

Effects of Light and Nutrients on Leaf Size, CO₂ Exchange, and Anatomy in Wild Strawberry (*Fragaria virginiana*)¹

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ABSTRACT

Plants of a single genotype of wild strawberry, *Fragaria virginiana* Duchesne, were grown with or without fertilizer in high (406 microeinsteins per square meter per second) and low (80 microeinsteins per square meter per second) light. High-light leaves were thicker than low-light leaves and had greater development of the mesophyll. Within a light level, high-nutrient leaves were thicker, but the proportions of leaf tissues did not change with nutrient level. Maximum net CO₂ exchange rate and leaf size were greatest in high-light, high-nutrient leaves and lowest in high-light, low-nutrient leaves. Changes in mesophyll cell volume largely accounted for differences in CO₂ exchange rate in low-light leaves, but not in high-light leaves.

Leaf size in these experiments was apparently determined by nutrient and carbon supply. This may explain the observation that the largest leaves produced by wild strawberries in the field occur in high-light, mesic habitats, rather than in shady habitats.

photosynthesis of *Lolium* spp. grown in several light/temperature treatments was expressed on a mesophyll volume basis. Despite the correlations between leaf anatomy and photosynthesis noted above, the effects of environmental conditions on the relationships among the anatomic and physiologic characteristics of leaves are still not clear. Also, control of leaf size has received inadequate attention even though it is a major variable in leaf carbon yield. For example, it is typically expected that leaves grown in shade will be larger and thinner than leaves grown in sunny conditions. However, we have observed that the largest leaves of wild strawberries occur in mesic, sunny habitats, not in shady areas (7).

This study investigated the effects of light and nutrients on leaf size, anatomy, and CO₂ exchange. The common wild strawberry of the northeastern U.S., *Fragaria virginiana*, was used because it exhibited apparently unusual patterns of leaf size, because its vegetative reproduction allowed the use of genetically identical plants, and because our previous experience with *Fragaria* (2, 3, 8) facilitated the design of these experiments.

MATERIALS AND METHODS

Plant Growth Conditions. Runner plantlets of a single genotype of *F. virginiana* Duchesne, taken from a natural population near Ithaca, NY, were rooted in a commercial artificial soil mixture (Jiffy-Mix), which consisted of peat, vermiculite, and nutrients adequate for plant establishment. When the first leaf produced after rooting was unfolding, all other leaves on that plant were removed. Plants were then placed in 500 cm³ plastic pots in fresh Jiffy-Mix and assigned to experimental treatments. This procedure maximized uniformity of plant size and physiologic condition, but required that individual plants be placed into the experimental conditions over a span of 1 week. Separate records for each plant were maintained, however, so that 'elapsed time' in each treatment was calculated separately for each individual.

All treatments were established in a single walk-in growth chamber (EGC M-12). A 12/12 h thermoperiod of 25/15°C was centered on a photoperiod of 15/9 h (light/dark). Light was supplied by a combination of 100-w incandescent, 400-w mercury vapor, and 400-w sodium vapor lamps (total wattage ratios of 3/4/4), with wire screen and lower racks used to achieve the lower flux density. All plants were watered daily with distilled H₂O. Relative humidity was uncontrolled.

Experimental Treatments. Two levels of nutrient supply in each of two light levels were used. Plants received either 0 ml or 150 ml/week of a complete balanced fertilizer, Peters General Purpose (14.39% ammoniacal nitrogen, 5.61% NO₃, 20% P₂O₅, 20% K₂O with trace elements; Robert B. Peters Inc., Allentown, PA). The fertilizer solution consisted of 2.53 g of dry fertilizer/1 distilled H₂O. The 150 ml were given in three doses of 50 ml two or three days apart. The high-light treatments had a PPFD³ of 406 ± 9 (n

Photosynthetic capacity of a leaf is a function of many factors, including the amount and configuration of the cells present and the CO₂ exchange potential of those cells. The source of differences in photosynthetic rate per unit area is often not apparent because leaves may vary considerably in number of cells, dry weight, Chl, etc., per unit blade area. Changes in amount of cellular material per unit area or in physiological capacity of the cells each might produce changes in whole-leaf CO₂ exchange rate. Hypothetically, even a change in the spatial relationships of cells could affect whole-leaf CO₂ exchange rate by altering gas diffusion patterns (17).

