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The Relation Between Chlorophyll Concentration and the Internal Surface of Mesomorphic and Xeromorphic Leaves Grown under Artificial Light

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THE RELATION BETWEEN CHLOROPHYLL CONCENTRATION AND THE INTERNAL SURFACE OF MESOMORPHIC AND XEROMORPHIC LEAVES GROWN UNDER ARTIFICIAL LIGHT[1]

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Geneau de Lamarlière in 1892 demonstrated that photosynthetic rate is greater per unit area of leaf surface in sun leaves than in shade leaves. Alexandrov is reported by Maximov (1929) to have found higher photosynthetic rates in upper xeromorphic leaves than in lower inserted, less xeromorphic leaves, while Haas and Halma (1932) obtained quicker and better rooting in citrus cuttings which have a high percentage of palisade. Willstätter and Stoll (1918) showed photosynthetic rate increased with chlorophyll content. They (Willstätter and Stoll, 1913) recorded data which, by the writer's calculations on the fresh weight basis, showed that sun leaves of *Sambucus nigra* and *Aesculus hippocastanum* had higher Chlorophyll (a + b) concentrations than shade leaves, while the sun leaves of *Platanus acerifolia* and *Fagus sylvatica* had lower concentrations than the shade leaves. Guthrie (1929) showed soybean and radish plants had higher chlorophyll concentrations on fresh weight basis when grown in low light intensity. Ulvin (1934) found soybean plants had a higher per cent of chlorophyll on the wet weight basis when grown in continuous light, but the writer noted no clear trend of his results calculated on the basis of leaf area. A review of literature by Porter (1937) includes some references which support the view that chlorophyll concentration increases with light intensity and many references which do not. Porter, however, found a greater chlorophyll concentration per square centimeter in plants grown under high light intensity.

It was reported by Turrell (1936) that the internal-external surface ratios were low for shade leaves as compared with sun leaves of the same plant, and high for xeromorphic sun leaves as compared with mesomorphic sun leaves in different species.

Photosynthetic rate is regulated by a number of factors, the more evident of which are (1) the rate of absorption of carbon

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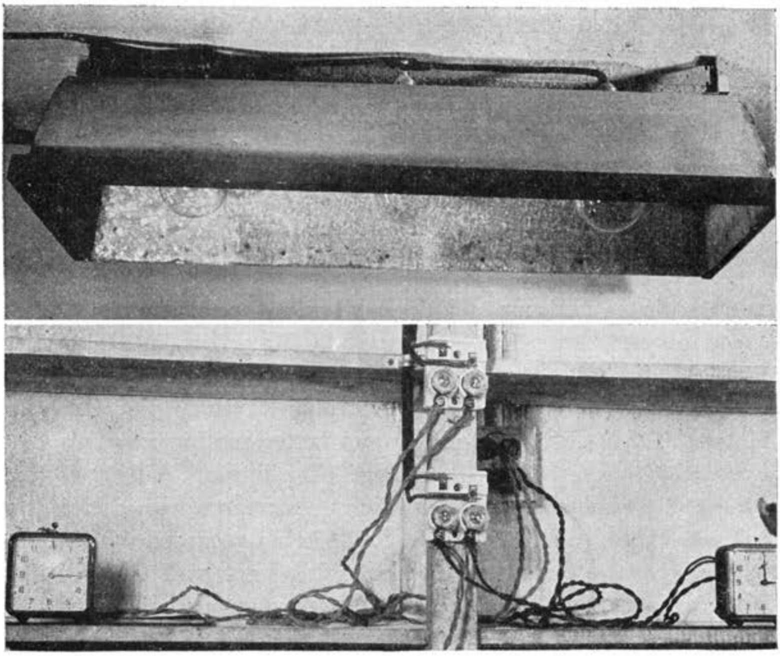

Fig. 1. One of two reflectors used in growing plants under artificial light and automatic clock control.

