The genus *Pistacia* is characterized by pinnately compound leaves. Paripinnate leaves predominate in some species while imparipinnate leaves are characteristic of others. Simple leaves occur rarely in *P. khinjuk* (29) and *P. vera* on juvenile growth and on bearing shoots produced subsequent to a mild winter during which the chilling requirement was not met completely (5). The number of leaflets per leaf varies from 1 to 20 pairs with more or less rounded apices.

Leaves of the pistachio nut tree, *P. vera*, are oriented at random; the lamina of some are horizontal, others are vertical, while the remainder occupy various intermediate positions. This random orientation raised questions regarding such leaf characteristics as distribution of stomata between the adaxial and abaxial leaf surfaces and the internal structure of the leaflets. These aspects were investigated for *P. vera* and several other *Pistacia* species which have horizontally oriented leaves. Differences among these species prompted subsequent photosynthetic studies of *P. vera*, *P. atlantica*, and *P. integerrima*, the results of which are reported here.

**Materials and Methods**

*Leaf morphology.* Leaves for this study were sampled during midsummer from mature trees growing at 2 locations. Leaves of *P. atlantica*, *P. chinesis*, *P. integerrima*, *P. khinjuk*, *P. mutica*, and *P. vera* were collected from experimental orchards of the Univ. of California at Davis. Leaves of *P. lentiscus*, *P. mexicana*, and *P. weinmannifolia* were collected from trees growing at the former USDA Plant Introduction Station, Chico, Calif. Ten to 20 leaves were collected for morphological studies from well-exposed areas of canopies of 2 to 4 trees of each species.

Portions of leaf lamina were dissected into 4–10 mm² sections prior to fixation to facilitate penetration of fixative and embedding resin. Tissues were fixed in either 1 formalin : 1 propionic acid : 18 70% ethanol (FPA, by volume) solution or 3% glutaraldehyde in 0.025 M phosphate buffer, pH 6.8. FPA-fixed samples were dehydrated in a tertiary butyl alcohol series, embedded in paraffin, and sectioned at 6–8 μm thickness. The sections were stained with safranin-fast green. Glutaraldehyde-fixed samples were washed with distilled water, dehydrated in acidified, 2,2-dimethoxypropane (15), and embedded in glycol methacrylate resin (Dupont-Sorvall). Plastic-embedded material was sectioned paradermally and transversely at 2 to 3 μm thickness with glass knives on a Sorvall JB-4 microtome. The sections were stained with 0.05% toluidine blue-O in 1% sodium tetaborate.

For studying stomatal distribution, epidermal replicas of leaflets were made by coating the adaxial and abaxial surfaces with clear fingernail polish. The dried films then were peeled and mounted on slides. Replicas were observed under a compound microscope and stomatal density was determined for each surface.

*Photosynthetic and leaf conductance studies.* Mature trees of *P. vera*, *P. atlantica*, and *P. integerrima*, growing adjacent to each other in a species collection, were selected and daily patterns of leaf CO₂ assimilation and leaf conductance rates were determined. Sixteen leaves of each species were used during a cloudless 4-day period (11–14 June 1983). Measurements were made on 2 fully expanded, well-exposed leaves on the SE quadrants of the trees at 2-hr intervals from dawn until noon. The same routine was followed in the afternoon until sunset on 2 leaves on the SW quadrants of the same trees.

