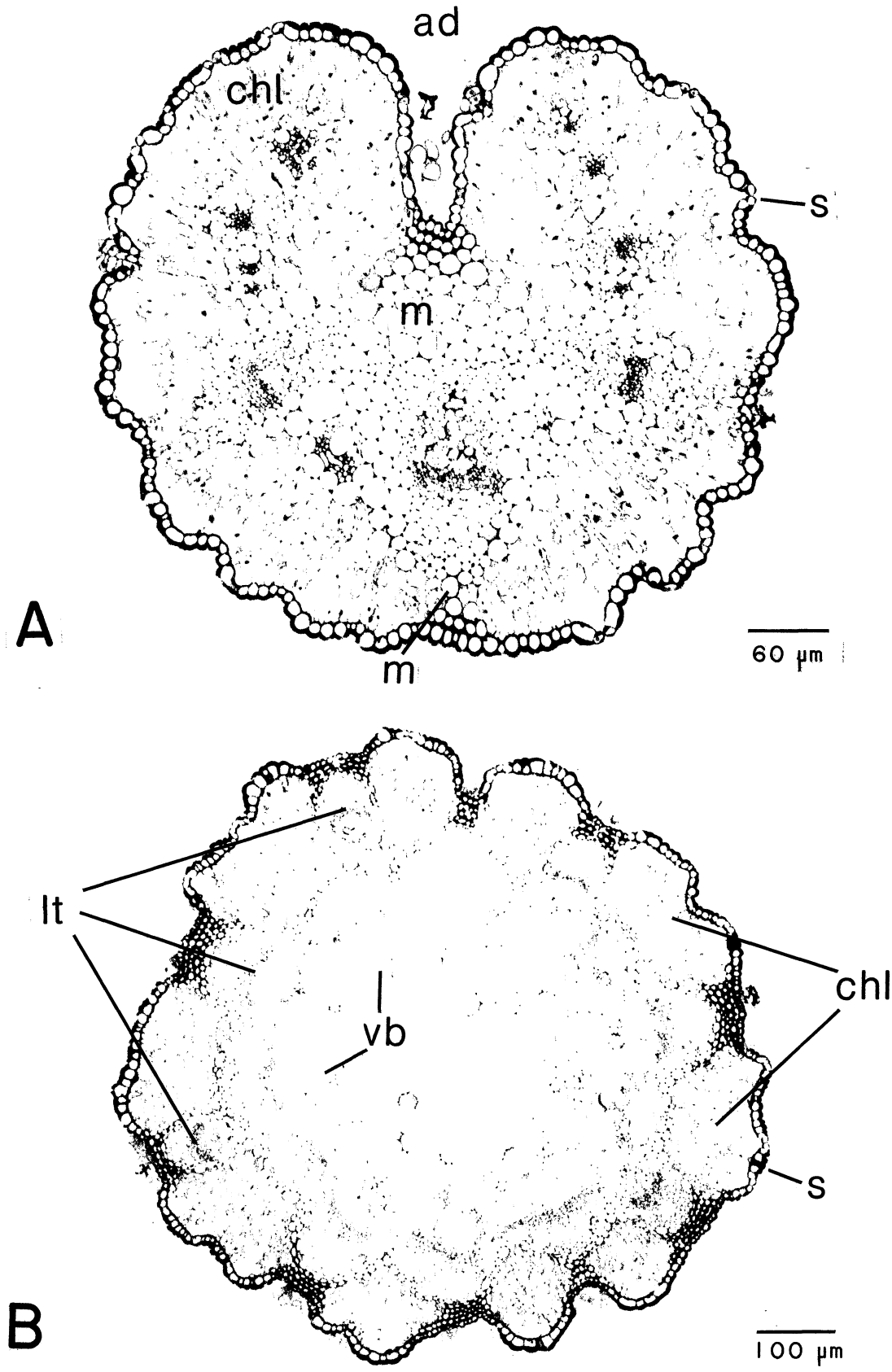
Fig. 1. Cross-sectional anatomy of **A)** a leaf and **B)** a green twig of *Hymenoclea salsola.* ad = adaxial leaf surface, chl = chlorenchyma, lt = leaf trace, m = leaf midrib, s = stomate, vb = vascular bundle.


ad
chl
s
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A
m
60 µm
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s
B
100 µm

