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Comparative histological basis of sun and shade leaf dimorphism in *Helianthus annuus*

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The development processes which give rise to the histological differences between leaves expanding under full daylight and 25% daylight are described for *Helianthus annuus*. Generally the pattern of cell division and cell enlargement in shaded leaves lags behind that in unshaded leaves by about 2 days. There is no significant difference in the amount of cell enlargement in the paradermal plane in the epidermis and palisade layers of shaded leaves as compared with leaves expanding in full sunlight. However, cell division in all cell layers occurs at greater rates in unshaded leaves, resulting in greater final leaf area. Cell elongation in both palisade layers is of longer duration and occurs at a greater rate in unshaded leaves and is closely correlated with increase in leaf thickness. The volumes of palisade and spongy mesophyll are significantly greater in unshaded leaves than in shaded leaves, but the relative proportions of each tissue type does not change significantly. However, in shaded leaves there are fewer spongy mesophyll cells per unit area and a greater proportion of intercellular space than in unshaded leaves. Stomata are formed over the same time period in leaves grown at both intensities, but differentiate at greater rates in leaves grown in full sunlight, giving greater final stomatal density. These observations indicate that a sun plant such as *Helianthus* responds phenotypically to lowered light intensity primarily by a reduction in cell division (resulting in reduced leaf area), and secondarily by modifying cell expansion in a plane perpendicular to paradermal resulting in the characteristic anatomy of shaded leaves.

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L'auteur décrit, chez *Helianthus annuus*, les processus morphogénétiques responsables des différences histologiques entre les feuilles qui se développent en pleine lumière et celles qui se développent à 25% de la pleine lumière. En général, le déroulement de la division et de la croissance cellulaires chez les feuilles ombragées est en retard d'environ 2 jours par rapport aux feuilles de pleine lumière. Il n'y a aucune différence significative entre les deux types de feuilles dans la quantité d'accroissement cellulaire dans le plan paradermique dans l'épiderme et les couches palissadiques. Cependant, la division cellulaire se poursuit à un taux plus élevé dans toutes les couches cellulaires chez les feuilles de pleine lumière, ce qui conduit à une surface finale plus grande. L'élongation cellulaire dans les deux couches palissadiques dure plus longtemps et se poursuit à un taux plus élevé chez les feuilles de pleine lumière et est en corrélation étroite avec l'augmentation de l'épaisseur de la feuille. Le volume de parenchyme palissadique et le volume de parenchyme lacuneux sont significativement plus élevés chez les feuilles non ombragées que chez les feuilles ombragées, mais la proportion relative des deux types de tissus ne diffère pas significativement. Cependant, il y a chez les feuilles ombragées moins de cellules de parenchyme lacuneux par unité de surface et une plus forte proportion d'espace intercellulaire. Les stomates se forment durant la même période chez les feuilles croissant sous les deux intensités lumineuses, mais ils se différencient à un taux plus rapide chez les feuilles de pleine lumière, ce qui conduit à une densité stomatique finale plus grande. Ces observations montrent qu'une plante de pleine lumière, comme *Helianthus*, réagit phénotypiquement à une intensité lumineuse faible, d'abord par une réduction de la division cellulaire (et donc une diminution de la surface foliaire) et, secondairement, par une modification de l'expansion cellulaire dans le plan perpendiculaire au plan paradermique; cela produit la structure anatomique caractéristique des feuilles ombragées.

[Traduit par le journal]

Introduction

One of the expressions of phenotypic plasticity characteristic of many plant species is the modification of leaf morphology and anatomy by light intensity. Generally, leaves which expand under low light levels (shade leaves) differ from those which develop at high light levels (sun leaves) by having a thinner lamina with a less well-developed palisade mesophyll, a higher proportion of intercellular space, a lower ratio of internal to external surface area, wider spacing between the veins, larger epidermal cells with more undulate radial walls, and a lower stomatal density (Hanson 1917; Büsgen and Münch 1926; Penfound 1931; Wylie 1951; Hughes 1959; Lewis 1972). The striking histological differences associated with the di-

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morphism of mature sun and shade leaves obviously have been documented many times; however, with the exception of the work of Evans and Hughes (1961) on *Impatiens*, there is almost no information on the developmental sequence which results in this well-known dimorphism.

Typical shade plants such as *Impatiens parviflora* adapt to low light intensities by being able to increase leaf area along with only a slight reduction in photosynthetic rate per unit area (Evans and Hughes 1961), while sun-adapted species generally respond to a decrease in light intensity by a reduction in both net assimilation rate and leaf area (Blackman and Wilson 1951). In either type of response the difference in final leaf size will reflect differences in the roles of cell division and cell enlargement during leaf expansion. One or both of these processes may be involved; for instance, observations of leaf expansion in *Cucumis sativus* under different light intensities indicated that the smaller leaves which developed under shaded conditions had fewer cells than leaves expanding under high light intensity (Milthorpe and Newton 1963). Cell size was not affected except where the combination of high light intensity and low mineral supply resulted in smaller cells. On the other hand Schoch (1972) found that in *Capsicum annuum*, the greater expansion of leaf area in the shade is accompanied by both higher cell number and by larger cell size.

This investigation was undertaken to determine if the plasticity in leaf area brought about by an environmental change such as reduction in light intensity was the result of differences in cell division and (or) cell enlargement and, specifically, to determine differences in the timing and magnitude of these events. *Helianthus annuus* was chosen for this study since Blackman and Wilson (1951) have shown a direct correlation between leaf area and light intensity in this species and Sunderland (1960) has demonstrated a long period of overlap between cell division and cell enlargement during leaf formation under normal growth conditions.

Materials and methods

Seeds of *Helianthus annuus* cv. Commander were sown in a garden plot at the Glendon Hall campus of York University, Toronto. Burlap screening "Plantacryl super" which reduced incident light by 75% was placed over one-half the plot. Measurements in full sunlight using an Eppley pyronometer gave an average reading of 14.2 cal cm^{-2} min^{-1} (1 cal = 4.184 J) for the shaded plot and 55.7 cal cm^{-2} min^{-1} for the unshaded plot. Both plots received the same watering schedule.

Forty plants were collected at random from each plot at 2-day intervals after sowing, and the second pair of foliage leaves was removed from each plot. Twenty leaves, each from a different plant, were photocopied for leaf area determination and subsequently cleared. Leaves were cleared by boiling in 80% ethanol for 15 min, soaking in 5% sodium hydroxide at 60°C for 2 to 3 days, and placing in a saturated chloral hydrate solution until clear. The cleared leaves were then dehydrated in an ethanol series, stained with 0.05% safranin in 1:1 ethanol–xylol, and mounted in Permount. Pieces of tissue, 1 mm in diameter, were cut from the midregion of the lamina of the second leaf of each pair. The tissue was fixed with 1.5% glutaraldehyde in 0.05 *M* sodium cacodylate buffer at pH 7.2, postfixed with buffered 2% osmium tetroxide, dehydrated through an acetone series, and embedded in Spurr's plastic. Tissue was sectioned at 2 μm and stained with toluidine blue. The second pair of foliage leaves of the remaining 20 plants was oven-dried and used for determination of leaf dry weight.