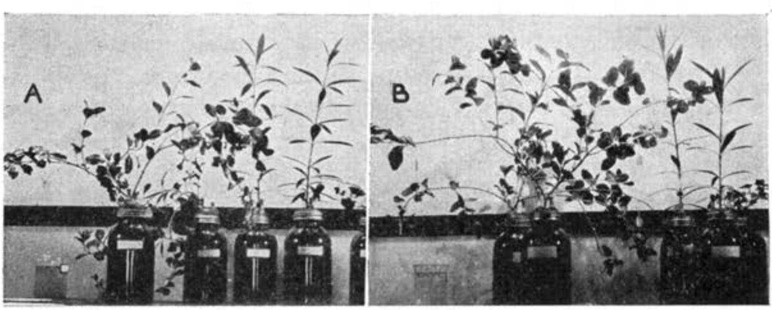

Fig. 2. (A) The plants grown under 76.8 foot candles of artificial light intensity. Periwinkle (*Vinca rosea* L.) plants on the left, oleander (*Nerium oleander* L.) plants on the right. (B) The plants grown under 175 foot candles of artificial light intensity. Periwinkles on the left, oleanders on the right.

dioxide; (2) the quantity of water available; (3) quantity of chlorophyll; (4) intensity of sunlight; and (5) rate of removal of photosynthate. In accordance with Blackman's law, that factor which is minimum regulates the rate of the process. Since the area of an absorbing surface is an important factor in the rate of absorption of a gas as indicated by the Adeney and Becker equation $\frac{dw}{dt} = SAp - F\frac{Aw}{V}$, where A is the area of the absorbing surface, (Taylor, 1924), and since it has been shown that leaf internal surface and photosynthetic rate are greater in xeromorphic leaves than mesomorphic leaves, and since chlorophyll reacts chemically in the photosynthetic process, concentration of chlorophyll should be greater in xeromorphic leaves. This investigation was initiated to determine if large internal surface areas of leaves are accompanied by higher chlorophyll concentrations.

Methods and Materials

Periwinkle (*Vinca rosea* L.) and oleander (*Nerium oleander* L.) plants potted in rich loam soil in two-quart Ball Mason jar potometers were stripped of their leaves and allowed to grow new foliage under two different intensities of artificial light. For the low light intensity, three 200 watt Mazda lamps were used which supplied 76.8 foot candles at the table top on which the plants rested, as measured with a Macbeth illuminometer. The high light intensity was 175 foot candles at the table top and was supplied by three 500 watt lamps (fig. 1).[2] Clocks controlled the daily twelve-hour illumination period and although outdoor temperatures ran over 100°F in the shade, temperatures in the growing room averaged approximately 87.7°F with a maximum of 93°F. All the plants were well watered.

The plants (fig. 2) made a vigorous growth and the periwinkles under high light intensity were in bloom at the time the leaves were stripped from the plants for analysis. Blueprints were made of the leaves using a 30-second exposure under a "daylight" arc lamp. As soon as printed the leaves were placed in covered beakers in a refrigerator and chlorophyll isolations and purifications were carried out as rapidly as possible by the method of Schertz (1928). Great care was exercised in grinding as recommended by Zscheile (1934). Concentration measurements were made with a photoelectric colorimeter (Turrell and Waldbauer, 1935), the calibration of which with American Chlorophyll Company's ("5X") purest

2 One set of plants was shielded from the other. Only artificial light was used in the experiment.

grade chlorophyll is shown in figure 3. Concentration measurements were duplicated with a standard Duboscq colorimeter using the Guthrie Standard (1928), consisting of an ammoniacal solution of copper sulphate and potassium dichromate.

One-fourth square centimeter areas were selected from upper and lower leaves, killed in F. A. A. and permanent microscopical

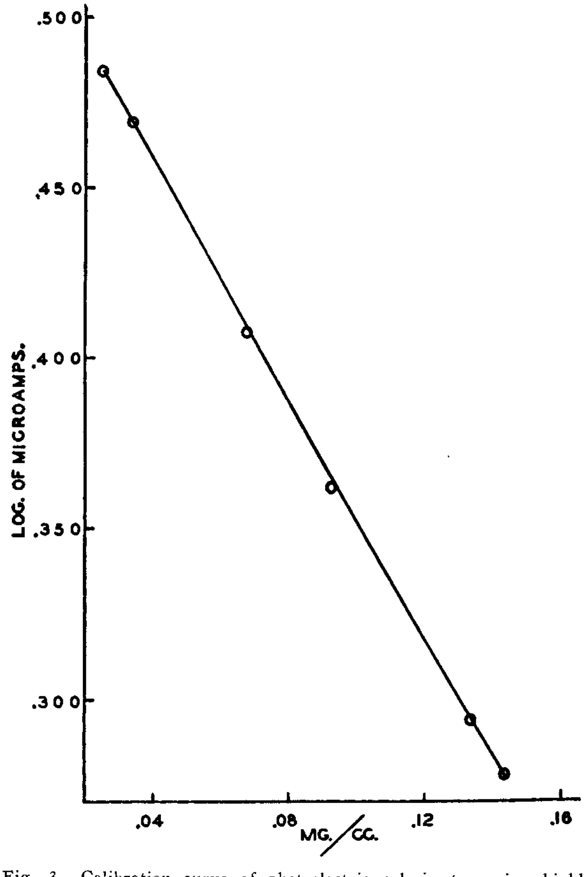

Fig. 3. Calibration curve of photoelectric colorimeter using highly purified chlorophyll ("5 X").

slides prepared. Internal surface measurements were made from these by the method described by Turrell (1936).