TABLE 1. *Anatomical comparisons of leaf and twig tissues. Significance values were determined using a t test for the difference between leaf and twig mean values. The values reported are the averages ± one SD*

| | Leaf | Twig | | Twig/leaf |
|---|---|---|---|---|
| Organ diameter, mm | 0.71 ± 0.08 | 1.20 ± 0.09 | * | 1.69 |
| Percent of circumference nonphotosynthetic | 13.3 ± 5.6 | 34.8 ± 5.3 | ** | 2.62 |
| Stomatal density, $mm^{-2}$ | 111 ± 20 | 108 ± 23 | ns | 0.98 |
| Stomatal aperture length, μm | 12.5 ± 1.9 | 13.3 ± 0.9 | ns | 1.06 |
| Percent of organ volume occupied by chlorenchyma | 42.7 ± 4.0 | 19.3 ± 2.2 | ** | 0.45 |
| Chlorenchyma volume per projected area, mm | 0.210 ± 0.018 | 0.173 ± 0.019 | ** | 0.82 |
| Chlorenchyma depth, μm | 82 ± 9 | 104 ± 9 | * | 1.27 |
| Internal diameter of tracheary elements, μm | 7.7 ± 0.3 | 15.5 ± 2.3 | ** | 2.09 |

$N = 4$ specimens of each organ type for tracheary element diameter. For all other measurements, $N = 6$ specimens (from six plants) for leaves and 7 for twigs. For stomatal density, stomatal aperture length, chlorenchyma depth, and internal diameter of tracheary elements numerous measurements were taken and averaged for each specimen, and values given in the table are averages of the specimen means. * = $P < 0.01$; ** = $P < 0.001$; ns = not significant.

per projected area of the whole organ was significantly lower in twigs than in leaves despite the greater thickness of the chlorenchyma layer in twig tissues. This was possible due to two contrasting anatomical features between leaves and twigs. The first factor was related to the vascular and supportive functions of the twigs. A large fraction of the twig epidermis was immediately subtended by vascular strands and bundles of fibers involved in the formation of leaf traces. Chlorenchyma did not occur in those radial sectors occupied by the leaf traces, and the chlorenchyma was thus divided into numerous longitudinal striations visible to the naked eye on the twig surface. In contrast, the nearly continuous chlorenchyma layer of the leaf was interrupted in only two places by the leaf midrib. The second anatomical feature was the "folding" of the leaf organ forming a channel out of the adaxial leaf surface. This invagination into the cylindrical shape increased the relative surface area of the leaf organ, and it was subtended throughout by chlorenchyma tissue under the epidermis. Stomata did not occur on those portions of the epidermis of either leaves or twigs which were not subtended by chlorenchyma tissues (leaf traces and midrib attachments). But in those regions of the respective organs where stomata did occur, the stomatal density was not significantly different between leaves and twigs. Within any given specimen of leaf or twig, stomatal size was highly variable encompassing about a 2-fold range. The mean size, however, was not different between organ types. The very narrow internal diameter of the tracheary elements is consistent with values reported for *H. salsola* by Carlquist and Hoekman (1985) and the trend toward small diameters observed in desert shrubs.

The curves describing photosynthetic response to irradiance were similar in shape for leaf and twig tissues in *H. salsola,* but leaves exhibited higher absolute rates of net photosynthesis than did twigs at any given irradiance (Fig. 2). Light saturation, if it was reached at all, did not occur until irradiance levels approached 2.0 mmol photons (400–700 nm) $m^{-2}$ $s^{-1}$. Although there was a tendency for twigs to saturate photosynthetically at slightly lower irradiances than leaves, this effect did not account for the large difference in absolute photosynthetic rates between leaf and twig tissues at high irradiance levels. Rather, the difference in rates at high irradiances was a reflection of the difference in the quantum efficiency observed at all irradiances. The relative difference in the incident quantum yield between leaves and twigs at irradiances between light compensation (approximately 0.05 mmol $m^{-2}$ $s^{-1}$) and 0.15 mmol $m^{-2}$ $s^{-1}$, was approximately