Measurement of carbon dioxide assimilation was made using the CO₂ depletion technique similar to that described by Clegg et al. (3) and Ehleringer and Cook (9). An intact leaf was enclosed in a hand-held, 2.8-liter plexiglass cuvette that was fitted with a dual trigger-activated syringe system so that cuvette air samples could be taken at timed intervals. The leaf was oriented to obtain maximum interception of available solar radiation. Two, small, battery-powered fans were mounted inside the cuvette to ensure adequate mixing of internal atmosphere and reduce leaflet boundary layer resistance. An air sample was withdrawn with a spring-activated glass syringe (10 ml) immediately after closing the cuvette, followed by a 2nd sample 30 sec later. The syringes were sealed immediately and placed...
in an insulated box. Within 2 hr of sampling, the contents of the syringes were injected into a continuous flow, infrared gas analyzer (Horiba, VIA 500R) designed and calibrated to measure CO₂ concentrations between 250 and 400 ppm. Compressed air of known carbon dioxide concentration was used as the carrier gas and the system was calibrated by injecting 10-ml samples of air containing various known concentrations of carbon dioxide. Assimilation rates were calculated according to Ehleringer and Cook (9).

**Leaf conductance, g.** Leaflet resistance was measured on both surfaces of P. atlantica, P. integerrima, P. khinjuk, P. mutica, and P. vera in the early afternoon of 8 Oct. 1981, with a LI-COR LI-65 Autoporometer. Leaf conductance was computed as the reciprocal of the resistance reading (cm·s⁻¹). The total leaf conductances for those leaves having stomata on both surfaces were computed as follows: Total g = g(adaxial) + g(abaxial).

**Results**

**Leaflet structure.** A single layer of thin-walled epidermal cells characterized both leaflet surfaces of all species. The epidermal cells were covered with a relatively thick layer of cutin in P. lentiscus, P. mexicana, and P. weinmannifolia, but little or no cutin was observed in other species. No trichomes were present on leaflets of any species.

Outwardly, the leaves of P. vera appear different from those of other species. The leaves are stiff, gray-green, and randomly oriented; their adaxial and abaxial surfaces are similar. Hence, they appear to be isolateral. The internal leaf morphology differs from that of the other species in degree of differentiation of mesophyll cells. The length of adaxial palisade cells of P. vera is about half to a third of those of P. atlantica and P. integerrima (Fig. 1). A 2nd layer of palisade cells which gradates to more isodiametric spongy parenchyma cells occurs in P. vera. The 2 layers of abaxial palisade cells of P. vera appear slightly longer than those of the other species (Fig. 1). These cells are less densely packed (Fig. 2c) than those of the adaxial layer (Fig. 2b).

Leaves of the other 8 species are distinctly dorsiventral in appearance. Their adaxial-most palisade layer make up 40% to 60% of the lamina thickness compared to about 20% for P. vera. Many cells in the spongy tissue were palisade-like in appearance, being slightly elongated, vertically arranged, and in close proximity of each other (Fig. 1b, c). Among these cells, there are typical isodiametric parenchymatous cells in all species. The 2 layers of abaxial palisade cells are very similar in appearance and density in these species.

**Stomatal distribution.** All species had actinocytic stomata (Fig. 2a, d). Guard cells were situated at the same level as adjacent epidermal cells (Fig. 1). Stomatal density in all species was higher on the abaxial than the adaxial surface (Table 1). The ratio of abaxial to adaxial stomatal density varied from a low of 1.3 in P. vera to a high of 13.3 in P. integerrima; the ratio was intermediate in P. atlantica. In P. atlantica and P. chinensis, adaxial stomata were located only in close proximity to the major and minor veins. Stomata were observed also on the adaxial and abaxial surfaces in P. khinjuk and P. mutica, but only on the abaxial surface in P. lentiscus, P. mexicana, and P. weinmannifolia.

**Leaflet conductance.** Leaflet water vapor conductance measurements taken in Oct. 1981 showed that the abaxial surfaces of all species tested had much higher conductances than adaxial surfaces, with the exception of P. vera in which the values were the same (Fig. 3). This response is not uncommon for isolateral

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**Fig. 1.** Cross-sections of leaves of a) P vera, b) P. atlantica, and c) P. integerrima. ×128. Note difference in leaf thickness and gradation in the amount and size of spongy parenchyma cells.

