

# DEVELOPMENT OF FALL FOLIAGE COLOR IN SUGAR MAPLE

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## Abstract

Fall foliage development is important to tourism and culture in the Northeast. However, few data exist on the control of the timing and brilliance of fall color. In this study, leaf tissue from 16 sugar maples (*Acer saccharum*) was collected periodically from June 30 through October 27, 1999 and analyzed for foliar nutrient, moisture and carbohydrate content, and the extent of color development. Data were analyzed to determine which, if any, parameters were significantly predictive of the timing and quality of fall coloration. Concentrations of moisture, nitrogen and xylose on June 30 were significantly positively correlated with the time to coloration, while concentrations of starch were significantly negatively correlated with the time to coloration ( $p \leq 0.05$ ). Concentrations of nitrogen throughout the growing season were significantly negatively correlated with the subsequent quality of fall coloration ( $p \leq 0.05$ ), while the opposite relationship was exhibited by starch. Patterns in the relationships between fall coloration and these parameters substantiate literature and anecdotal accounts that suggest stress may promote anthocyanin production and premature senescence. Although further research is required to determine the true causal influences on timing and quality, these identified parameters appear to be useful in the prediction of fall coloration.

## Résumé

Le développement du feuillage d'automne est important pour le tourisme et la culture dans le nord-est. Cependant, il existe peu de données sur le contrôle du moment de l'apparition des couleurs d'automne et sur leur brillance. Dans la présente étude, des tissus de 16 érables à sucre (*Acer saccharum*) ont été récoltés périodiquement du 30 juin au 27 octobre 1999 et leur teneur en éléments nutritifs, humidité et hydrates de carbone a été analysée, ainsi que le degré de développement de la couleur. Les données ont été analysées pour déterminer quels paramètres, s'il y en a, peuvent avoir une utilité pour prédire le moment de l'apparition et la qualité des couleurs d'automne. Les concentrations en humidité, en azote et en xylose au 30 juin étaient très positivement corrélées avec le temps restant avant l'apparition des couleurs, tandis que les concentrations en amidon étaient très négativement corrélées avec le temps restant jusqu'à la coloration d'automne ( $p \leq 0.05$ ). Les concentrations en azote tout au long de la saison étaient très négativement corrélées avec la qualité des couleurs d'automne ( $p \leq 0.05$ ), tandis que la relation est inversée pour l'amidon. Les patrons dans les relations entre les couleurs d'automne et ces paramètres corroborent les publications et les anecdotes suggérant que le stress pourrait stimuler la production d'anthocyanine et la sénescence prématurée. Bien que d'autres études soient nécessaires pour déterminer les véritables influences causales sur le moment de l'apparition et la qualité des couleurs, les paramètres identifiés semblent avoir une utilité dans la prédiction des couleurs d'automne.

## Introduction

The display of fall foliage by sugar maple (*Acer saccharum*) in the northeast is valued highly by both residents and non-residents. Despite its many benefits, few substantiated data exist regarding the exact mechanisms of fall color development. If available, data relating to the causes of differential timing and brilliance of fall foliage color development could be used to make more accurate predictions, and to possibly develop procedures for manipulating fall color development on selected trees. The objective of our study was to identify leaf constituents valuable in predicting the timing and/or quality of fall coloration.

Most of the basic physiological processes involved in color development in fall foliage are known. The chlorophyll molecule begins to break down in response to lower temperatures and shorter daylengths associated with the approaching winter months (Kozlowski and Pallardy, 1997). As chlorophyll breaks down, the yellow and orange carotenoid pigments are revealed. These yellow pigments are present in leaves during the entire growing season, as they aid chlorophyll in light absorption for photosynthesis (Dey and Harbourne, 1997). However, their presence is masked by the green chlorophyll pigment. What is unclear is the cause for the formation of the red anthocyanin pigments in late summer and early fall. These pigments yield the highly valued mosaic of colors in species such as sugar maple. This part of the process of fall color development, the formation of anthocyanins, was the focus of our study.

## Material and methods

Sixteen open-grown sugar maples at the U.S.D.A Forest Service Northeastern Research Station were sampled monthly during the period of June through July 1999, biweekly through August, and then weekly from August 30 to October 27, for a total of 12 sample periods. Samples were only collected from the lower half of the southern aspect of each tree's crown, and only obviously sun-exposed leaves were used. The order in which trees were sampled was varied for each sampling date to avoid possible bias caused by diurnal carbohydrate fluctuations. A total of five randomly selected subsamples (branchlets) were collected from each tree during each collection period.