There have been numerous studies of the photochemical and biochemical aspects of photosynthesis, photorespiration, and dark respiration of leaves. Most such studies have either examined processes directly at an intracellular level or did not distinguish anatomic and physiologic effects on CO₂ exchange. However, in some cases changes in whole-leaf CO₂ exchange have been correlated with changes in leaf anatomy. Wilson and Cooper (22–25) found a negative correlation of photosynthetic rate with mesophyll cell size in different genotypes and experimental treatments of *Lolium perenne* L., although the relationship was not completely consistent. Nobel *et al.* (15) found that changes in mesophyll cell surface area accounted for differences in CO₂ exchange rates of *Plectranthus parviflorus* grown at several irradiances. Charles-Edwards *et al.* (4) found differences in rates to be reduced when

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³ Abbreviations: g^{cell}, intracellular liquid-phase CO₂ conductance; PPFD, photosynthetic photon flux density (400–700 nm); RuBP, ribulose 1,5-bisphosphate; SLW, specific leaf weight (weight/area).

= 12) $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, or 21.9 $\text{E}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, at leaf level; low-light treatment PPFd was 80 ± 8 ($n = 12$) $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, or 4.3 $\text{E}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Strawberry leaf development is determined by the integral of light received during the day, up to values of 20 to 30 $\text{E}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Daily PPFd above 22 $\text{E}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ result in little further increase in leaf thickness or SLW (3, 7). Our low-light treatment matched the daily PPFd received by wild strawberries in one of the shadier habitats studied by Jurik (7). Daily integrated PPFd in our high-light treatment was slightly greater than the daily PPFd in the mesic habitat in which Jurik (7) observed the largest strawberry leaves. A cloudless day in early summer in Ithaca will have integrated light values of about 50 $\text{E}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Values less than 5 $\text{E}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, including light flecks, occur commonly in *Fragaria* populations under forest and herbaceous canopies (B. F. Chabot, unpublished data).

Gas Exchange Measurements. Leaves used for gas exchange and leaf anatomy analysis were from leaf buds appearing 2 to 4 weeks after the plants were placed in the experimental conditions. Leaves were individually tagged on appearance and were analyzed 9 to 12 d after unfolding to minimize age effects on metabolism. Leaves of this age have just completed expansion and have maximum photosynthetic capacity (8). Single attached leaves were measured using a Beckman 315 IR gas analyzer modified for differential analysis in an open system using ambient levels of CO_2 and O_2 . Light was supplied by two to four GE Quartzline 500-w lamps filtered through 11 cm of water and variable amounts of wire screen and cotton cheesecloth. PPFd was measured using a LI-COR quantum sensor in the gas-exchange cuvette. Air temperature, leaf temperature, air dew point, and wind speed were controlled and monitored. Air stream flow rates were measured with a Hastings-Raydist mass flow meter, whereas air stream humidity was measured with Vaisala Humicap polymer-film type sensors. Leaf temperatures were measured with a fine-wire thermocouple (40 gauge) and maintained to within one degree of the nominal 25°C measurement temperature. RH at 25°C was 90%. Leaf area was calculated by comparing total leaf weight to weight of leaf disks of known area. Dry weight was determined after drying to constant weight in a forced-air oven at 70°C. Stomatal conductance was calculated from the transpiration rate divided by the difference in water vapor concentration between leaf and air (13).

Anatomic Measurements. After gas exchange analysis, plants were placed back in the growth chamber overnight to permit the hydrolysis and translocation of starch formed during the day. Early the next morning discs of known area were taken from the leaves used in gas exchange analysis for determination of SLW. Samples for anatomy were taken from the same leaves and fixed in 2.5% glutaraldehyde-2.0% (para)formaldehyde in 0.1 M Na-K phosphate buffer. After washing in buffer the tissues were post-fixed in 1% OsO_4 in the same buffer, dehydrated in an ethanol and propylene oxide series, and embedded in plastic. Sections

(less than 0.5 μm thick) for light microscopy were stained with aqueous toluidine blue. Anatomic features were quantified using a micrometer scale and a grid in one eyepiece of a microscope. Percentages of tissue volumes were calculated by using the number of grid points over each cell type on five different sections from each treatment. The area occupied by each tissue in thin sections should be equivalent to the volume of each in the leaf (21). Stomatal frequency of the lower leaf surface was measured using cellulose acetate (nail polish) replicas mounted on slides with double-sided tape. Counts were made with a grid reticule in one eyepiece of a microscope using a 10× objective lens. Treatment means were calculated from three leaves per treatment using counts from three areas per leaf.