RESULTS

The periwinkle and oleander plants grown under the higher intensity of artificial light were more xeromorphic than the plants grown under the lower light intensity as indicated by greater leaf thicknesses, palisade depths, and epidermal and cuticle thicknesses (table I), characteristics which are indicative of xeromorphy (Maximov, 1929). Leaves of *Nerium oleander* plants were more

Table I. Characteristics of the leaves of Nerium oleander *L. and* Vinca rosea *L. Plants grown under different intensities of artificial light*

Plants	Illumination in Foot Candles	Leaf Thickness in Microns	Palisade Depth in Microns	Thickness of Epidermis in Microns (Upper)	Thickness of Cuticle (Upper) in Microns
Vinca rosea	175.0	130	37	21	<1
Vinca rosea	76.8	108	25	15	<1
Nerium oleander	175.0	239	64	48	3.5
Nerium oleander	76.8	203	55	31	1.8

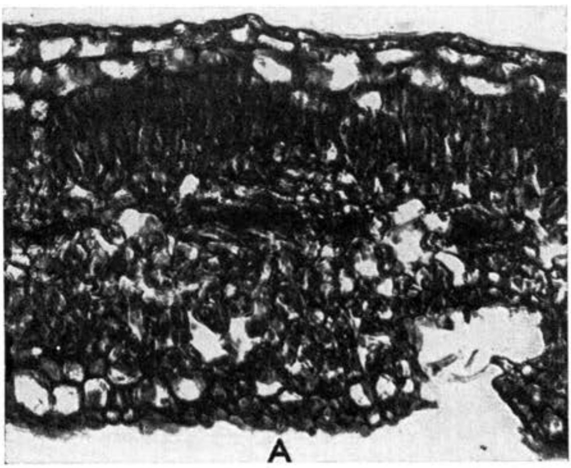

Fig. 4. (A) Transverse section of a thick xeromorphic oleander (*Nerium oleander* L.) leaf grown under high light intensity (175 foot candles), showing excessive development of palisade tissue and stomatal cavity in which stomata are located. Magnification 240 X.

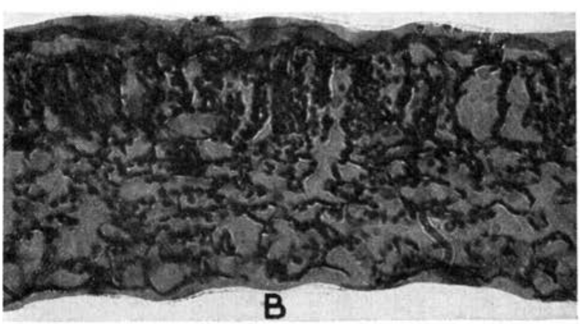

Fig. 4. (B) Transverse section of a thin mesomorphic periwinkle (*Vinca rosea* L.) leaf grown under high light intensity (175 foot candles), showing moderate development of palisade tissue. Magnification 240 X.

Table II. Chlorophyll concentration of leaves of Vinca rosea *L. and* Nerium oleander *L. plants grown in two intensities of light. Chlorophyll concentration in terms of leaf area, internal surface area and wet weight, as measured by Duboscq and photoelectric colorimeters*

Plant	Light Intensity in Foot Candles	Internal Leaf Surface / External Leaf Surface	Chlorophyll in mg/cm² of Leaf, Duboscq Colorimeter	Chlorophyll in mg/cm² of Leaf, Photocolorimeter	Chlorophyll in mg/g Wet Weight, Duboscq Colorimeter	Chlorophyll in mg/g Wet Weight, Photocolorimeter	Chlorophyll in gammas/cm² of Internal Leaf Surface, Duboscq Colorimeter *	Chlorophyll in gammas/cm² of Internal Leaf Surface, Photocolorimeter *
Vinca rosea	175.0	8.72	0.044	0.045	2.44	2.53	4.97	5.14
Vinca rosea	76.8	7.45	0.041	0.036	2.53	2.23	5.50	4.78
Nerium oleander	175.0	16.87	0.107	0.107	3.64	3.66	6.49	6.51
Nerium oleander	76.8	14.22	0.106	0.102	4.34	4.15	7.45	7.16

* Values calculated from individual items. Calculations based on the means in columns 2, 3, and 4 only roughly correspond.

xeromorphic than those of *Vinca rosea* plants (table I). Photomicrographs of their transverse leaf sections (figures 4 A & B), show the relative xeromorphy.