About five leaves were selected from each branchlet to comprise a subsample. To avoid color bias, leaves were collected systematically, first from the terminal positions and then moving down the branch. Leaves with large visual amounts of damage from insects or disease were not selected.

One leaf from each branchlet was retained for moisture analysis. The remaining four leaves were used in all subsequent chemical and color analyses. Immediately following field collection, 12 standard hole-punches were collected from each of the four-leaf subsamples and

preserved in 80% ethanol for carbohydrate analysis. The location of each hole-punch was selected randomly, although obviously damaged tissue and veins were avoided and samples were taken alternately from near the edge and towards the inside of the leaf.

Immediately following field collection (after hole-punch sampling), samples were taken to the Aiken Center at The University of Vermont. There, all leaves of each four-leaf subsample were scanned into the computer at a resolution of 250 dots per inch (dpi) and saved as image files.

After scanning was complete, the leaves were dried in an oven at 70° C. Each dried subsample (consisting of 3 to 4 leaves) was subsequently ground to a 0.5-mm particle size using a Wiley Mill. This ground tissue was used for nutrient (including nitrogen) analysis.

## Laboratory Analyses

**Carbohydrates.** Foliar carbohydrate analysis was completed at the U.S.D.A. Forest Service Northeastern Experiment Station. Soluble sugars were extracted with 80% ethanol. This portion was subsequently separated and quantified on a high pressure liquid chromatograph (HPLC) with a Waters Sugarpak™ column and 0.1 mM Ca EDTA at a flow rate of 0.6 ml min<sup>-1</sup> at 90°C. Sugars were detected with a Waters model 410 refractive index detector and identified with known standards (Kelly Baggett, personal communication, adapted from Hinesley *et al.*, 1992). Carbohydrates assayed included raffinose, sucrose, glucose, fructose and xylose.

The ethanol-insoluble pellet left after removing the soluble supernatant was analyzed for starch content. After the pellet was boiled in potassium hydroxide followed by acetic acid, amyloglucosidase was added. The amount of glucose liberated was assayed with the INT assay and read at 492 nm. Samples were compared with known glucose standards and the amount of starch was calculated from glucose standard curves.

**Nutrients Other than Nitrogen.** Concentrations of aluminum, iron, calcium, magnesium, boron, potassium, phosphorous, manganese and zinc within leaves were determined with inductively coupled plasma atomic emission spectroscopy (ICP-AES). The oven-dried and subsequently mill-ground leaf samples were first digested using a nitric acid/hydrogen peroxide tissue digest (Gary Hawley, personal communication). Following digestion, the solutions were analyzed with ICP-AES to yield the concentration of each element of interest in milligrams per kilogram of leaf tissue.

**Nitrogen.** Because the digest for ICP-AES analysis used nitric acid, the nitrogen content of the digested samples was altered and a separate assay was necessary for nitrogen. Approximately 2- to 4-mg portions of each oven-dried and mill-ground subsample were placed in tin capsules. The percentage of nitrogen by weight for each subsample was determined through combustion analysis.

**Moisture.** Leaf moisture (percentage by weight) was determined by recording the fresh and dry weights of one leaf from each branchlet.

**Color.** Quantification of color in each subsample was performed with NIHImage, public domain imaging software developed by the National Institute of Health. Each subsample scanned during field collection was analyzed for its percentage of green, yellow and red/orange.

## Data Analysis

Following the completion of laboratory analyses, the data were analyzed as follows:

- 1 Simple linear regression was used to determine if any leaf constituent might be valuable in predicting the subsequent timing of fall color development. "Timing" was defined for each tree as the number of days between budbreak and the first date when the tree's leaf color was greater than 5% yellow plus red.
- 2 Simple linear regression was also used to test for significant relationships between quality of coloration for each tree and physiological data from previous sample dates. "Quality of fall coloration" for each tree was expressed as the maximum recorded percent of red, regardless of when that value occurred.

For both types of regression analyses, data from the 5 subsamples of each tree were averaged; thus, there were a total of 16 observations, one for each tree per sample date. A p-value of  $\leq 0.10$  was accepted as significant for all analyses.

## Results and discussion

Although many relationships were tested, only the constituents most promising for use as indicators are included below.

### Leaf Constituents that may Predict the Timing of Fall Coloration

Relationships were tested between timing values for each tree and their leaf constituent concentrations on June 30, the only sample date when no tree had begun to exhibit color. Concentrations of moisture, nitrogen and xylose were positively correlated with the timing of

coloration, while starch concentrations were negatively correlated (Table 1). Thus, the higher the concentrations of leaf moisture, nitrogen, and xylose and the lower the concentrations of starch leaves of a tree contained on June 30, the longer a tree took to begin exhibiting coloration.

