# VARIATIONS IN LEAF COLORATION USING A REFLECTANCE COLORIMETER[1]

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**Abstract.** Leaf coloration for 9 plant species measured with a Hunter colorimeter showed major variations between plants and leaves within a plant, but minor variations associated with reading position within leaves and instrument. The lower leaf surface was more sensitive to color differences. The magnitude of variation between leaves was dependent on species. The colorimeter provides quantitative information for comparing plant leaves.

Although leaf color is used for studying plants, quantitation is generally based on standard color samples or rating scales. Plant materials subject to treatment, genetic, or growth differences may be compared on this basis (2, 3, 6). Color comparisons may also facilitate varietal selection in plant breeding (7, 8) or provide indication of plant disease, nutrient deficiencies, and maturity (1, 3, 4, 5).

A quantitative basis for color evaluation of plant leaves is obtained by use of the colorimeter. Colorimeters are widely used in industrial and food applications but have not been used for leaf color measurements. Colorimeter measurement of reflected light provides numerical values for lightness (L), the red vs. green (a), and yellow vs. blue components (b) of color. Principal advantages include speed, objectivity, and numerical results. Quantitative leaf color values may be correlated to genetic information, biochemical measurements, and plant treatments. Since readings are easily obtained, it is possible to improve precision by sampling more material or revising sampling methods. An additional advantage for the colorimeter is color specification without preparation of a standard reference color series.

The purposes of this study are to determine colorimetric values of leaves from a wide range of species, and to examine sources of variation when using a Hunter colorimeter for leaf color measurement to determine precision associated with the L, a, and b measurements.

## Materials and Methods

Leaves were collected in Peoria from 2 areas about 8 km apart. About 70 leaves were collected at one time, and color values were taken from 1 to 5 hr after collection. From 1 to 8 different trees of each species were sampled between June 9 and 13, 1980. The same species were sampled on different days when possible from widely scattered collection areas. Five leaves were collected around the lower portion of the tree or from different branches of the plant and placed in brown paper bags. Leaves were sampled and reflected light values read on the same day.

Measurements were taken with a Hunterlab Model D-25 Tristimulus Colorimeter. The color difference meter was calibrated using a white standard. Leaves were placed directly over the 2.54-cm diam aperture for reading and 3 values were recorded: **L** for lightness on a scale of 0 to black to 100 for white; **a** associated with positive values for red and negative for green; and **b** a value more negative for yellower and more positive for bluer samples. Hunter values are related to tristimulus values X, Y, Z (6).

On each day a leaf from each species in random order was read before proceeding to succeeding leaves in order to balance possible order effects. The instrument was recalibrated after each sequence of species. A spot near the center of the leaf was placed over the instrument aperture and the reading was recorded. Effort was made to assure readings from blemish-free areas at least 2.54 cm in diam. About 60 leaves, upper and lower surfaces, were measured for color in an hour.

A single area on each surface was read for each leaf except for catalpa, grape, and silver maple.

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Two areas were read on each leaf of these species to assess variation within a leaf. Tree species were used since single leaves were large enough to cover the light aperture.

Data consisted of the Hunter **L, a,** and **b** readings for the upper and lower leaf surfaces of each leaf. Analyses of variation were computed for each measurement. All possible combinations of L, a, and b values from upper and lower surfaces were examined for correlations.

**Results and Discussion**

Analyses of variance computed from the leaf measurements are shown in Table 1. Based on ratios of mean squares and taking into account data imbalance, highly significant variation is observed between leaves, trees, and species. Similar results are observed for all 6 measurements. The lower leaf surface appears more sensitive to species differences since ratios of species variation to tree variation are considerably higher for lower leaf surface measurements. The analyses are an average for the species considered since there was evidence that variation between leaves is species dependent. This point is considered later.

Separate analyses using 8 species, each sampled on 4 different days, indicated no significant variation attributable to reading times. Thus, instrumental variation appears of minor importance, particularly with checking of calibration after each leaf series is read.

For 3 species — silver maple, grape, and catalpa — 2 readings were taken from each leaf surface to assess within-leaf variation. Clearly, within-leaf variation, designated *Readings* in Table 1, is a minor source of variation relative to leaves and plants for the 3 species examined.

The means for each species appear in Table 2. Since between species comparisons are complicated by unequal number of observations, a least significant difference (LSD) assuming 5 trees and 5 leaves per tree is shown. Where only single trees are involved, the LSD would be 2.24 times as large. Although species differences are evident, they may reflect growth conditions. The main point is that significant quantitative differences between means are observed for at least one measurement for all species pairs. Further examination of the variation indicated that about 10% of the variation in a measurement was due to readings, 35% to leaves, and 45% to trees.

In general, since plant-to-plant variation is such a significant contributor to variation, taking more plants with a minimum of leaves per plant generally leads to the best precision in estimating a species mean. However in sampling experimental plots, number of replicates would provide an upper limit to the number of plants that could be sampled.