**Fig. 2.** Paradermal sections of a P. vera leaflet showing upper epidermis (a), palisade parenchyma near the adaxial (b) and abaxial (c) sides, and lower epidermis (d).
Table 1. Stomatal density on the adaxial and abaxial leaf surfaces of *Pistacia* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stomata per mm²</th>
<th>Ratio adaxial abaxial</th>
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<tbody>
<tr>
<td><em>P. atlantica</em></td>
<td>84 ± 2</td>
<td>26 ± 8</td>
</tr>
<tr>
<td><em>P. chinensis</em></td>
<td>175 ± 52</td>
<td>1005 ± 113</td>
</tr>
<tr>
<td><em>P. integerrima</em></td>
<td>60 ± 10</td>
<td>800 ± 95</td>
</tr>
<tr>
<td><em>P. khinjuk</em></td>
<td>91 ± 25</td>
<td>601 ± 141</td>
</tr>
<tr>
<td><em>P. lentiscus</em></td>
<td>0</td>
<td>377 ± 56</td>
</tr>
<tr>
<td><em>P. mexicana</em></td>
<td>72 ± 26</td>
<td>339 ± 74</td>
</tr>
<tr>
<td><em>P. mutica</em></td>
<td>226 ± 29</td>
<td>304 ± 42</td>
</tr>
<tr>
<td><em>P. vera</em></td>
<td>0</td>
<td>699 ± 85</td>
</tr>
<tr>
<td></td>
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*Limited to region of major vein.

*Limited to region of minor veins.

leaves (26). Leaves of *P. integerrima* exhibited the lowest abaxial conductance even though they had the highest stomatal density. Adaxial conductance of *P. vera* was from 2 to 15 times greater than that of other species.

**Photosynthesis.** Leaf temperature in *P. vera* (Fig. 4), *P. atlantica* (Fig. 5), and *P. integerrima* (Fig. 6) increased from 15° to 16°C in the morning to about 30° maximum at 1500 HR in the afternoon. The daily trends of photosynthetic photon flux density in the open (PPFD₀) were typical for a clear day. PPFD perceived by the leaf surfaces (PPFDₖ) followed a similar trend as that in the open but actually decreased at 1100 HR due to shading. Subsequent increase in PPFD (Fig. 4, 5, 6) was the result of using a different set of leaves in the afternoon.

A and g increased rapidly in the early morning with increasing PPFDₖ. Responding to PPFDₖ, values of A in each species increased progressively until 1500 HR, except for the brief period when shading occurred in *P. vera* and *P. atlantica*. Maximum values of A and g corresponded in all species to the time of maximum temperature.

Statistical analyses of the distribution plot of A vs. g for the 3 pistachio species reveal that the curvilinear regression curves had r² values of 0.57, 0.64, and 0.77, respectively, for *P. vera*, *P. atlantica*, and *P. integerrima*. The r² values for a straight line regression for the same distribution were 0.52, 0.54, and 0.61, respectively. Although there was an improvement in fit when the curvilinear models were used instead of a linear model, this difference was not statistically significant. The initial slopes of *P. vera* and *P. atlantica* are equally steep and steeper than that of *P. integerrima* under field conditions.

**Discussion**

The genus *Pistacia* has been referred to as a xerophyte (24, 28). However, with the exception of more advanced development of palisade tissue, none of the characteristics commonly associated with xeromorphic leaves (23) were found in the species studied. It may be assumed that other adaptations, such as extensive root growth (19), contribute to the xerophytic characteristic. The occurrence of stomata on both the adaxial and
Fig. 5. Photosynthetic parameters in *P. atlantica*. Upper: Average daily patterns of photosynthetic photon flux density (PPFD) in the open (O), at leaf surface (L), and of leaf temperature (T). Lower: Average daily patterns of CO₂ assimilation rate (A) and leaflet conductance (g). All measurements were made in the field on 11–14 June 1983. Vertical bars indicate standard error of the means.