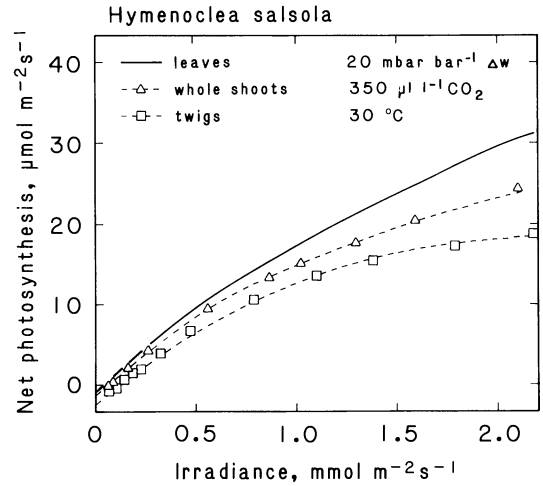


Fig. 2. The effect of irradiance level on net photosynthetic rate in leaves and twigs of *Hymenoclea salsola.* Ambient $CO_2$ concentration = 350 μl $l^{-1}$, tissue temperature = 30 C, leaf-air vapor pressure difference (Δw) = 20 mbar $bar^{-1}$, and oxygen concentration = 21%. Leaf values are calculated from twig and whole shoot values at the same irradiance.

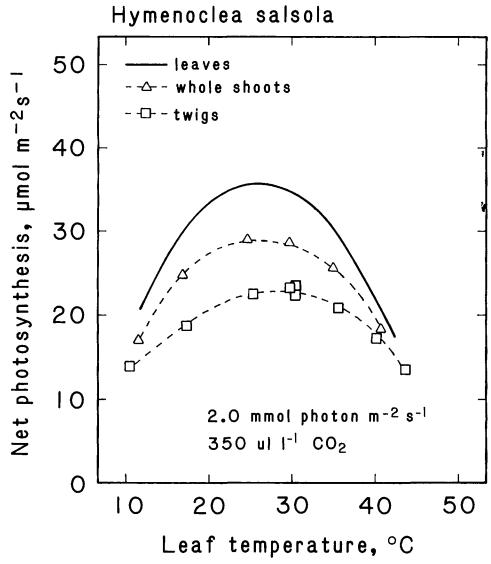


Fig. 3. The effect of tissue temperature on net photosynthetic rate in leaves and twigs of *Hymenoclea salsola*. Ambient $CO_2$ concentration = 350 $\mu$l $l^{-1}$, irradiance = 2.0 $\mu$mol $m^{-2}$ $s^{-1}$, leaf-air vapor pressure difference ($\Delta w$) = 20 mbar $bar^{-1}$, and oxygen concentration = 21%. Leaf values are calculated from twig and whole shoot values at the same tissue temperature.

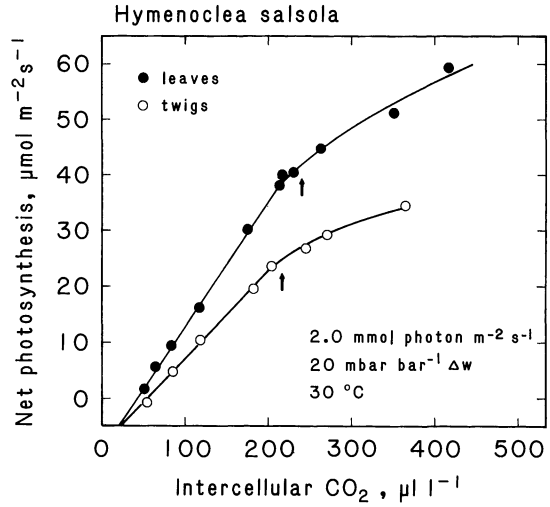


Fig. 4. The effect of internal CO;2 concentration ($c_i$) on net photosynthetic rate in leaves and twigs of *Hymenoclea salsola*. Arrows indicate the mean $c_i$ calculated under measurement conditions of $A_{max}$ (ambient $CO_2$ = 350 $\mu$l $l^{-1}$). Tissue temperature 30 C, irradiance = 2.0 $\mu$mol $m^{-2}$ $s^{-1}$, leaf-air vapor pressure difference ($\Delta w$) = 20 mbar $bar^{-1}$ and oxygen concentration = 21%. Ambient $CO_2$ was varied to control $c_i$. Both leaf and twig tissues were measured directly.

the same as the difference in net photosynthesis measured at 2.0 mmol $m^{-2}$ $s^{-1}$ ($A_{max}$) (Table 2).