Leaf area was determined by cutting out photocopies of leaves and weighing the photocopies in comparison to a standard. Leaf thickness was determined from 20 leaf cross sections per stage. Cell number was determined from leaf clearings (20 leaves per sample) by focussing on a single cell layer and counting the number of cells per ocular grid. Counts of cell number for leaves collected 4, 6, and 8 days after sowing were made from leaf paradermal sections. Values for cell number per layer per leaf were determined by dividing the leaf area by the area of the ocular grid and then multiplying by cell number. Height of epidermal and mesophyll cells was measured on photographic enlargements of leaf cross sections (20 leaves per sample, 10 cells per leaf). Cell cross-sectional area was determined from photographic enlargements of optical sections of cleared leaves and (or) paradermal leaf sections by cutting out cells and weighing the cutouts in comparison to a standard (20 leaves per sample, 10 cells per leaf). Tissue volume and volume of intercellular space were estimated by weighing cutouts from photographic enlargements of leaf cross sections representing a portion of leaf 100 μm across in an intercostal region. Counts of stomata were made from cleared leaves. Calculations of the significance of differences between mean values were done using either a "*d*"-test or *t*-test, where appropriate, at a $P \leq 0.05$ level.

Observations

Leaf area, leaf thickness, and leaf dry weight

Measurements of leaf area, leaf thickness, and leaf dry weight of the second foliage leaf of plants grown in full daylight show similar patterns with the highest rates of growth occurring between 6 and 16 days after sowing although leaf thickness and leaf dry weight continue to increase after growth in area ceases (Figs. 1, 2, 3). In contrast, expansion in area of leaves from plants grown in 25% sunlight lags behind that of leaves grown in full sunlight: the highest rates of growth occur between 10 and 20 days (Fig. 1). Expansion occurs over a shorter time period, and apparently at lower rates, giving a smaller final leaf area (9 cm^2, as compared with 21.6 cm^2, Table 1). The highest rates of increase in lamina thickness in shaded leaves occur between 8 and 12 days (Fig. 2); leaf dry weight increases slowly between 10 and 20 days (Fig. 3). Specific leaf area (leaf area per leaf dry weight) for leaves of plants grown in full daylight increases between 6 and 8 days and then remains more or less constant (0.3 cm^2/mg), while specific leaf area for leaves of plants grown at 25% daylight increases rapidly from

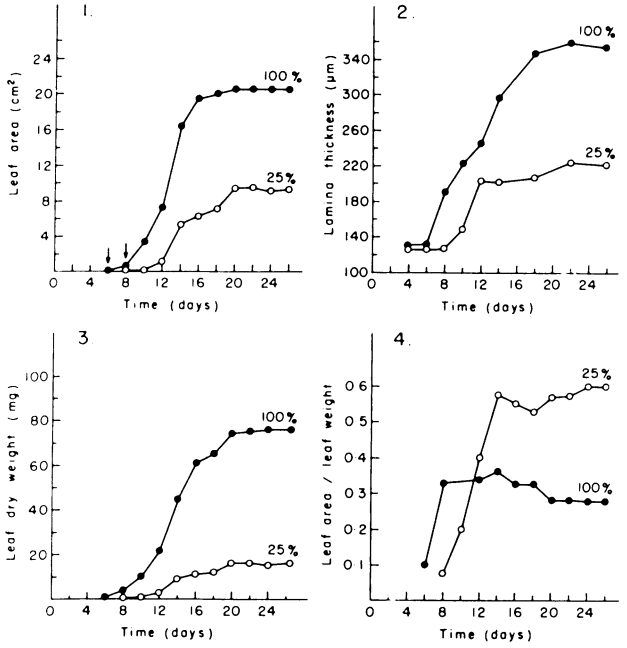


FIG. 1. Average area of the second leaf of plants grown in 100% and 25% daylight expressed as a function of time in number of days from sowing. Arrows indicate time of expansion from bud, 6 days in 100% daylight and 8 days in 25% daylight. FIG. 2. Average thickness of the second foliage leaf of plants grown in 100% and 25% daylight. FIG. 3. Average dry weight of the second foliage leaf of plants grown in 100% and 25% daylight. FIG. 4. Specific leaf area (leaf area per dry leaf weight) for the second foliage leaf of plants grown in 100% and 25% daylight.

10 days to 14 days, and then stabilizes between 0.55 and 0.69 cm^2/mg (Fig. 4). The greater specific leaf area of shaded leaves is a result of the reduced leaf thickness and dry weight which accompany reduced area; however, the increase in specific leaf area with shading in plants such as *Helianthus* is much less than that observed in typical shade plants (e.g., Blackman and Wilson 1951).

Tissue differentiation

Initiation of growth of the lamina of the second foliage leaf takes place between 2 and 4 days after germination in plants grown under conditions of full sunlight. By 4 days, the lamina consists of seven layers of cells; expansion at this stage apparently takes place by plate meristem activity, as mitotic figures with an anticlinal orientation are commonly observed (Fig. 15). Most of the cells of the leaf are meristematic with thin walls, small vacuoles, and relatively large nuclei. However, some trichome cells in both epidermal layers and a few cells associated with the large minor veins are enlarged and vacuolated. Two types of trichomes are present: multicellular trichomes consisting of two tiers of six to eight cells, and elongated unicellular trichomes with a multicellular base (Figs. 12, 13, 15, 17, 18). New trichomes are initiated through 6 days and occur with a relatively high density; after this stage no trichomes are initiated and leaf expansion results in the wider separation of trichomes. The epidermal cells become vacuolated by 8 days and also contain the first differentiated guard cells by this stage (Fig. 13). This characteristic is apparent in the lower epidermis by 10 days (Fig. 25) and in the upper epidermis by 12 days. At the earliest stages of lamina growth examined (6 days after sowing), cells of both palisade mesophyll layers are somewhat elongated, while cells of the three spongy mesophyll layers are more cuboidal in cross section (Fig. 12). Vacuolation becomes extensive in the mesophyll when intercellular space begins to develop, at about 8 days (Fig. 13). At this stage elongation of the cells of the uppermost layer of spongy mesophyll also becomes apparent. These elongated cells are more lobed at maturity than the true palisade parenchyma, but the presence of this "third palisade layer" generally distinguishes leaves grown at high light intensity from those grown at low light intensity (Figs. 13, 16). Numerous chloroplasts are observed in the peripheral cytoplasm of all mesophyll cells from 10 days onwards. Periclinal and oblique cell divisions in the lowermost palisade mesophyll layer and the uppermost spongy mesophyll layer give rise to the procambium of the minor veins. All procambium appears to be laid down by 6 days in leaves grown at high light intensity; mature tracheary elements are observed in some minor veins in clearings of leaves at 6 days and all minor veins have at least one mature tracheary element by 10 days. At maturity the smallest minor veins consist of a parenchymatous bundle sheath, one to two tracheary elements, a vacuolated xylem parenchyma cell, usually three sieve tube elements, and three to six darkly stained phloem parenchyma cells (Fig. 20).