Abaxial surfaces of leaves of some species and not on others is not readily attributable to any particular selection pressure under which the species originated. Parkhurst (20) proposed that leaves with thick mesophyll tend to be amphistomatic but no clear relationship was apparent between these parameters in the *Pistacia* species studied. The values of A obtained from the different species were not related to amphistomaty.

The maximum A values of *P. vera* and *P. atlantica* were in the midrange for C₃ plants. Although the A values are affected by environmental and endogenous factors, the maximum values obtained in this study were higher than those reported for other temperate fruit tree species such as apple (14), peach (6, 8), plum, cherry, apricot (8), and walnut (27), but similar to that of almond (8). Values obtained for *Pistacia* species were similar to the theoretical maximum value for apple trees grown under optimum conditions (1). By contrast, the CO₂ assimilation rate of *P. integerrima* is at the lower range of C₃ plants.

*P. vera* leaves appear isolateral. Their internal leaf structure is quite unlike the dorsiventral ones of apple (16), pear (10), and peach (11). The isolateral leaf appearance found in *P. vera* leaves is not surprising in view of their random leaflet orientation. The latter may enhance light penetration into the interior of the leaf canopy.

Nobel et al. (18) found that, in any given species, the light-saturated photosynthetic rate is related to the mesophyll cell wall area per unit leaf area, Aₘₑₛ/ₐₐₑₙₐₙ. The highly developed palisade parenchyma toward the abaxial sides of leaflets of *P. vera* and *P. atlantica* could increase the Aₘₑₛ/ₐₐₑₙₐₙ ratio and be involved in the attainment of high photosynthetic rates. However, the development of palisade parenchyma appears not to be the sole factor responsible for the high photosynthetic capacity in these species because *P. integerrima* had a comparatively low A value even though it had some palisade-like parenchyma cells toward the abaxial side of its leaflets. As Björkman (2) pointed out, an increase in photosynthetic rate can only occur if an increase in Aₘₑₛ/ₐₐₑₙₐₙ is accompanied by increases in other constituents determining the photosynthetic rate at the chloroplast level.

Higher values of A in *P. vera* and *P. atlantica* than those in *P. integerrima* may be related to plant origin. *P. vera* and *P. atlantica* originated in relatively open, arid areas of Central Asia (30); whereas, *P. integerrima* is indigenous to regions of relatively high, summer rainfall in northern Pakistan and northwest India (7, 25). Maximov (17) pointed out that survival of plants in dry areas depends upon their ability to profit from comparatively short favorable periods—during rains and in the short period following when water is available for plant growth. The ability to do this presupposes that they have high assimilation rates. *P. vera* and *P. atlantica* apparently have this potential.

If the curvilinear correlations between A and g in *Pistacia* species are real, they are in contrast to the linear correlations reported for walnut (27) and apple (13) but similar to that reported for *Fagus silvatica* (21). The optimization theory of Cowan and Farquhar (4), which proposed that stomata maximize the
rate of carbon gain while minimizing water loss, would be achieved if stomatal aperture varied linearly with CO$_2$ assimilation. It may be that _Pistacia_ species did not exhibit optimization under the nonsteady field conditions in this study because of fluctuations in radiation level, leaf temperature, and/or humidity during the measurement period (12). Under conditions of no water stress, many plants exhibit stomatal opening with increasing temperature (22). Although the effects of temperature on g independent of vapor pressure deficit effects had not been tested in _Pistacia_, maximum g values in _P. vera_, _P. atlantica_, and _P. integerrima_ coincided with the highest leaf temperatures. High transpiration due to high g values in the hot afternoon leads to evaporative cooling of leaves. This cooling mechanism may be important, especially for _P. vera_ which has relatively large leaves, to maintain leaf temperatures near the favorable range for photosynthesis. Further research under controlled conditions is necessary to determine the potential effects of vapor pressure deficit, leaf temperature, and transpirational leaf cooling on net CO$_2$ assimilation of _P. vera_ under natural field conditions.