In both leaf and twig tissues, the photosynthetic response to temperature exhibited an optimum between approximately 26 and 28 C (Fig. 3). There was a small upward shift of the temperature optimum of twigs but this difference was not statistically significant. Similarly, the depression of the photosynthetic rate at extreme temperatures relative to the temperature optimum was not significantly different for twigs than for leaves. Both tissues showed a rapid decline in photosynthetic rate at temperatures below 15 or above 35 C; these declines became irreversible at tissue temperatures approaching 10 or 40 C.

Leaves also exhibited consistently higher photosynthetic rates than twigs when photosynthesis was measured as a function of the intercellular $CO_2$ concentration of the respective tissues (Fig. 4). The ratio of the slopes of the initial, apparently linear portions of the $CO_2$ response curves was very similar to the ratio of photosynthetic rates at an internal $CO_2$ concentration of 350 $\mu$l $l^{-1}$ (Table 3). This ratio was very similar to the ratio of leaf and twig gas exchange rates in both the irradiance and temperature response curves (Fig. 2, 3).

A very interesting physiological difference between leaf and twig photosynthetic behavior was found when comparing $c_i$ of leaves and twigs under ambient $CO_2$ concentrations (Table 4). In well-watered plants, the $c_i$ of twigs was 27 $\mu$l $l^{-1}$ lower than that of leaves (Table 4). It should be noted that this $c_i$ difference was in the opposite direction to that which would occur as an artifact of wound respiration or bleeding. After leaf removal, both wound respiration decreasing apparent twig photosynthetic rates relative to stomatal transpiration and additional water loss from the open leaf scars would cause the calculation of abnormally high rather than low $c_i$ in the twig tissues.

TABLE 2. *Comparison of photosynthetic response to irradiance in leaf and twig tissues of* Hymenoclea salsola. *Significance values were determined from a t test on the difference between mean values for leaf and twig tissues. Data are means ± one SD*

| | Leaf | Twig | | Twig/leaf |
|---|---|---|---|---|
| Incident quantum yield, mol $mol^{-1}$ | 0.045 ± 0.014 | 0.022 ± 0.008 | * | 0.50 |
| $A_{max}$, $\mu$mol $m^{-2}$ $s^{-1}$ | 42.2 ± 9.4 | 21.6 ± 4.9 | ** | 0.51 |

$N$ = 5 for each tissue in both measurements; * = $P < 0.02$; ** = $P < 0.0001$.

TABLE 3. *Comparison of photosynthetic response to intercellular $CO_2$ concentration ($c_i$) in leaf and twig tissues of* Hymenoclea salsola. *Significance values determined from a t test on difference between mean values for leaf and twig tissues. Data are means ± one SD. $A_o$ = photosynthetic rate at $c_i$ = 350 μl $l^{-1}$, relative stomatal limitation = $(A_o - A_{max})/A_o$*

| | Leaf | Twig | | Twig/leaf |
|---|---|---|---|---|
| Initial slope, mol $m^{-2}$ $s^{-1}$ | 0.24 ± 0.08 | 0.16 ± 0.02 | * | 0.64 |
| $A_{max}$, μmol $m^{-2}$ $s^{-1}$ | 36.9 ± 10.2 | 22.9 ± 6.6 | ** | 0.62 |
| $A_o$, μmol $m^{-2}$ $s^{-1}$ | 50.5 ± 12.7 | 33.7 ± 7.8 | ** | 0.67 |
| Relative stomatal limitation | 0.24 ± 0.04 | 0.33 ± 0.06 | ** | 1.35 |

$N$ = 5 for each tissue for measurements of initial slopes and $N$ = 8 for all other measurements; * = $P < 0.05$; ** = $P < 0.001$.