Development of the second foliage leaf in plants grown at 25% daylight generally lags behind those grown in full daylight by about 2 days; otherwise the process of tissue differentiation is very similar. The epidermal cells become slightly more lobed and the outer epidermal walls are not as thickened as in leaves grown in full sunlight (Figs. 20, 23, 26, 27, 30, 31). Elongation of the palisade parenchyma cells is not as pronounced, especially in the second palisade layer which gives the appearance of spongy parenchyma in some cross sections (e.g., Figs. 22, 23). Vacuolation of the mesophyll also accompanies the beginning of rapid cell enlargement and formation of intercellular space (Figs. 16,

TABLE 1. Comparison of mature leaves of plants grown under 25% and 100% sunlight

Character	100% daylight	25% daylight
Leaf area, cm^2	21.6	9.0
Leaf thickness, μm	354.9 ± 9.1*	222.2 ± 8.5
Leaf dry weight, mg	76	15
Specific leaf area, mg/cm^2	0.28	0.60
Cell number × 10^4		
Upper epidermis	86.5 ± 2.1	44.1 ± 1.4
Palisade mesophyll	199.3 ± 3.9	88.7 ± 3.1
Spongy mesophyll	95.6 ± 2.4	34.9 ± 1.1
Lower epidermis	93.2 ± 3.2	41.6 ± 1.2
Cell height, μm		
Upper epidermis	22.8 ± 1.2	18.2 ± 0.7
Palisade mesophyll	79.4 ± 2.1	63.9 ± 2.3
Lower epidermis	18.5 ± 0.7	14.8 ± 0.4
Cell cross-sectional area, μm^2		
Upper epidermis	2245.6 ± 77.3	1921.2 ± 91.3
Palisade mesophyll	826.2 ± 13.1	893.5 ± 32.4
Lower epidermis	2310.5 ± 93.0	2459.5 ± 41.4
Tissue volume, μm^3†		
Upper epidermis	$18.69 \times 10^4 \pm 0.95 \times 10^4$(5.3%)	$14.89 \times 10^4 \pm 0.71 \times 10^4$(6.6%)
Palisade mesophyll	$148.8 \times 10^4 \pm 2.73 \times 10^4$(42.5%)	$92.20 \times 10^4 \pm 3.48 \times 10^4$(43.0%)
Spongy mesophyll	$164.1 \times 10^4 \pm 3.14 \times 10^4$(46.8%)	$96.87 \times 10^4 \pm 3.13 \times 10^4$(44.1%)
Lower epidermis	$19.05 \times 10^4 \pm 1.41 \times 10^4$(5.4%)	$14.00 \times 10^4 \pm 0.72 \times 10^4$(6.3%)
Intercellular space volume, μm^3†		
Palisade mesophyll	$62.23 \times 10^4 \pm 1.53 \times 10^4$(42%)	$27.99 \times 10^4 \pm 0.78 \times 10^4$(32%)
Spongy mesophyll	$85.23 \times 10^4 \pm 2.56 \times 10^4$(58%)	$58.57 \times 10^4 \pm 1.6 \times 10^4$(68%)
Total mesophyll	147.47×10^4	86.56×10^4
Stomatal density per mm^2		
Upper epidermis	94.9 ± 3.3	61.8 ± 3.2
Lower epidermis	102.6 ± 2.8	90.2 ± 2.4
Total stomata per leaf × 10^3		
Upper epidermis	205.0 ± 7.1	55.6 ± 2.8
Lower epidermis	221.6 ± 6.0	81.2 ± 2.2
Millimetre minor vein per square millimetre of leaf surface	5.6 ± 0.1	6.04 ± 0.13

*± standard error of the mean.
†Volume per 10 000 μm^2 leaf surface area (intercostal).

17). Differentiation of vascular tissue follows the same pattern as in leaves grown in full sunlight and structure of the minor veins is identical. Although differences between sun and shade leaves in the density of minor venation have been reported for other species, measurements of the length of minor veins per unit leaf surface in *Helianthus* show no significant difference in vein density between leaves of plants grown under light conditions (Table 1).

Cell number

Counts of numbers of cells for both epidermal layers, the uppermost palisade layer, and lowermost spongy mesophyll layer per leaf were made from leaf clearings and (or) paradermal sections using an ocular grid. Since vein density does not differ between the two treatments and the gradation in size of minor veins appears to be similar, measurements of cell number and size have been made for dermal and ground tissue only. At 6 days the second foliage leaves of plants grown in full daylight are about 5 mm in length and are just expanding from the bud. At this stage the leaves have reached 1% of the final area, and cell division before this stage has produced about 2% of the final number of cells in the uppermost palisade layer, 4% of the final number of cells in the lowermost spongy layer, and 5% of the final number of cells in both epidermal layers (Fig. 5). Cell division continues over the next 6 to 8 days in high light intensity leaves with the highest rates of increase in cell number occurring in the uppermost palisade layer between 8 and 12 days. Cell division apparently ceases at 14 days when the leaf has reached 74% of its final area. Final cell numbers in the epidermal layers and spongy mesophyll of the 100% light treatment are not significantly different, while

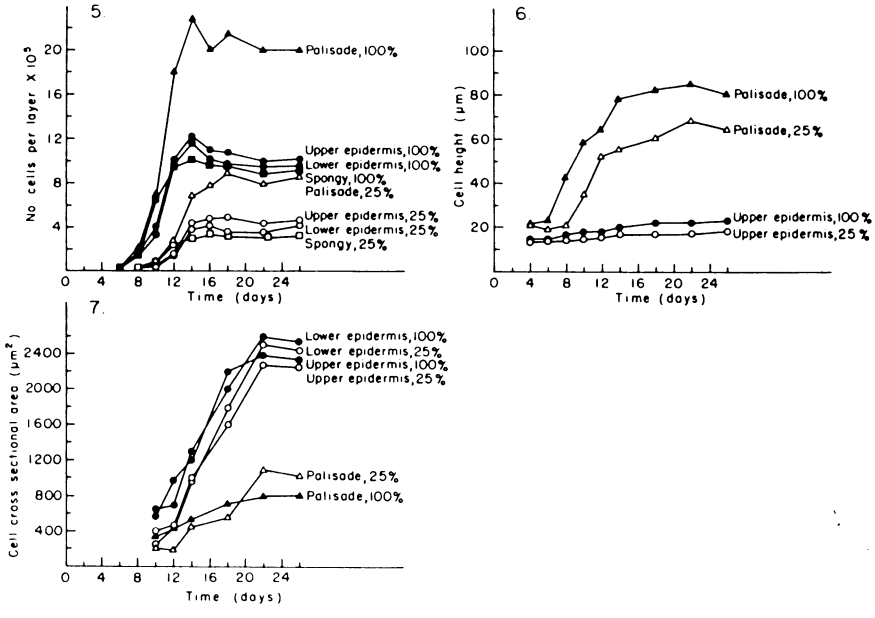


FIG. 5. Average cell number per upper epidermis, upper palisade mesophyll layer, lowermost spongy mesophyll layer, and lower epidermis in plants grown in 100% and 25% daylight. FIG. 6. Average height of cells of the upper epidermis and uppermost palisade layer of leaves of plants grown in 100% and 25% daylight. Pattern for the lower epidermis is similar to that for the upper. FIG. 7. Average cell area in the paradermal plane (cell cross-sectional area) for the upper epidermis, uppermost palisade layer, and lower epidermis of leaves of plants grown in 100% and 25% daylight.

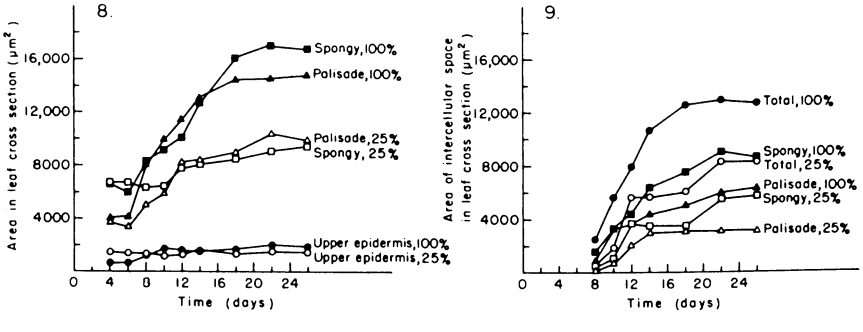


FIG. 8. Average tissue volume in an intercostal region (expressed as area of leaf cross section 100 μm wide) of upper epidermis, palisade mesophyll, and spongy mesophyll of leaves of plants grown in 100% and 25% daylight. Pattern for the lower epidermis is similar to that for the upper epidermis. FIG. 9. Average volume in an intercostal region (expressed as area of leaf cross section 100 μm wide) of intercellular space of the palisade and spongy mesophyll of leaves of plants grown in 100% and 25% daylight.

higher rates of cell division in the palisade mesophyll give rise to about 2.2 times as many cells per layer.