Leaf Biomass. Leaves on the mother crown were individually tagged on appearance, whereas plantlets produced on runners were not included. Means for number of leaves per plant, area per leaf, total canopy leaf area, and leaf biomass were based on leaf buds appearing during the first 35 d of growth. Area of fully expanded leaves was calculated from measurements of blade length and width using the regression equation given by Jurik (7). Total canopy leaf area was based on mature leaf area. Total leaf blade weight was based on actual weight of mature leaves harvested at the end of the experiment.

Chemical Analysis. After 5 to 6 weeks of plant growth in high light and 8 weeks of growth in low light, all completely expanded leaves showing no signs of senescence were harvested and oven dried. Leaf blade material was combined with the disks used for SLW determinations and ground in a Wiley mill. Samples were analyzed for nitrogen using a micro-Dumas procedure (Coleman Nitrogen Analyzer). Two determinations were made on the composite sample of each experimental treatment.

Statistics. Treatment means were compared by using analysis of variance and the Student-Newman-Keuls procedure for multiple comparisons (19).

RESULTS

The number of leaves produced per plant in the first 35 d of the experiment was greatest under high light-150 ml (H150) conditions (Table I); the high light-0 ml (H0) plants produced substantially fewer leaves. Low-light plants (L150 and L0) produced only one-fourth as many leaves as the H150 plants; there was no difference between nutrient treatments under low light.

Mean mature leaf area varied widely both among and within treatments (Table I). The H150 treatment had the greatest mean area, whereas the H0 treatment had the lowest. Low-light treatments had intermediate means, with the L150 mean higher than the L0 mean. In all treatments, there were no obvious changes in shape with size except in a few very small leaves produced from axillary buds; such leaves were narrower and had fewer marginal serrations.

Within nutrient levels, high-light leaves generally were thicker

Table I. Number, Size, and Biomass of Leaves Produced in the Light \times Nutrients Experiment

High and low light levels were 406 and 80 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. High nutrient treatment plants received 150 ml/week of Peters 20-20-20 (2.53 g/l), while low nutrient treatment plants received 0 ml/week. Values in the same row followed by the same letter are not significantly different ($P > 0.05$). Numbers in parentheses indicate sample sizes.

	High Light-High Nutrients	High Light-Low Nutrients	Low Light-High Nutrients	Low Light-Low Nutrients
No./plant	19.7 ^a \pm 3.8 (7)	11.7 ^b \pm 1.4 (7)	5.4 ^c \pm 1.1 (7)	5.4 ^c \pm 1.8 (7)
Area/leaf (cm ²)	52.1 ^a \pm 26.8 (61)	21.5 ^c \pm 13.6 (55)	35.2 ^b \pm 11.4 (36)	27.7 ^{b,c} \pm 15.5 (39)
Total canopy leaf area (cm ²)	951 ^a \pm 180 (5)	260 ^b \pm 47 (5)	195 ^b \pm 35 (5)	167 ^b \pm 26 (5)
Average biomass/leaf (g)	0.24 ^a \pm 0.15 (97)	0.14 ^b \pm 0.10 (59)	0.14 ^b \pm 0.04 (27)	0.11 ^b \pm 0.05 (27)
Total canopy leaf bio- mass (g)	4.75 ^a \pm 1.00 (5)	1.66 ^b \pm 0.26 (5)	0.75 ^b \pm 0.12 (5)	0.58 ^b \pm 0.07 (5)

Table II. Anatomies of Leaves Grown at Various Levels of Light and Nutrients (See Text for Growth Conditions)

Data are from the leaves used for CO₂ exchange measurements. Treatments are as in Table I. Values in the same row followed by the same letter are not significantly different ($P > 0.05$). Numbers in parentheses in first row indicate sample size for all means in the column, except for stomatal frequency and specific leaf weight for which the numbers in parentheses indicate sample sizes.