Photosynthesis was not $CO_2$ saturated in the range of $c_i$ concentrations spanning the mean twig and leaf values obtained during measurement of $A_{max}$ (Fig. 4). Consequently, low twig $c_i$ resulted in an increased twig WUE compared to leaves, but at the cost of a decrease in the absolute rate of twig photosynthesis. The ratio of net photosynthetic rates of twigs to leaves at midday irradiances was 0.62 and 0.67 during measurement of $A_{max}$ (ambient $CO_2$ = 350 μl $l^{-1}$) and $A_o$ (intercellular $CO_2$ equal 350 μl $l^{-1}$), respectively ($A_o$ and $A_{max}$ were paired measurements always taken on the same tissues). The slightly lower ratio obtained when comparing $A_{max}$ as opposed to $A_o$ values was due to the lower $c_i$ of twigs during the $A_{max}$ measurement. The ratio of 0.62 differs from the ratio of 0.51 reported in the comparison of irradiance response curves most likely because of chance differences in the rates of the leaves measured in the two samples. One way to express the effect of operating at low $c_i$ on potential productivity is by calculating the relative stomatal limitation to photosynthesis (defined as the percent limitation to net photosynthesis due to gas-phase diffusional barriers) (Table 3). The relative stomatal limitation to photosynthesis was 9% greater in twigs than in leaves. The lower $c_i$ found in twigs could also explain the tendency of twigs to become light-saturated at slightly lower irradiances.

There were even greater differences between leaf and twig tissues in their relative photosynthetic capacities if rates were expressed as $CO_2$ assimilation per unit of plant biomass or nitrogen content. Because of the much higher specific weight of twig tissues (Table 5), the ratio of twig to leaf net photosynthesis declined from 0.48 to 0.21 when photosynthetic rates were converted from projected area to biomass units ($g^{-1}$ dry weight). Nitrogen contents were considerably higher in leaf tissues on a biomass basis, but far higher in twig tissues on a projected area basis, again because of the much higher specific weight of twigs. The photosynthetic assimilation rate per mole nitrogen in the twig tissues was only 0.28× that of the leaves.

The effect of water stress on photosynthetic gas exchange was also studied for both leaf and twig tissues (Table 4). Plants watered twice daily had predawn plant water potentials of about −1.0 MPa, which is similar to the water potential of plants in the field during early springtime peak productivity. A predawn water potential of −2.3 MPa corresponds to a level of water stress at which plants in the field shed many of their leaves. For greenhouse grown plants, photosynthesis declined substantially in both tissues over this range of plant water potentials (Table 6). The percent decline in photosynthesis between predawn water potentials of −1.0 MPa and −2.3 MPa was much greater for leaves (65%) than for twigs (38%). The predicted photosynthetic rates of leaves and twigs are significantly different from each

TABLE 4. *Gas exchange parameters of leaves and twigs of well-watered individuals of* Hymenoclea salsola *operating under favorable environmental conditions ($A_{max}$): irradiance = 2.0 mmol quanta $m^{-2}$ $s^{-1}$, leaf temperature = 30 C, $\Delta w$ = 20 mbar $bar^{-1}$, and ambient $CO_2$ = 350 μl $l^{-1}$. Data are means ± one SD. Significance levels were determined by a t test on the difference between the means for leaf and twig tissues*

| | Leaf | Twig | | Twig/leaf |
|---|---|---|---|---|
| $A_{max}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$ | 300 ± 89 | 64 ± 23 | *** | 0.21 |
| $A_{max}$, μmol $CO_2$ $m^{-2}$ $s^{-1}$ | 43.3 ± 9.1 | 20.8 ± 6.0 | ** | 0.48 |
| Conductance, mol $m^{-2}$ $s^{-1}$ | 0.776 ± 0.274 | 0.290 ± 0.113 | ** | 0.37 |
| Intercellular $CO_2$, μl $l^{-1}$ | 247 ± 19 | 220 ± 23 | * | 0.89 |
| Water use efficiency, mol $CO_2$ (mmol $H_2O$)$^{-1}$ | 2.85 ± 0.63 | 3.63 ± 0.72 | * | 1.27 |

$N$ = 14 and 12 for leaf and twig tissues respectively in all measurements; * = $P < 0.01$; ** = $P < 0.001$; *** = $P <$ 0.0001.