Cell division in the leaves of plants grown under 25% daylight occurs at a lower rate than in plants grown in full daylight in all cell layers. Eight days after sowing leaves expanding from the bud are 6 mm in length and have attained about 1% of their final area. At this stage both epidermal layers contain about 8% of final cell number, the spongy mesophyll about 9%, and the uppermost palisade contain about 6% of the final cell number. Cell division continues over the next 6 days in both epidermal layers and the spongy mesophyll and apparently for the next 10 days in the uppermost palisade layer (Fig. 5). Because of the lower rates of cell division final cell numbers are lower in all cell layers counted in leaves of plants grown under 25% daylight: about 36% of the final number found in the full sunlight plants for the upper epidermis, about 45% for the palisade mesophyll and lower epidermis, and 37% for the spongy mesophyll. When cells per unit area are compared in full daylight and shaded leaves there is no significant difference

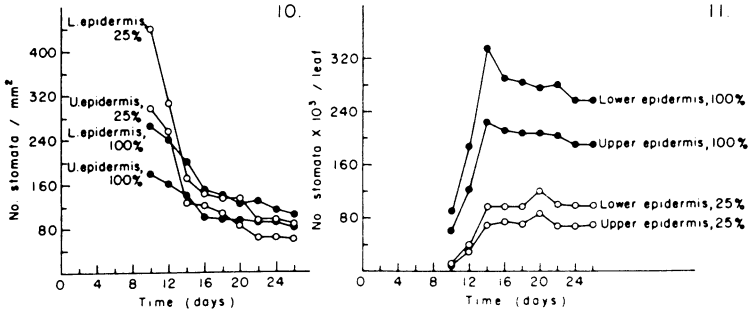


FIG. 10. Average density of stomata of the upper and lower epidermis of leaves of plants grown in 100% and 25% daylight.
FIG. 11. Average total number of stomata in the upper epidermis and lower epidermis of leaves of plants grown in 100% and 25% daylight.

between the two in upper epidermis, lower epidermis, and palisade mesophyll. However, there are significantly fewer spongy mesophyll cells per unit area in shaded leaves.

Cell size

Cell height was determined for upper and lower epidermis and for both palisade layers. Cell cross-sectional area was determined for the uppermost palisade layer and both epidermal layers. Measurements of the spongy mesophyll were not made because of the irregularity of cell shape after 8 days.

The uppermost two layers of mesophyll are differentiated as palisade parenchyma in leaves grown in full daylight (Fig. 20). Elongation of cells of the uppermost layer has begun at 6 days (Figs. 6, 12) and continues through 14 days. Elongation in the second palisade layer was found to occur through 18 days; cell height in both palisade layers was significantly greater in full daylight leaves. Cells of the uppermost spongy mesophyll layer also become elongated in leaves grown at high light intensity (Figs. 13, 19). Elongation of these mesophyll layers correlates well with increase in leaf thickness (Fig. 2).

Leaves grown in 25% sunlight characteristically have only one strongly differentiated layer of palisade parenchyma, although cells of the second layer are usually longer than wide (Figs. 21–23). Cells of the abaxial three layers of mesophyll differentiate as typical spongy parenchyma. Elongation of the uppermost palisade layer lags behind that in full daylight leaves with the period of most rapid elongation occurring between 8 and 12 days. This correlates closely with increase in leaf thickness in leaves grown at the lower light intensity (Figs. 2, 6).

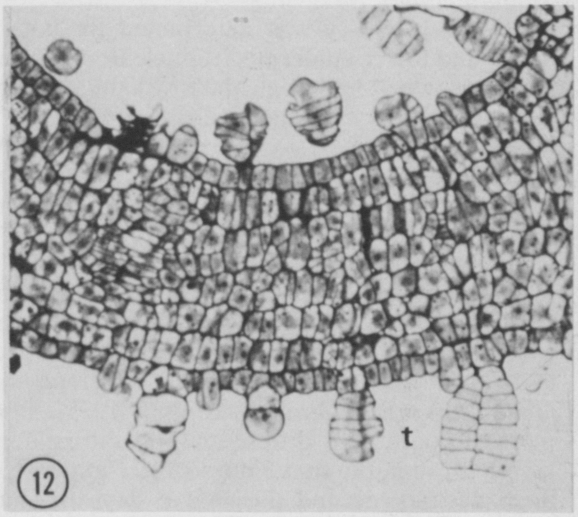
Most of the growth in size of epidermal cells occurs in the horizontal plane, with little increase in cell height during leaf expansion (Figs. 6, 7). Both upper and lower epidermal cells of leaves grown in high light intensity show significantly greater mean cell height compared with the same layers in low light intensity leaves throughout leaf expansion. Rapid growth in area occurs over a 12-day interval, from 10 days to 22 days (Fig. 7). There is generally good correlation between the pattern of cell enlargement in the epidermal layers and growth in leaf area for leaves from plants grown at both light intensities (Figs. 1, 7). There is no significant difference between average cell area of the upper and lower epidermis in either treatment, or between treatments throughout development.

Comparison of growth in cell area of the uppermost palisade layer shows that enlargement in a paradermal plane takes place earlier in leaves grown at high light intensity, but reaches a higher final value in leaves grown at 25% sunlight (Fig. 7). However, when cell volume (cross-sectional area × height) is calculated for the upper palisade layer, cells from leaves grown at high light intensity have a larger average volume, 65 550 μm^3, as compared with 57 080 μm^3 in low light intensity leaves.