![Graph](image)

**Fig. 7.** Relationship between CO$_2$ assimilation rate, A, and concurrent transpirational leaf conductance, g. Data were gathered during a 4-day cloudless period. The coefficients of correlation, r$^2$, for _P. vera_ (A), _P. atlantica_ (B), and _P. integerrima_ (C) are 0.57, 0.64, and 0.77, respectively, for the line of best fit.

**Literature Cited**

Quantification of Free ABA and Free and Conjugated IAA in Strawberry Achene and Receptacle Tissue during Fruit Development

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Abstract. Indoleacetic acid (IAA) was identified by gas chromatography-mass spectrometry in extracts of achene and receptacle tissue of 'Midway' strawberry (Fragaria Xananassa Duch.). Free, ester-conjugated, and amide-conjugated IAA present in both tissues from anthesis to maturity were quantified by a double-standard isotope dilution method using 14C-IAA and 14C-indolebutyric acid (14C-IBA) as internal standards. Whole fruit at anthesis contained 6.2 µg/g dry weight free IAA, 2.0 µg/g dry weight ester-conjugated IAA, and no detectable amide-linked IAA. Maximum concentrations of free IAA in achenes were 1.2 times higher than those in receptacle tissue; maxima occurred simultaneously in the 2 tissues 14 days after anthesis. The maximum concentration of ester-conjugated IAA in achene tissue 8 days after pollination and subsequently declines. Fruit growth, however, as measured by fresh or dry weight gain, is not well-correlated with hormone concentrations. The concentration in the achenes declined until midway through development, then increased as fruit approached maturity. The total quantities in both achenes and receptacle increased as fruit ripened. The ratio of free IAA to free ABA changed during fruit development but was not well-correlated with fruit growth rate.

The strawberry provides a classic model for studies of seed and hormonal control of fruit development (9, 10, 11). IAA is believed to exert primary control over strawberry fruit development (10, 11). Exogenously applied auxins can stimulate parthenocarpy (16, 17), enhance fruit development of pollinated fruit, and increase yields (1, 14, 18). Nitsch (9, 10) demonstrated that removal of achenes stopped fruit enlargement and that treatment with synthetic auxins caused continued growth. Numerous auxins are capable of replacing achenes and each differs in its efficacy (8). IAA was identified tentatively as the major auxin present in strawberry achenes, representing 1/2 to 3/5 of the auxin activity as measured by bioassay (11); however, only Dreher and Pooovaiah (5) have provided mass spectral evidence for its occurrence in achenes only. Bioassays (5, 7, 10, 11) and spectrofluorimetry (5) have detected the presence of auxin-like compounds in both achenes and receptacle extracts, the former being more active (5, 7, 10, 11). Auxin or auxin-like activity increases in both achenes and receptacle tissue through 8 to 12 days after pollination and subsequently declines. Fruit growth, however, as measured by fresh or dry weight gain, is not well-correlated with hormone concentrations. ABA has been identified tentatively in extracts of ripe and unripe strawberry fruit (12) and quantified by bioassay (7). ABA-like activity was not detectable at anthesis and the concentration was greater in achenes than receptacle tissue throughout development (7). At maturity, however, activity in achenes extracts rose 10-fold.

The poor correlation between fruit growth and hormone content raises questions as to the role of these compounds in controlling fruit development. Cohen and Bandurski (3) suggested that conjugates of IAA play crucial roles in many physiological processes which could include fruit development. Following exogenous application of 14C-IAA to strawberry fruit, 14C-IAA-aspartate can be recovered in vitro and 14C-IAA-glucose in vivo (6). Thus, mechanisms for conjugation exist in strawberry.

The objectives of this research were: (a) to identify IAA in strawberry fruit tissue by gas chromatography-mass spectrometry (GC-MS) and (b) to quantify IAA and its conjugates, as