TABLE 5. *Nitrogen content and maximum instantaneous nitrogen use efficiency of leaves and twigs on well-watered* Hymenoclea salsola. *Data are means ± one SD. Significance levels were calculated from t tests on the difference between leaf and twig means*

| | Leaf | Twig | | Twig/leaf |
|---|---|---|---|---|
| Unit weight, g $m^{-2}$ | 157 ± 31 | 381 ± 98 | *** | 2.48 |
| Kjeldahl nitrogen, mmol $m^{-2}$ | 263 ± 45 | 461 ± 109 | *** | 1.75 |
| Kjeldahl nitrogen, mmol $g^{-1}$ | 1.80 ± 0.32 | 1.36 ± 0.28 | ** | 0.76 |
| Photosynthetic nitrogen use efficiency, $\mu$mol $CO_2$ (mol N)$^{-1}$ $s^{-1}$ | 166 ± 31 | 47 ± 16 | *** | 0.28 |

$N$ = 21 and 22 for leaf and twig tissues respectively for all measurements; ** = $P < 0.001$; *** = $P < 0.0001$.

other only for the well-watered plants, while during water stress the photosynthetic superiority of leaves (expressed on a projected area basis) is lost. A similar relationship exists between leaves and twigs when examining the conductance to water vapor and the $c_i$. When well watered, leaves have higher photosynthetic rates than twigs, much higher conductances to water vapor, and higher $c_i$ values. At low predawn water potentials photosynthesis, conductance and $c_i$ all declined and converged so that leaves and twigs were no longer significantly different from each other.

Combining the data for plants at all water potentials, the photosynthetic capacity of leaves was more tightly correlated with leaf nitrogen content than photosynthetic capacity of twigs was with twig nitrogen content (Table 7). For both tissues, the correlation was much better when photosynthesis and nitrogen content were both expressed on a biomass rather than a projected area basis. Consistent with the high correlation of leaf photosynthesis with leaf nitrogen content, there was also a significant decline in leaf nitrogen content during water stress (Table 6).

Somewhat surprisingly, there was no significant relationship between twig nitrogen content and plant water potential. The photosynthetic nitrogen use efficiency (defined in the limited sense of instantaneous ratio of $CO_2$ assimilation to nitrogen content) decreased in both tissue types during water stress. Due to the insensitivity of twig nitrogen content to water stress, twig photosynthetic nitrogen-use efficiency remained only 0.4 times that of the leaves at low water potentials.

DISCUSSION—The maximum photosynthetic rates reached by leaves of *Hymenoclea salsola* were very high compared to those of $C_3$ plants in general, but were similar to those reached by many desert annuals (Seeman, Tepperman, and Berry, 1980; Werk et al., 1983) and some drought deciduous leaved shrubs (Ehleringer and Mooney, 1983) of the same

TABLE 6. *The effect of predawn leaf water potentials (MPa) on net photosynthesis and related parameters in* Hymenoclea salsola. *Values at −1.0 and −2.3 MPa are predicted from linear regressions. The column labeled P gives the probabilities that the slopes of the regressions are different from zero. Asterisks indicate where leaf and twig regression statistics are significantly different from each other: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant*

| Dependent variable | Tissue | Intercept | Slope | $r^2$ | $P$ | Predicted values of dependent variables = −1.0 MPa | = −2.3 MPa |
|---|---|---|---|---|---|---|---|
| | | *** | ** | | | *** | ns |
| Net photosynthesis, $\mu$mol $m^{-2}$ $s^{-1}$ | leaf | 63.8 | 21.2 | 0.47 | 0.001 | 42.6 | 15.0 |
| | twig | 26.6 | 6.0 | 0.29 | 0.01 | 20.6 | 12.8 |
| | | *** | ** | | | *** | ns |
| Conductance, mol $m^{-2}$ $s^{-1}$ | leaf | 1.307 | 0.537 | 0.46 | 0.001 | 770 | 72 |
| | twig | 0.398 | 0.114 | 0.34 | 0.005 | 284 | 137 |
| | | *** | ** | | | *** | ns |
| Intercellular $CO_2$, $\mu$l $l^{-1}$ | leaf | 303 | 53.4 | 0.57 | 0.001 | 250 | 180 |
| | twig | 246 | 25.9 | 0.27 | 0.025 | 220 | 187 |
| | | *** | ns | | | *** | * |
| Kjeldahl nitrogen, mmol $g^{-1}$ | leaf | 2.19 | 0.42 | 0.26 | 0.25 | 1.76 | 1.22 |
| | twig | 1.46 | 0.15 | 0.11 | ns | 1.31 | 1.12 |
| | | *** | ** | | | *** | *** |
| Photosynthetic nitrogen use efficiency, $\mu$mol $CO_2$ (mol N)$^{-1}$ $s^{-1}$ | leaf | 234 | 7.11 | 0.47 | 0.001 | 163 | 70.6 |
| | twig | 62 | 1.43 | 0.25 | 0.025 | 47 | 29 |

$N$ = 22 and 24 for leaf and twig tissues, respectively, for all regressions.