Tissue and intercellular space volume

The volume of epidermal and mesophyll tissue

FIGS. 12–14. Cross section of leaves from plants grown in 100% daylight. Fig. 12. Leaf 6 days after sowing showing uniform appearance of cell layers and multicellular trichomes (*t*) in the epidermis. Fig. 13. Leaf 8 days after sowing showing vacuolation of epidermis and mesophyll, formation of intercellular space, differentiation of guard cells (*g*), and elongation of palisade parenchyma. Fig. 14. Cross section of leaf 10 days after sowing. FIGS. 15–17. Cross section of leaves from plants grown in 25% daylight. Fig. 15. Leaf 6 days after sowing showing anticlinal division at arrow. Fig. 16. Cross section of leaf 8 days after sowing. Fig. 17. Cross section of leaf 10 days after sowing. Note cell vacuolation and formation of intercellular spaces. The bases of two elongated trichomes (*t*) are seen in the upper epidermis.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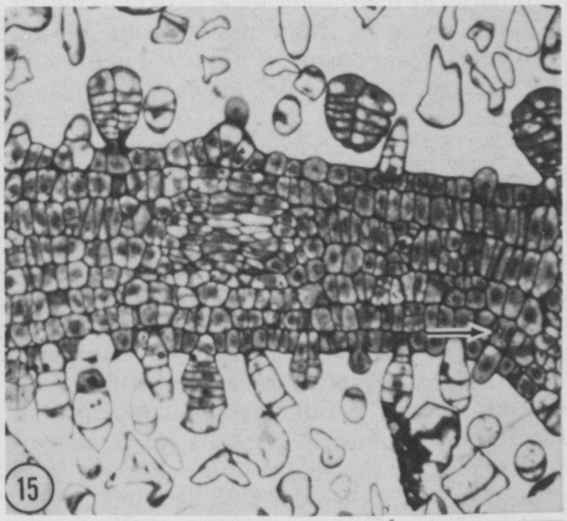
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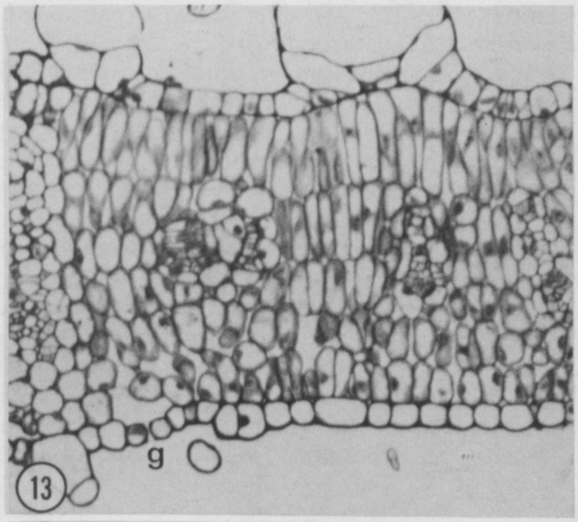
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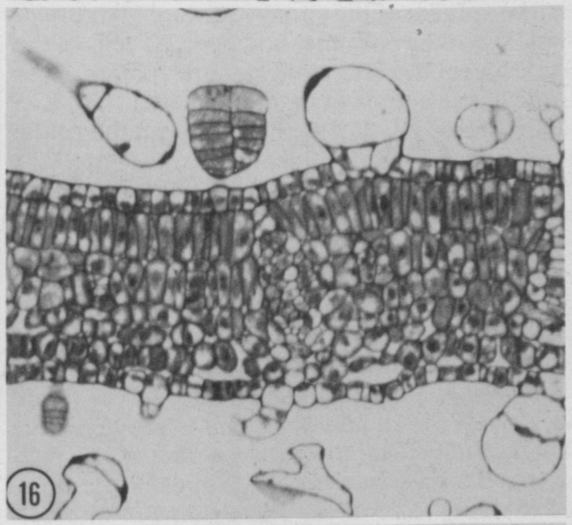
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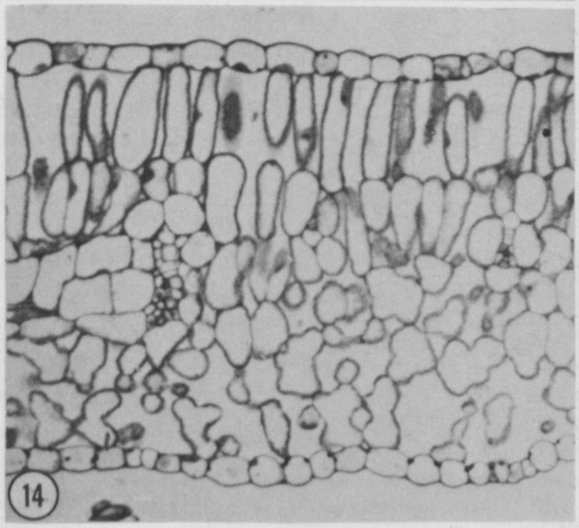
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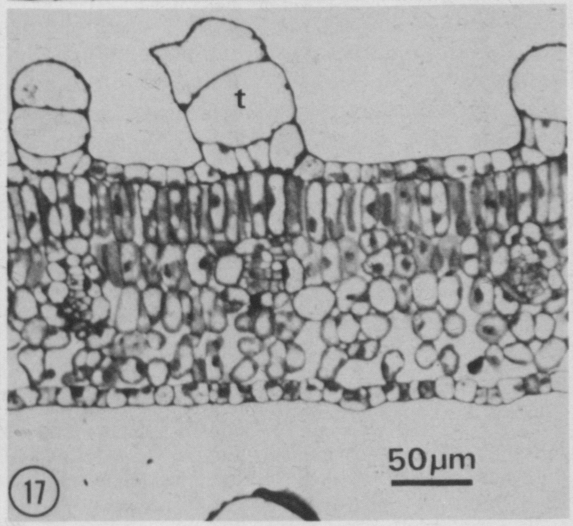
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was estimated by measuring the area of cells and of intercellular space in leaf cross sections 100 μm wide. No significant change is observed in the volume of the epidermal layers of leaves of plants grown under either light intensity during leaf expansion (Fig. 8). In leaves from plants grown at high light intensity, the volume of palisade and spongy mesophyll increases between 6 and 18 days, the period of time during which the leaf increases in thickness and area (Figs. 1, 2). Spongy mesophyll occupies a significantly greater final volume than palisade mesophyll. In leaves from plants grown at lower light intensity, palisade and spongy mesophyll show a rapid increase in volume between 6 and 12 days, paralleling a similar pattern of growth in leaf thickness. There is no significant difference between the final volumes of spongy and palisade parenchyma. Under both light intensities, the initial volume of the spongy mesophyll is greater than that of the palisade; however, as cells of the palisade layers begin to elongate, the volume of the palisade tissue comes to be more similar to that of the spongy mesophyll (Fig. 8). Although the actual volumes of palisade and spongy mesophyll are significantly greater in leaves of plants grown at high light intensity as compared with low light intensity; the proportion of total leaf volume occupied is similar: palisade occupies about 42% and spongy mesophyll occupies about 45% of total leaf volume in mature leaves.

The volume of intercellular space was measured separately using the same techniques as for tissue volume (Fig. 9). In leaves of plants grown under both light intensities, the total volume of intercellular space increases from the earliest stage measured (8 days) to 22 days. The volume of intercellular space in the spongy mesophyll is significantly greater than the volume of intercellular space in the palisade mesophyll. And the volume of intercellular space is significantly greater in leaves grown under high light intensity. However, in leaves grown in 25% daylight, intercellular space of the spongy mesophyll accounts for a higher proportion of the total intercellular space in the leaf (68.2% as compared with 58.6%). Also, a higher proportion of the total tissue volume of the spongy mesophyll is occupied by intercellular space in shaded leaves (59% as compared with 51%).