	High Light-High Nutrients	High Light-Low Nutrients	Low Light-High Nutrients	Low Light-Low Nutrients
Thickness (μm)	188.0 ^a \pm 11.1 (10)	148.3 ^b \pm 7.1 (10)	148.8 ^b \pm 21.7 (8)	130.0 ^b \pm 9.3 (8)
Mesophyll cell volume/ leaf surface area ($\mu\text{m}^3/\mu\text{m}^2$)	75.3 ^a \pm 12.8	63.0 ^b \pm 7.3	48.2 ^c \pm 8.8	39.4 ^c \pm 4.5
Tissue volumes (%)				
Epidermis	21.40 ^a \pm 3.75	24.70 ^a \pm 1.95	28.38 ^a \pm 7.77	27.12 ^a \pm 3.00
Mesophyll	40.00 ^a \pm 6.02	42.50 ^a \pm 4.50	32.62 ^b \pm 2.32	30.87 ^b \pm 4.20
Vascular	18.40 ^a \pm 11.45	17.90 ^a \pm 4.43	10.62 ^b \pm 4.63	15.88 ^a \pm 6.71
Air space	20.10 ^b \pm 4.70	15.10 ^b \pm 3.73	28.12 ^a \pm 4.42	26.12 ^a \pm 4.22
Stomatal frequency (no./ mm^2)	116 ^b \pm 26 (9)	207 ^a \pm 30 (9)	114 ^b \pm 19 (9)	124 ^b \pm 13 (9)
Specific leaf wt (g/m^2)	56.0 ^b \pm 9.4 (5)	76.0 ^a \pm 2.6 (5)	28.6 ^c \pm 1.6 (4)	31.4 ^c \pm 2.7 (4)

Table III. Leaf Nitrogen Content and CO₂ Exchange Rates

Treatments are as in Table I. Where standard deviations are given, values in the same row followed by the same letter are not significantly different ($P > 0.05$). Numbers in parentheses indicate sample sizes.

	High Light-High Nutrients	High Light-Low Nutrients	Low Light-High Nutrients	Low Light-Low Nutrients
N Content				
Dry wt basis (mg/g)	24.6 ^b \pm 2.3 (2)	11.7 ^c \pm 0.1 (2)	35.6 ^a \pm 2.4 (2)	19.0 ^{b,c} \pm 2.6 (2)
Areal basis (g/m^2)	1.38	0.89	1.02	0.60
N/mesophyll cell volume (kg/m^3)	18.32	14.13	21.17	15.23
Net CO ₂ exchange rates ^a				
Dark respiration ($\text{mg}\cdot\text{dm}^{-2}\cdot\text{h}^{-1}$)	-1.68 ^a \pm 0.48 (5)	-1.26 ^{a,b} \pm 0.08 (5)	-0.72 ^b \pm 0.14 (4)	-0.67 ^b \pm 0.15 (4)
CO ₂ /leaf dry weight ($\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$)	31.1 ^b \pm 6.7 (5)	12.1 ^c \pm 1.3 (5)	50.0 ^a \pm 4.7 (4)	34.9 ^b \pm 3.9 (4)
CO ₂ /mesophyll cell volume ($\text{kg}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$)	22.8 ^b \pm 2.6 (5)	14.6 ^c \pm 1.2 (5)	29.9 ^a \pm 4.0 (4)	27.7 ^{a,b} \pm 1.7 (4)
CO ₂ /N ($\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$)	1.23	1.03	1.39	1.81
CO ₂ /leaf area at growth chamber PPF (mg·dm ⁻² ·h ⁻¹)	13.5	8.0	5.0	4.5

^a Rates at 1,010 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ except for dark respiration and for values at growth chamber PPF. Rates are based on measured whole-leaf CO₂ exchange rate; the derived values thus include respiration of all leaf tissues, etc.

than low-light leaves and had greater development of mesophyll (Table II). Within a light level, high-nutrient leaves were thicker, but proportions of mesophyll cells, air space, epidermis, and vascular tissue did not change with nutrient level. However, within each nutrient level there were differences between light levels in mesophyll development, as reflected in % air space, % mesophyll, and mesophyll cell volume per leaf surface area. Since the H150 leaves were thickest and had a high % mesophyll, they had the greatest mesophyll cell volume per leaf surface area, whereas the low-light treatments had the lowest values. Number of stomata per area of the lower side of the leaf was highest in the H0 treatment, with no differences among the other treatments. In both nutrient treatments, high-light leaves had higher SLW than the low-light leaves. In high light, low-nutrient leaves had higher SLW while the means for low-light treatments were not significantly different. The higher SLW of low-nutrient leaves, as com-

pared to high-nutrient leaves within a light level, apparently resulted in part from the accumulation of starch and, especially in the H0 leaves, phenolic materials (Chabot *et al.*, manuscript in preparation).

The differences among treatments in leaf area and thickness were a result of changes in both cell number and cell size. The mesophyll cell volume data reflect those changes as well as differences in cell shape. Further data on leaf anatomy and chemical composition are given in Chabot *et al.* (manuscript in preparation).