TABLE 7. *The dependency of light-saturated photosynthetic rate on nitrogen content. The column labeled P gives the probabilities that the slopes of the regressions are different from zero. Asterisks indicate significant differences between leaf and twig regression statistics: *** = 0.001, ns = not significant*

| | Tissue | Intercept | Slope | $r^2$ | P |
|---|---|---|---|---|---|
| Net photosynthesis, $\mu$mol $m^{-2}$ $s^{-1}$ | | *** | *** | | |
| vs. | leaf | −4.64 | 0.163 | 0.34 | 0.01 |
| Nitrogen content, mmol $m^{-2}$ | twig | 25.29 | 0.016 | 0.05 | ns |
| Net photosynthesis, nmol $g^{-1}$ $s^{-1}$ | | *** | *** | | |
| vs. | leaf | −202.9 | 271.5 | 0.73 | 0.001 |
| Nitrogen content, mmol $g^{-1}$ | twig | −12.5 | 51.1 | 0.28 | 0.025 |

$N$ = 21 and 22 for leaf and twig tissues, respectively, for both regressions.

desert communities. The photosynthetic rates measured in twigs also fit in these groupings of plants adapted to utilize the high irradiance conditions of the warm deserts, but the measurements made on *H. salsola* leaves fell at the high end while those on *H. salsola* twigs fell at the low end of the range. Compared to leaves of $C_3$ plants in general, the photosynthetic rate of the twigs would be considered to be high. They are also quite high (Table 4) compared to the twigs of other photosynthetic-twigged species which have been studied: 32.8 nmol $g^{-1}$ $s^{-1}$ in *Gutierrezia sarothrae* (DePuit and Caldwell, 1975) and 15.8 $\mu$mol $m^{-2}$ $s^{-1}$ in *Cercidium floridum* (Adams and Strain, 1968).

Much of the difference in photosynthetic performance between leaf and twig tissues at high water potentials appears to be related to the differing anatomical constraints of the two organ types. When operating at equivalent $c_i$ values, the ratio of photosynthetic rates twig: leaf is 0.67 (Table 4). The analogous ratio of chlorenchyma volume per projected area (Table 1) is 0.82. Further, the organization of the chlorenchyma layer in the twigs is such that intercepted light may not be absorbed as efficiently as in the leaves. A substantial fraction of the twig surface is nonphotosynthetic. Where a chlorenchyma layer occurs, it is thicker in the twigs, and twigs also have far more structural tissues separating chlorenchyma on opposite sides of the organ. Both these factors will contribute to greater self-shading effects within the twig organ than within the leaf.

Young twigs were found to have a greater instantaneous WUE ($\mu$mol $CO_2$ $mmol^{-1}$ $H_2O$) than their associated leaves due to their lower $c_i$. The meaning of these differences in $c_i$ between two organs of the same plant at the same moment in time is not yet understood. If whole shoot transpiration rate is held constant, then the observed inequality in $c_i$ between tissue types should actually impair both productivity and water use efficiency at the whole-shoot level. This is because the shape of the $A[c_i]$ response is convex, with diminishing photosynthetic returns for each subsequent increase in $c_i$, while the stomatal conductance required to permit an increase in $c_i$ is a concave function with increasing transpirational costs for each subsequent $c_i$ increment. By finding a simultaneous solution of equations defining 1) the supply of $CO_2$ via the stomata and 2) the consumption (as a function of $c_i$) of $CO_2$ in photosynthesis (Jones, 1973; Farquhar and Sharkey, 1982), it is possible to calculate what would happen if two *H. salsola* leaves were to operate, one at a $c_i$ of 247, and the other at a $c_i$ of 220. Their combined productivity would be 3.5% lower than it would be if both leaves were to operate at a $c_i$ of 235 while using the identical amount of water between them. This result is predicted by Cowan and Farquhar's (Farquhar, 1977) theoretical analysis of maximizing productivity for a given expenditure of water. The marginal cost $\delta E/\delta A$ should be constant across the canopy which means equivalently illuminated leaves at a constant temperature and possessing an identical $A[c_i]$ response should operate at the same $c_i$. This result also holds if the $A[c_i]$ response differs between tissues only by a scalar multiple at all $c_i$ which appears to be the case between leaves and twigs of *H. salsola.* If the conductance of leaves and twigs is allowed to vary but total water use is held constant in a whole shoot of *H. salsola,* and again assuming the $A[c_i]$ response for plants at high water potential (Fig. 4), the whole-shoot photosynthetic rate would be increased by a maximum of 0.4% when all tissues were operating at a $c_i$ of 242. This difference is small but inevitable, and illustrates that having an inequality in $c_i$ between the two tissue types does not improve instantaneous whole plant water use efficiency.