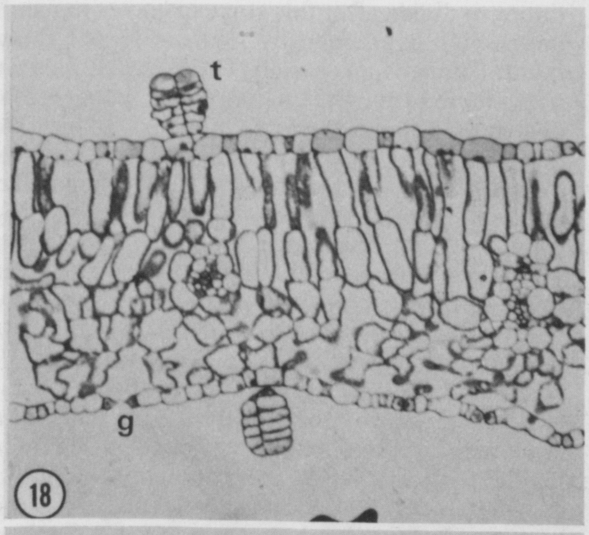
Stomatal index

Stomatal density was determined for both the upper and lower epidermis from cleared leaves. In leaves grown at both high and low light intensities, stomatal density is significantly greater on the lower epidermis as compared with the upper epidermis: about $221.6 \pm 6.0 \times 10^3$ stomata/mm^2 as compared with $205.0 \pm 7.1 \times 10^3$ stomata/mm^2 in leaves grown at high light intensity, and $81.2 \pm 2.2 \times 10^3$ stomata/mm^2 as compared with $55.6 \pm 2.8 \times 10^3$ stomata/mm^2 in leaves grown in low light intensity (Fig. 10). At maturity, stomatal density on both leaf surfaces is significantly higher in leaves grown at high light intensity. Stomatal development in *Helianthus* is asynchronous (Figs. 28, 29), and new pairs of guard cells differentiate in the epidermal layers through the first 8 days of leaf expansion in unshaded leaves and the first 6 days in shaded leaves (Figs. 11, 25); overall leaf expansion, however, continues through 20 days. Since differentiation of stomata occurs over the same time period in both epidermal layers in leaves of plants grown at both light intensities, rate of initiation must be higher in the lower epidermis as compared with the upper, and in leaves of plants grown at high light intensity as compared with those grown at low intensity. It might be expected that leaves which show the greatest increase in area might have the same total number of stomata, but a lower stomatal density than in leaves which undergo less expansion. This is not the case in *Helianthus*: leaves grown under high light intensity show the greatest amount of leaf expansion and the higher stomal density, and therefore, a larger total number of stomata.

Discussion

The leaf dimorphism induced by such environmental cues as light, temperature, and day length provide one of the simplest situations in which to study differential expression of leaf form by the same genotype since leaf size and histology are altered, while leaf form often is not. By changing the environment it is possible to alter the timing and rates of cell division and cell enlargement, the processes by which final leaf size and the characteristic differences between tissue types are achieved. While the final result of the diverging

FIGS. 18–20. Cross section of leaves from plants grown in 100% daylight. Fig. 18. Leaf 12 days after sowing showing multicellular glandular trichomes (*t*) and pairs of guard cells (*g*) in the lower epidermis. Fig. 19. Leaf 14 days after sowing. Fig. 20. Leaf 26 days after sowing. Note tracheary element (*x*) and sieve tube element (*p*) of the minor vein. Figs. 21–23. Cross sections of leaves from plants grown in 25% daylight. Fig. 21. Leaf 21 days after sowing. Fig. 22. Leaf 14 days after sowing. Fig. 23. Leaf 26 days after sowing.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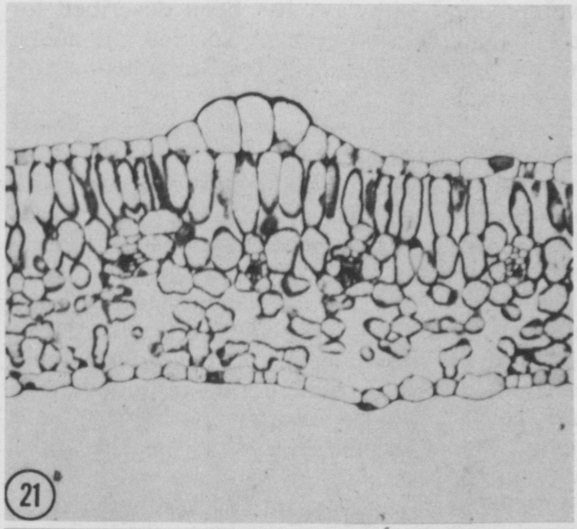
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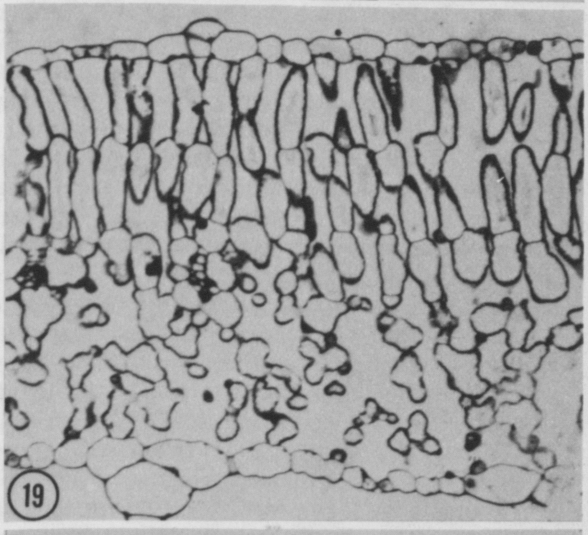
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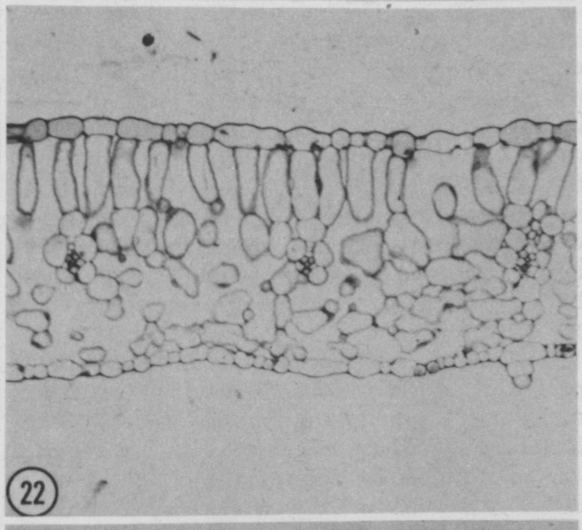
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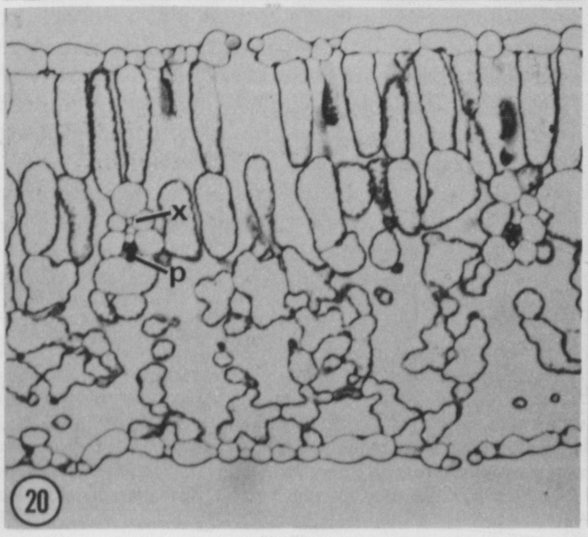
x
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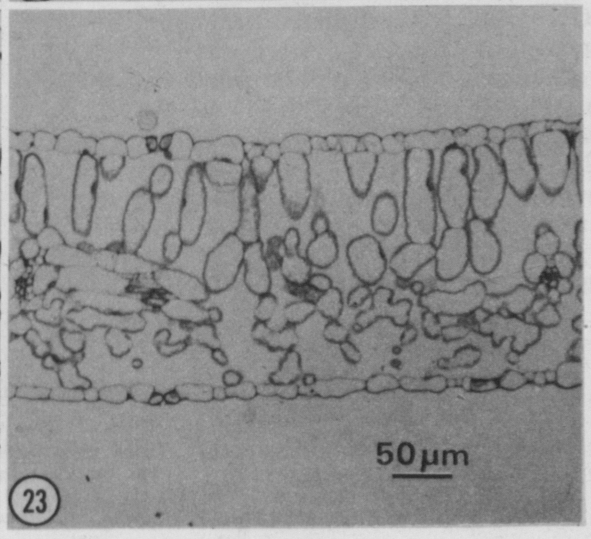
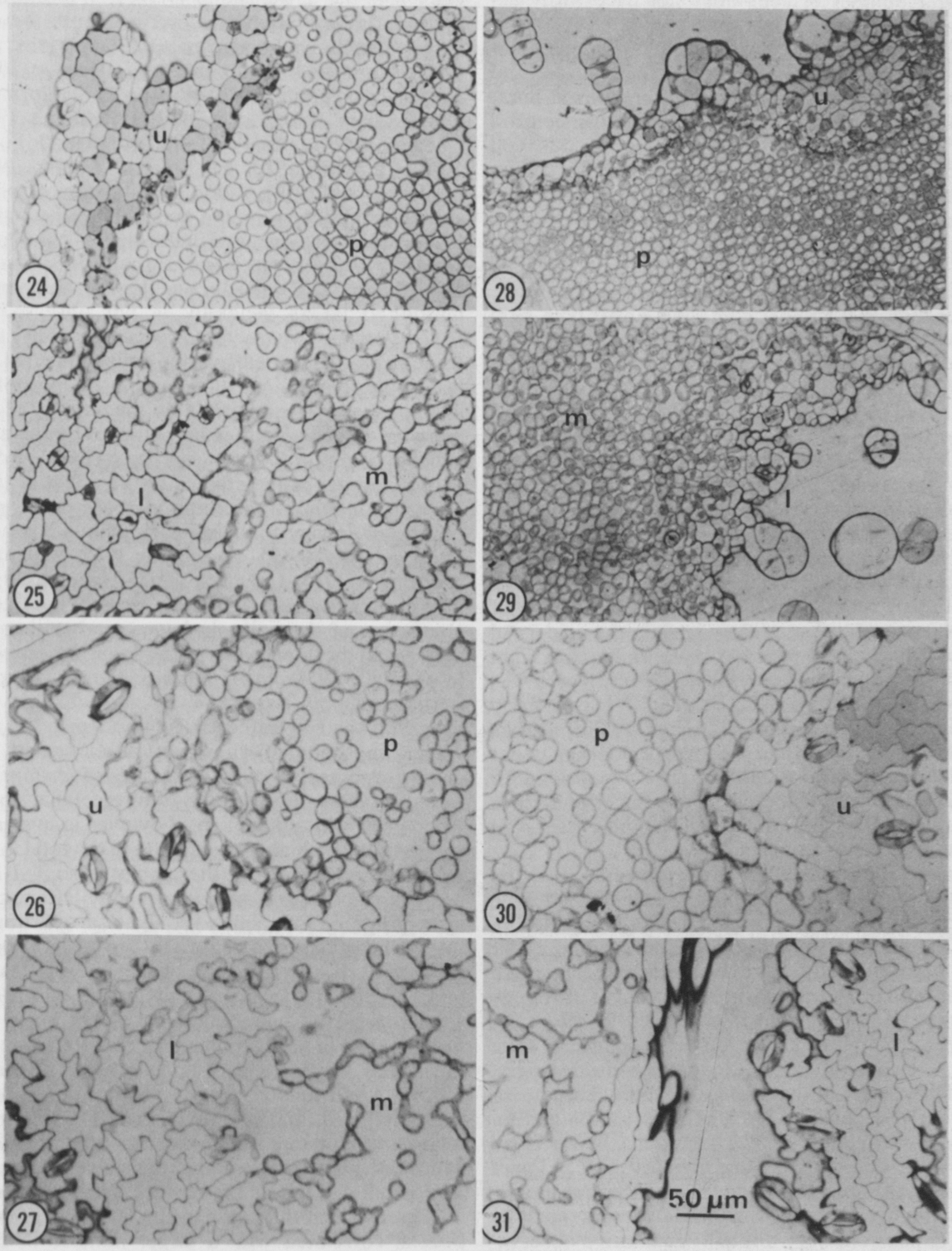