The mean internal-external surface ratios of the leaves of the periwinkles and oleanders were higher in those grown under the high light intensity. The chlorophyll concentration also was higher per square centimeter of leaf area in those plants which were grown under high light intensity as indicated by measurements with both the Duboscq and photoelectric colorimeter, (table II).

On the basis of wet weight, and the more reliable photoelectric colorimeter readings,[3] the leaves of the periwinkles grown in the higher light intensity had the greater concentration of chlorophyll (table II). As indicated by both methods of estimation, the chlorophyll concentration was higher in the oleander plants under low light intensity (table II).

Chlorophyll concentration per unit area of internal leaf surface was higher in the *Vinca rosea* plants grown under high light intensity than grown under low light intensity, as indicated by the more accurate photoelectric colorimeter readings (table II). However, in the *Nerium oleander* plants, the chlorophyll concentration per unit area of internal leaf surface was higher under the low light intensity growing conditions, as indicated by both the Duboscq and photoelectric colorimeter methods, which are in fair agreement (table II).

Reference to table 1 indicates that the leaves of *Nerium oleander* plants are much more xeromorphic than those of *Vinca rosea* plants. The various tissue thicknesses are nearly twice as great in the former as in the latter. Likewise, the internal-external surface ratios in the oleanders are approximately twice (1.92 times) as great as in the periwinkles (table II). The mean chlorophyll concentrations per square centimeter leaf surface in the oleanders is approximately twice (2.56 times) as great as in the periwinkles. On the wet weight basis the mean chlorophyll concentration in the oleanders is 1.62 times as great as in the periwinkles. When calculation of chlorophyll concentration per square centimeter of internal leaf surface is made, the oleander foliage contains 1.38 times as much chlorophyll per unit as the periwinkle.

[3] Difficulty in obtaining a satisfactory match in color between standard and unknown by the Duboscq method is responsible for its lower accuracy, although 0.4 per cent deviation from the mean may be due to differences in brightness of field, (Lowry, 1931). For example, readings on sample 1 were 26.8, 28.3, and 29.0, a difference of .058 mg. of chlorophyll per cc. between maximum and minimum reading. The mean of a number of readings reduces the probable error somewhat. However, with the photoelectric colorimeter, the reading consistently obtained on sample 1 was 244 $\pm$ 5 and the sensitivity of the instrument was 0.0004 mg. per cc. per division of the microammeter scale.

Table III. The relative xeromorphy developed in Vinca rosea L. *and* Nerium oleander L. *by high light intensity*

Plants	Leaf Thickness High Light — Low Light	Palisade Depth High Light — Low Light	Upper Epidermal Thickness High Light — Low Light
Vinca rosea	22 μ	12 μ	6 μ
Nerium oleander	36 μ	9 μ	17 μ
Plants	$\frac{\text{Difference in Leaf Thickness}}{\text{Leaf Thickness}}$	$\frac{\text{Difference in Palisade Depth}}{\text{Palisade Depth}}$	$\frac{\text{Difference in Epidermal Thickness}}{\text{Epidermal Thickness}}$
Vinca rosea	$\frac{22}{108} = 0.204$	$\frac{12}{25} = 0.48$	$\frac{6}{15} = 0.40$
Nerium oleander	$\frac{36}{203} = 0.177$	$\frac{9}{55} = 0.16$	$\frac{17}{31} = 0.55$
Plants	$\frac{\text{Diff. in Leaf Thick./Leaf Thick.}}{\text{Diff. in Leaf Thick./Leaf Thick.}}$	$\frac{\text{Diff. in Palisade Depth/Palisade D.}}{\text{Diff. in Palisade Depth/Palisade D.}}$	$\frac{\text{Diff. in Epidermal Thick./Epi. Thick.}}{\text{Diff. in Epidermal Thick./Epi. Thick.}}$
Vinca rosea / *Nerium oleander*	$\frac{0.204}{0.177} = 1.15$	$\frac{0.48}{0.16} = 3.00$	$\frac{0.40}{0.55} = 0.73$

Discussion

The foliage of *Nerium oleander* L. is much more xeromorphic than that of *Vinca rosea.* From these naturally different types of plants, the data presented show that the greater internal surface of the former was accompanied by the higher chlorophyll concentration.