It is possible that differing anatomical constraints are influencing $c_i$ in the two organs. Stomatal density and aperture size are not significantly different between the leaves and twigs, but the chlorenchyma layer is thicker in the twigs (Table 1). The ratio of twig to leaf chlorenchyma depth is 1.27, and the ratio of the drawdowns of $CO_2$ between the ambient air (350 $\mu$l $l^{-1}$) and the intercellular airspaces is

1.26 (tissue $c_i$ values taken from Table 4). The similarity of these ratios suggests a possible anatomical mechanism causing different $c_i$ values in the two tissues, but does not, even if correct, address the evolutionary question of why the disparate $c_i$ values are not selected against producing, for instance, a greater density of stomata on the twigs. It may be that the interruptions of the chlorenchyma by the vasculature of the twig result in a thicker chlorenchyma layer, and that stomatal density does not compensate because the twigs normally remain active further into the drought period than the leaves and are specialized for survival under more stressful conditions. Further study is needed to clarify both the underlying cause and significance of the $c_i$ difference between leaves and twigs.

Using instantaneous measures of efficiency and ignoring for the moment potential differences in longevity between the two tissue types, leaves appear to be consistently more efficient than twigs at utilizing limiting resources for achieving carbon assimilation during favorable seasons. Leaves achieve a 2-fold superiority over twigs with respect to assimilated $CO_2$ per intercepted quanta, a 5-fold superiority per biomass investment in the assimilating organ, and a 3.5-fold superiority with respect to nitrogen content of the assimilating organ. The longer expected lifespan of twigs may slightly erode the superiority of leaves in these instantaneous measures, by allowing a longer period of assimilatory activity after initial investments have been made (Rundel, 1982; Vitousek, 1982). When sufficient data become available to make such a quantitative analysis of lifetime efficiency, it will, however, alter the above conclusions only slightly. This is because leaves remain an important part of the canopy of *H. salsola* and continue to have either higher or at least equal photosynthetic rates compared to the twigs until the rates of both organs are quite low. Plants are aphyllus only during periods of very high stress during which little contribution to whole-plant annual productivity is made (Comstock, Cooper, and Ehleringer, 1988).

If considered as two alternative organs designed solely for photosynthesis, leaves appear to be far superior to twigs in the assimilatory return per resource use. This perspective is inaccurate, however, because twigs play important structural roles as well as photosynthetic ones. The separation of costs associated with photosynthesis, transport, and support is difficult, but it is clear that much of the investment in twig structures is related to the functions of support, storage, and vascular transport.

The cost/benefit analyses of making existing twigs photosynthetically active would be an extremely different matter from asking the relative cost/benefit of leaves vs. twigs as the primary canopy structures for photosynthetic carbon gain. The data presented here suggest that twigs are rarely photosynthetically superior to leaves, but are much more expensive to build. It is quite likely, however, that when other factors involved in determining canopy structure (such as leaf display, elevation of inflorescences, etc.) require the production of a large twig surface area, the additional cost of photosynthetic investment in these existing twigs is quite low relative to the photosynthetic returns. It is also likely to be much lower than the cost of building an equivalent new leaf surface area (plus associated nonphotosynthetic twigs to put it on) from scratch.

It is of interest to note that of 15 species of plants with photosynthetic twigs at a warm desert field site in Oatman, Arizona, only one species, the gymnosperm *Ephedra nevadenses,* was aphyllus at all times of the year (Comstock et al., 1988). One important area of future inquiry, therefore, will be to analyze some of the cost/benefit trade-offs of high allocation to twigs during plant growth, which are independent of the photosynthetic capacity of the twigs. The implications of whole-canopy growth dynamics to allocation to twigs, as well as more detailed studies of the stress tolerance of leaves and twigs, are the subject of current research efforts.

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