Leaf nitrogen content expressed as percent of dry weight was greatest in high-nutrient treatments (Table III). Within nutrient levels, low-light plants had higher % N content. N per unit leaf area was greatest in the high-nutrient treatments. Within nutrient treatments, high-light leaves had higher area N content. N content per mesophyll cell volume was estimated from N/area divided by mesophyll volume/leaf area. High-nutrient leaves had the greatest

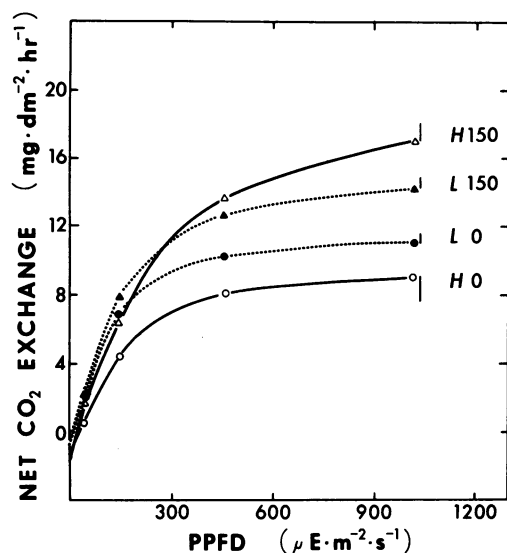


FIG. 1. Effects of nutrient and light levels during growth on light response of leaf net CO₂ exchange per unit area. Curves are based on the mean of four or five leaves. Vertical bar indicates one standard deviation at 1010 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. (●), low light, 0 ml of fertilizer solution (L0); (▲), low light, 150 ml of fertilizer solution (L150); (○), high light, 0 ml of fertilizer solution (H0); (Δ), high light, 150 ml of fertilizer solution (H150).

N/mesophyll cell volume (Table III).

Within a given growth light level, high nutrient level resulted in higher net CO₂ exchange rates per unit leaf surface area (Fig. 1). The H150 leaves had the highest light-saturated net CO₂ exchange rates of all treatments. The H0 leaves had net CO₂ exchange rates lower than all other treatments. The H150 leaves had the highest respiration rates of all treatments, with H0 rates slightly lower (Fig. 1, Table III). The L0 and L150 leaves had almost identical dark respiration rates. Their light response curves were about the same until 100 to 200 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Fig. 1). The major difference occurred at light saturation, with the L150 leaves having greater photosynthetic capacity at high PPFD. The H0 and H150 leaves had the same general pattern, except that the differences between the response curves were greater at all but very low PPFD. Both the H0 and H150 leaves had lower net CO₂ exchange rates at low PPFD than did the L0 and L150 leaves.

Stomatal conductance varied from 2 to 7 mm/s within all treatments. There were no significant differences among the treatments.

Net CO₂ exchange rate expressed per unit mesophyll cell volume was greatest in the low-light treatments (Table III). Low nutrient level led to decreased rates in high light but not in low light. CO₂ exchange expressed in terms of total N was highest in low-light treatments (Table III). The L0 leaves had the highest rates per g N, while the H0 leaves had the lowest rates, so that the effects of nutrient treatments were reversed in the different light levels.

DISCUSSION

Stomatal conductance values were similar for all treatments in this study, whereas the measurement conditions created uniformly high boundary layer conductances. Observed differences in net CO₂ exchange rates thus resulted primarily from differences in internal leaf characteristics affecting CO₂ exchange.

Depression of maximum net CO₂ exchange rate in the H0 leaves matches the results of previous studies of nutrient effects. Low levels of NO₃⁻, PO₄²⁻, or K⁺ depressed photosynthesis in cotton (9). Osman and Milthorpe (16) found that low nutrient levels lowered maximum photosynthetic rate in wheat. In a study of *Atriplex patula*, Medina (10, 11) showed nitrogen level to have little effect at low light, while considerably altering the photosyn-

thetic capacity of leaves grown in high light. The lower rates of high light-low nitrogen leaves were attributable to lower levels of carboxydismutase (RuBP-carboxylase). We also observed decreased effect of nutrients on plants grown in low light. Osman and Milthorpe (16) and Gulmon and Chu (6) found maximum photosynthetic rate to be correlated with nitrogen content of leaves, which is consistent with Medina's (11) association of RuBP-carboxylase with maximum rate. Although our experimental design limits tests of statistical significance, we found generally higher maximum photosynthetic rate in leaves with higher N content (cf. Fig. 1; Table III).