50µm
23

developmental pathways has been described for sun and shade leaves in many species, the actual timing and magnitude of these developmental processes has not.

The leaves of *Helianthus annuus* which expand under low light intensities are characterized by having reduced (42%) surface area compared with leaves expanding in full sunlight. Such differences in leaf area between leaves expanding under different environmental conditions may be the result of differential cell division, cell enlargement, or a combination of the two processes. The reduction in leaf area in the shaded leaves of *Helianthus* largely is accounted for by a reduction in cell number; in mature leaves grown at low light intensity there are roughly 40% of the number of cells in the upper epidermis, lower epidermis, and adaxial palisade layer found in leaves grown at high light intensities. Before expansion from the bud, there is no significant difference in the number of cells per layer; however, differential cell division rapidly results in significant differences in cell number as the leaf expands. Although cell division occurs over roughly the same time period (0 to 14 days) in both light intensities, numbers of cells per cell layer in shaded leaves lag 2 to 4 days behind values for unshaded leaves, and higher rates of division must occur in leaves expanding in full daylight, contributing to the higher final cell number observed in these leaves. This is correlated with the observation that there is no significant difference in paradermal area of epidermal cells between leaves expanding at different light intensities in *Helianthus*, indicating that cell enlargement is not the mechanism involved in the expression of leaf size in *Helianthus*.

Generally, it appears that cell number is the main determinant of leaf size and that cell size is relatively unimportant (Humphries and Wheeler 1963). This is true for *Cucumis sativus*, a sun plant in which shading produces smaller leaves with few cells (Milthorpe and Newton 1963; Wilson 1966). In *Cucumis*, in contrast to *Helianthus*, the ultimate number of cells appears to be primarily determined by the rate of division before the leaf expands from the bud; however, a higher proportion of cells continues to divide after this stage in leaves expanding under high light intensity. Wilson (1966) showed that cell dimensions parallel to the leaf surface were not affected by light intensity and that therefore, final areas depended on cell numbers. In his extensive study of the growth of sun and shade leaves in *Populus euamericana*, Pieters (1974) found that cell size remains relatively constant irrespective of light treatment and concluded that leaf size is determined primarily by cell number. Dale (1965) found that high light intensity resulted in larger leaves with a greater number of cells in *Phaseolus vulgaris* but did not measure cell size. More recently, Verbelen and DeGreef (1979) have shown that, in *Phaseolus vulgaris*, both cell division and cell enlargement are significantly reduced in leaves expanding in total darkness as compared with those growing under continuous light of intensity similar to that of Dale. Schoch (1972) compared number of epidermal cells and leaf area in the shade plant, *Capsicum annuum*, grown under sun and shade conditions and found greater expansion of leaf area (1.8 × sun leaves) in the shade accompanied by higher cell number (1.4 × sun leaves). Calculations from his data show that shade leaves must have epidermal cells that are about 1.3 times the size of those of sun leaves, indicating that both cell number and cell size are correlated with leaf area. Njoku (1956) found that a reduction in light intensity resulted in a change in leaf shape in *Ipomoea caerula*, as well as reduction in leaf area. Leaves grown at higher light intensities had more epidermal cells of smaller size than leaves grown at low light intensity. Isanogle (1944) indicates that more divisions of the plate meristem occur during expansion of unshaded leaves of *Cornus florida* and *Acer platanoides* resulting in greater cell numbers; however, she also illustrates larger cell size in mature unshaded leaves. Slade (1970) found that in *Poa alpina* the wider spacing of minor veins in shaded leaves was the result of greater cell size rather than an increase in cell number. These observations that leaf size and shape can be altered either by changing cell number or by changing cell size lend support to the concept that the control of size is at the level of the whole organ and that partitioning into a

FIGS. 24–27. Paradermal sections of leaves from plants grown in 100% daylight. Fig. 24. Upper epidermis (*u*) and palisade mesophyll (*p*) of leaf 10 days after sowing. Fig. 25. Spongy mesophyll (*m*) and lower epidermis (*l*) of leaf 10 days after sowing. Note lobing in anticlinal walls of epidermis and asynchronous development of stomata. Fig. 26. Upper epidermis (*u*) and palisade mesophyll (*p*) of leaf 26 days after sowing. Fig. 27. Spongy mesophyll (*m*) and lower epidermis (*l*) of leaf 26 days after sowing. FIGS. 28–31. Paradermal sections of leaves from plants grown in 25% daylight. Fig. 28. Upper epidermis (*u*) and palisade mesophyll of leaf 10 days after sowing. Fig. 29. Spongy mesophyll (*m*) and lower epidermis (*l*) of leaf 10 days after sowing. Fig. 30. Upper epidermis (*u*) and palisade mesophyll (*p*) of leaf 26 days after sowing. Fig. 31. Spongy mesophyll (*m*) and lower epidermis (*l*) of leaf 26 days after sowing.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31

greater number of cells does not necessarily accompany increase in leaf surface area.