Comparison of values given in table I shows that the degree of xeromorphy induced in *Vinca rosea* plants by the higher light intensity was relatively greater than that induced in the *Nerium oleander plants* (table III). The chlorenchyma in particular, as indicated by the palisade layer thickness was relatively greatly increased (3.0 times) in *Vinca* as compared with *nerium,* by the higher light intensity, while the leaf thickness was increased approximately 1.15 times as much relatively in *Vinca* as in *Nerium.* The high light intensity however, stimulated a relatively greater epidermal development in *Nerium* than in *Vinca* (table III).

Under the high light intensity the xeromorphy which was induced in *vinca rosea* plants was accompanied by a higher internal-external surface ratio, and higher chlorophyll concentration on the basis of leaf area, wet weight, and internal surface area.[4] Failure of the *Nerium oleander* plants to show a similar response on the basis of wet weight and internal surface in increased light intensity is believed to be owing to the low light intensities of the artificial light (175 foot candles) which was not sufficient to cause a significant increase in the xeromorphy of the already strongly xeromorphic foliage. A great increase in epidermal thickness did occur in *Nerium,* but the palisade tissue was not greatly affected probably because insufficient light penetrated to the palisade. The thickening of the cuticle in high light intensity (table I) may have reduced the penetrating radiation as did the increased epidermal thickness. Direct sunlight, it must be recalled, would have an intensity on clear days more than twenty times the highest intensity, in foot candles, obtained in this experiment at the level of the lowest leaves. The chlorophyll concentration per square centimeter of leaf was very slightly higher in *Nerium oleander* in high light intensity than in low, but increase in chlorophyll did not keep pace with the increase in net weight and internal surface.

These data strongly suggest that variance in the results of studies of chlorophyll concentration under various light conditions ob-

4 Chlorophyll concentration per unit internal surface was expected to reach the same value in boh high and low light intensities. The more rapid increase in chlorophyll than in internal surface in the periwinkles in high light intensity was not anticipated. Explanation is not possible at this time.

tained by various investigators is because the different species of plants which were used had different tendencies toward xeromorphy and may or may not have been significantly affected by the environments imposed.

Numerous investigators have used artificial light alone in growing plants or in supplementing sunlight. These experiments have brought to the writer's attention one particular disadvantage of artificial illumination for growing plants for experimental purposes. It should be mentioned here as it has been given little attention before.

Although readings with the Macbeth illuminometer showed some variation of the light intensity at different positions at the same level below the lamps, these differences were quite insignificant as compared with readings at 1-foot intervals in a vertical direction toward the lamps. The intensities in foot candles may readily be calculated from the inverse square law. A 500 watt lamp without a reflector would be expected to give $\frac{500}{5^2}$ foot candles, five feet from the source, or 20 foot candles. At three feet one would expect the intensity to be 55.5 foot candles. A plant, therefore, four or five feet in height would have foliage along its stem exposed to very different intensities of light. A plant, exposed to sunlight, on the contrary, would not have foliage affected by such an intensity gradient. An increment of 10 to 100 feet in the distance of the sun to the earth (4.9×10^{23} feet) would be insignificant when the intensity of light at the source approximates 3×10^{27} foot candles.

The author wishes to express his gratitude to Professor W. F. Loehwing who supplied the light room facilities, to Professor L. Waldbauer in whose laboratory the chlorophyll analyses were made, and to Professor R. B. Wylie in whose laboratory the internal leaf surface measurements were completed.

Summary

1. In the mesomorphic leaves of *Vinca rosea* L., xeromorphy was induced under high artificial light intensities and was accompanied by higher chlorophyll concentration and higher internal-external surface ratios.

2. The xeromorphic foliage of *Nerium oleander* L. showed little increase in xeromorphy under the "high intensity of artificial light" and although the internal surface ratio increased, the chlorophyll content did not increase proportionally, owing to the comparatively low value of the high light intensity.

3. The xeromorphic leaves of *Nerium oleander* L. had a higher internal-external surface ratio and higher chlorophyll concentration than the mesomorphic *Vinca rosea* L. leaves.

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