Nobel (14) found that irradiation level during leaf development led to changes in maximum net CO₂ exchange rate by causing changes in mesophyll cell surface area rather than in intracellular liquid-phase CO₂ conductance, g^{cell} . In contrast, low nutrient level during leaf development of *Gossypium hirsutum* (cotton) led to decreased net CO₂ exchange rate and lower g^{cell} , whereas mesophyll surface area remained constant (9). Our results indicate that light level was more important than nutrient level in influencing mesophyll development as reflected in mesophyll cell volume per leaf area (Table II). Within a light level, mesophyll cell volume tended to be greater in the high-nutrient treatment, with the differences reduced in low light. Although no precise estimates of g^{cell} are available, maximum net CO₂ exchange rate expressed per unit mesophyll cell volume (Table III) showed little effect of nutrients in low light, whereas in high light low nutrient level reduced the rate by one-third. The differences in whole-leaf net CO₂ exchange rate between nutrient treatments in high light thus apparently were attributable to changes at the photochemical or biochemical level inside the cell (g^{cell}), as found by Longstreth and Nobel (9) for cotton. In low light, changes in mesophyll cell volume largely accounted for differences in whole-leaf net CO₂ exchange rates, although there may have been some decrease in intracellular CO₂ exchange capacity of low-nutrient leaves.

Calculations of net CO₂ exchange based on N content suggest some possible bases for the differences in net CO₂ exchange per mesophyll cell volume. Compared to the H0 leaves, the H150 leaves had greater N/mesophyll cell volume and slightly higher net CO₂ exchange/N, with a correspondingly greater net CO₂ exchange/mesophyll cell volume (Table III). In low light, the L150 leaves had greater N/mesophyll cell volume but the L0 leaves had a greater net CO₂ exchange/N, so that the low-light treatments had similar net CO₂ exchange/mesophyll cell volume. Inasmuch as a large part of a plant's N may be in enzymes involved with the carboxylation process in photosynthesis (12), the greater efficiency of low-light leaves in CO₂ capture per g N suggests they had relatively greater investment (per unit mesophyll cell volume) in other parts of the photosynthetic system than did the high-light leaves (cf. 1).

There are several hypotheses relating to leaf size. Large, thin leaves in understory plant species would increase the probability of intercepting light flecks. Alternatively, leaf size has been explained as contributing to the optimization of photosynthesis/transpiration (18), leaf temperature and photosynthesis (20), or the difference between photosynthetic profits and transpirational costs (5). All of these latter hypotheses are based upon the role of leaf size in determining boundary layer resistances and hence the rate of energy and mass exchange between a leaf and its environment. All of these hypotheses predict large leaf size in low radiation environments and small leaf size in high radiation environments.

Jurik (7) has observed that the largest leaves of wild strawberry occur in mesic, high-light habitats, not in shady habitats. Our laboratory results and field observations indicate that leaf size is determined in a complex manner and runs counter to existing predictions. Both light environment and available nutrients can affect leaf sizes. Where either is limiting, leaf size is reduced.

Medina (10) and Gulmon and Chu (6) have also reported reduced leaf area under limiting nitrogen.

Total canopy leaf area, total leaf biomass, biomass per average leaf, and leaf number per plant all varied consistently between treatments (H150 > H0 > L150 > L0). These characteristics were also consistently and perhaps causally related to an estimate of photosynthesis rates under the growth environments (Table III). The high light-high nutrient treatment produced considerably higher values than the other treatments, which tended to show insignificant variation between limiting environments. The proportional allocation of biomass to mesophyll tissue on an area basis is determined primarily by light environment. The decreased allocation under low light may relate to a developmental mechanism whereby biomass is distributed laterally rather than contributing to leaf thickness. Consequently leaf sizes under low light conditions may be somewhat larger than would be predicted from amount of biomass in each leaf. Such a mechanism could contribute to explaining the higher leaf sizes in L0 and L150 in comparison with the H0 treatment. Absolute leaf size may be a function of substrate supply to the developing leaf moderated by the effects of light on mesophyll cell distribution.

Leaf size of wild strawberry plants in the field might often be largely determined by substrate supply, with environmental limitation of substrate supply being commonplace. Such limitation may explain the disparity between observed leaf sizes in strawberry and the predictions of the various leaf size hypotheses (5, 18, 20).

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