The characteristic histological differences between upper and lower epidermis and palisade and spongy mesophyll arise from differences in duration and rate of cell division and cell expansion and the direction of cell expansion in various cell layers. For instance, cell division normally occurs at higher rates and for a longer duration in the palisade parenchyma than in the epidermal layers (e.g., Maksymowych 1963; Denne 1966; Dengler *et al.* 1975). This is true in *Helianthus* where cell division occurs at higher rates in the upper palisade layer than in the upper epidermis under both light intensities, and apparently occurs over a longer time period under low light intensity. Many studies of the effect of light intensity on leaf development are based on measurements of the upper epidermis only (e.g., Njoku 1956; Schoch 1972; Pieters 1974); however, because the epidermis ceases dividing and starts enlarging before underlying tissues, these observations only partly indicate the processes taking place in the whole leaf. In *Helianthus* palisade cells show considerable increase in cell height during leaf development while the epidermal cells show very little. Cell expansion in the plane of the lamina occurs at greater rates in the epidermal layers than in the palisade layer under both light conditions, while a decrease in mean cell area was observed in the palisade of shaded leaves early during cell division. The upper epidermis has slightly more cells than the lower epidermis under both light intensities; this is likely compensated for by greater mean cell areas in the lower epidermis although the difference measured was not significant in our samples.

In *Helianthus* leaf thickness increases over the same time period in which leaf expansion is taking place and is closely correlated with elongation of the palisade mesophyll. Leaves expanding under full light typically have two elongated palisade layers and often cells of the adaxial layer of spongy mesophyll are somewhat elongated. In contrast, leaves expanding under shaded conditions have one, and sometimes two, elongated palisade layers, while the cells of the spongy mesophyll are never obviously elongated. Both cell elongation and increase in leaf thickness lag behind that in unshaded leaves by about 2 days and also level off at lower values. Although the lobed shape of typical spongy mesophyll cells makes accurate measurement of cell dimensions very difficult; qualitative observation of cross sections indicate that the volume of spongy mesophyll cells in sun leaves may be greater. The greater elongation of individual palisade cells and the presence of a greater number of palisade layers has been described both for sun leaves developing under natural conditions and under controlled conditions (e.g., Hanson 1917; Büsgen and Münch 1926; Watson 1942; Cormack and Gorham 1953; Cormack 1955; Hughes 1959; Cooper and Qualls 1967; Ballantine and Forde 1970; Cameron 1970; Chabot and Chabot 1977; Chabot *et al.* 1979). Some investigators also describe a reduction in the number of mesophyll layers in leaves expanding under shaded conditions (Hanson 1917; Cormack and Gorham 1953; Chabot and Chabot 1977). Reduction in the number of mesophyll layers generally is not observed in *Helianthus*, but where it does occur it is likely that these changes occur early in the development of the leaf primordium since the number of mesophyll layers usually is established by the activity of the marginal meristem and is perpetuated by the anticlinal divisions of the plate meristem (Esau 1965).

In a number of the studies of the effect of light intensity on leaf structure an increase in the proportion of spongy to palisade mesophyll has been observed (Wylie 1951; Anderson 1955; Ballantine and Forde 1970; Cormack 1955; Isanogle 1944). This generally appears to be the result of the lack of elongation of the palisade layers, rather than an increase in the dimensions of the spongy mesophyll (e.g., Wylie 1951). In *Helianthus*, the relative proportions of both types of mesophyll change very little with shading, rather cells of both palisade and spongy mesophyll tissues are less elongated, resulting in thinner leaves. The leaves of *Helianthus* have a greater total volume of intercellular space when grown in full sunlight; however in leaves of shaded plants a higher proportion of the total intercellular space is found in the spongy mesophyll and a larger proportion of spongy mesophyll tissue consists of intercellular space. A higher proportion of intercellular space, especially in the spongy mesophyll, is characteristic of many shade leaves (e.g., Wylie 1951; Ballantine and Forde 1970; Cameron 1970; Chabot and Chabot 1977), but the total volume of intercellular space is often reduced in shaded leaves (e.g., Isanogle 1944; Cormack 1955). Unfortunately the shape of spongy mesophyll cells makes it very difficult to determine whether the increase in proportion of intercellular space is the result of greater expansion of individual mesophyll cell lobes, smaller cells, or fewer cells. The greater leaf thickness in sun leaves of *Helianthus* is correlated with a larger number of cells which are more elongated giving a larger internal surface area which, on a per unit external surface area basis, is greater than that for shaded leaves.

Stomatal development during leaf expansion has been described for *Helianthus* by Rawson and Craven (1975). They found mean values of 182.5 ± 24.9 stomata/mm² on the adaxial epidermis and 276.7 ± 30.5 stomata/mm² on the abaxial epidermis of the 14th foliage leaf, as compared with values reported here, 205.0 ± 7.1 stomata/mm² and 221.6 ± 6.0 stomata/mm², for the 2nd. foliage leaf. They describe the decrease in stomatal density during leaf expansion similar to the pattern described in this paper, but indicate that this dilution was largely offset by an increase in the size of the guard cells with the result that the stomatal area per unit leaf changed little after leaves reached 50% of their final area. In the observations of *Helianthus* reported here, it was found that the density of stomata on both the abaxial and adaxial epidermal layers is significantly higher in leaves expanding under full daylight than leaves expanding under 25% daylight, and since the unshaded leaves reached a larger final area, they have higher total numbers of stomata on both epidermal layers. The lower density of stomata in leaves expanding under shaded conditions appears to be a general phenomenon and has been reported in *Medicago* (Cooper and Qualls 1967), *Capsicum* (Schoch 1972), *Phaseolus* (Knecht and O'Leary 1972; Crookston *et al.* 1975), and *Solidago* (Holmgren 1968), amongst others. The lower density of stomata in the shade may result from either the greater enlargement of epidermal cells or from lower rates of formation of stomatal initials during the phase of cell division. In *Helianthus*, the greater density of stomata is due to a higher rate of initiation of guard cell pairs. Leaves expanding under low light intensities have lower stomatal densities because of a low rate of initiation of guard cells, and epidermal cell expansion is slightly less than that in high light intensity leaves. Penfound (1931), Schürmann (1959), and Rawson and Craven (1975) all have reported similar observations for *Helianthus*. In contrast, Knecht and O'Leary (1972) found that the total number of stomata per leaf did not vary in *Phaseolus* and that the lower density observed in leaves grown at the lowest light intensities was the result of greater leaf expansion.

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