Lilac and poplar showed the least variation between leaves from the same plant, and wild grape and silver maple the most. In sampling and taking readings of some species, obvious visual differences between leaves were apparent. This was the case with silver maple and appears to reflect maturation of leaves on the limb being sampled. Variation between leaves for some species can be controlled by more precise definition of the leaves to be sampled. To obtain equal precision, however, up to 3 times as much sampling of a more variable species may be required.

The correlation of "a" and "b" measurements from the same surface was −0.96 for the upper

**Table 1. Analyses of variance for Hunter measurements of tree leaves.**

| Source of variation | df | Upper leaf | | | Lower leaf | | |
|---|---|---|---|---|---|---|---|
| | | *L* | *a* | *b* | *L* | *a* | *b* |
| Species | 14 | 272.71 | 93.56 | 233.71 | 706.46 | 71.23 | 266.34 |
| Trees | 47 | 25.11 | 8.47 | 20.78 | 17.64 | 3.22 | 9.00 |
| Leaves | 248 | 3.89 | 1.10 | 3.30 | 2.14 | 0.43 | 1.45 |
| Readings | 40 | 0.60 | 0.16 | 0.26 | 0.80 | 0.09 | 0.19 |

**Table 2. Mean Hunter color values for leaves of 15 plant species**

| Scientific name | t[z] | n | Upper leaf L | Upper leaf a | Upper leaf b | Lower leaf L | Lower leaf a | Lower leaf b |
|---|---|---|---|---|---|---|---|---|
| *Malus sylvestris* | 1 | 5 | 34.58 | −10.07 | 11.74 | 48.92 | −9.29 | 14.05 |
| *Catalpa speciosa* | 6 | 45 | 35.65 | −12.39 | 15.69 | 43.12 | −11.87 | 17.90 |
| *Prunus cerasus* | 1 | 5 | 29.23 | −8.18 | 9.54 | 42.45 | −9.49 | 14.15 |
| *Vitis* spp. | 6 | 40 | 37.65 | −12.06 | 17.10 | 43.49 | −11.67 | 18.23 |
| *Crataegus* spp. | 5 | 25 | 29.58 | −9.09 | 10.08 | 41.20 | −9.35 | 12.85 |
| *Syringa vulgaris* | 6 | 30 | 31.45 | −8.31 | 10.18 | 42.81 | −9.72 | 13.93 |
| *Tilia americana* | 6 | 30 | 30.00 | −8.74 | 10.61 | 38.47 | −8.93 | 12.53 |
| *Acer platanoides* | 6 | 30 | 28.14 | −7.70 | 8.65 | 38.44 | −9.37 | 13.19 |
| *Acer platanoides 'Schwedleri'* | 1 | 5 | 25.13 | −5.13 | 5.68 | 35.15 | −4.77 | 9.55 |
| *Acer saccharinum* | 7 | 50 | 32.56 | −10.00 | 12.24 | 53.26 | −7.13 | 8.49 |
| *Philadelphus coronarius* | 5 | 25 | 33.80 | −11.20 | 13.70 | 44.95 | −10.21 | 14.12 |
| *Quercus velutina* | 1 | 5 | 24.33 | −6.20 | 7.07 | 38.37 | −10.35 | 15.51 |
| *Quercus rubra* | 2 | 10 | 25.10 | −6.67 | 7.23 | 38.85 | −9.54 | 13.70 |
| *Phlox paniculata* | 1 | 5 | 32.22 | −10.32 | 12.57 | 41.84 | −9.24 | 12.51 |
| *Populus deltoides* | 8 | 40 | 30.92 | −7.35 | 8.89 | 35.12 | −7.66 | 9.79 |
| Overall mean | | | 32.07 | −9.57 | 11.83 | 42.61 | −9.44 | 13.37 |
| LSD[y] (5,5) | | | 2.83 | 1.65 | 2.58 | 2.38 | 1.02 | 1.70 |

[z]t = number of plants; n = number of readings.

[y]LSD (5,5) = least significant difference (5% level) assuming 5 trees and 5 leaves per tree.

and −0.95 for the lower. When "a" and "b" were from opposite surfaces, the correlations were −0.64 and −0.70. Lightness of the upper surface correlated with "a" (−0.89) and "b" (−0.95) but was less correlated with "a" (−0.61) and "b" (0.60) of the lower surface. Lightness of the lower surface was uncorrelated with "a" (0.00) and "b" (−0.03) of the lower surface. Correlations were based on 350 points ignoring species.

Based on similar correlations for "a" and "b", results suggest that "L" and "a" and "b" are sufficient to assess plant color differences.

## Conclusions

Hunter L, a, and b values provide a quantitative basis for assessing leaf color differences. Reading position on the leaf is a minor source of variation relative to leaf and plant variation for 8 species. Single readings per leaf are recommended. The lower leaf surface is more sensitive to color differences between species. Plant-to-plant variation was the major source of color variation suggesting use of more plants at the expense of leaves per plant to improve precision with least additional observations. The colorimeter is a fast reliable instrument for quantitative assessment of leaf coloration.

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