### Leaf Constituents that may Predict the Quality of Fall Coloration

Correlations were assessed between the quality of fall coloration and foliar constituent concentrations on each sample date before the first peak value of red was observed. Nitrogen (negative) and starch (positive) were significantly correlated with the subsequent quality of coloration on 7 of 8 dates tested and xylose (negative) was significantly correlated on 4 of 8 dates tested (Table 2). Thus, the more nitrogen and xylose present in leaves, the less likely a tree was to develop high quality coloration; starch exhibited the opposite relationship. Moisture concentrations on June 30 were significantly correlated (positive) with the quality of fall coloration (Table 2). Although significantly correlated on only one date, this parameter may be an important early-season indicator of the quality of subsequent coloration.

### Hypothetical Role of Stress in the Development of Coloration

Biochemical studies have indicated that stress may promote anthocyanin formation (Hussey, 1963; Bongue-Bartelsman and Phillips, 1995; Trull *et al.*, 1997). Thus, the larger story that emerges from our data may involve physiological stress and, to an extent, the molecular

**Table 1.** Correlation coefficients (*r*) for leaf constituents on June 30 versus the length of time between budbreak and the onset of fall color development (when yellow plus red > 5%) (*n*=16); \* indicates  $0.05 < p \leq 0.10$ , \*\* indicates  $0.01 < p \leq 0.05$  and \*\*\* indicates  $p \leq 0.01$ .

Leaf Constituent	<i>r</i>
Moisture	+0.55**
Nitrogen	+0.77***
Starch	-0.66***
Xylose	+0.46*

**Table 2.** Correlation coefficients (*r*) for the regression of parameters on each sample date before peak color development and the peak red values for the corresponding tree (*n*=16); \* indicates  $0.05 < p \leq 0.10$ , \*\* indicates  $0.01 < p \leq 0.05$  and \*\*\* indicates  $p \leq 0.01$ .

Leaf Constituent	30-Jun	2-Aug	17-Aug	30-Aug	7-Sep	14-Sep	21-Sep	28-Sep
Moisture	-0.63***	+0.32	-0.06	-0.05	+0.06	0.14	-0.32	-0.17
Nitrogen	-0.74***	-0.63***	-0.72***	-0.76***	-0.74***	-0.73***	-0.47	-0.68***
Xylose	-0.38	-0.56**	-0.02	-0.42	-0.61**	-0.60**	-0.23	-0.47*
Starch	+0.49*	+0.46*	+0.76***	+0.64***	+0.32	+0.59**	+0.61***	+0.63***

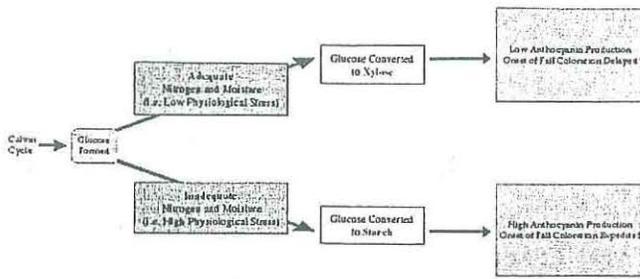


Figure 1. Simplified pathway of a glucose molecule from the Calvin Cycle through fall senescence, illustrating the effects of stress on the level of anthocyanin production. See text for discussion of this figure.

precursors of anthocyanin molecules. To comprehend the bigger picture regarding the quality of coloration, it helps to think of plant processes in very simplified terms. Figure 1 illustrates this process. A molecule of glucose is the main product of carbon fixation in the Calvin Cycle. First, presume this molecule of glucose can experience one of two fates. It can be either: 1) used for growth (as exemplified by soluble xylose concentrations); or 2) stored for later use (as exemplified by starch concentrations). If a tree is under physiological stress, especially from limiting factors such as low moisture and low nitrogen, it is unable to fully utilize the glucose produced in the Calvin Cycle for growth. Consequently, glucose is stored as starch. If the level of physiological stress is low, glucose can be utilized for growth, and higher levels of structural carbohydrates, such as xylose, are formed.

When fall senescence begins, cellular materials begin to break down. Starch is broken down into glucose subunits. There is no indication, however, that structural carbohydrates like xylose have the same fate. Thus, the larger the amount of starch present in a leaf before fall senescence begins, the more glucose will be liberated. Glucose molecules are precursors of red anthocyanin pigments. Although the mechanism is not clear, it would seem that the more glucose present during senescence, the greater the amount of anthocyanins formed. In essence, high levels of growing-season stress appear to initiate starch production, which eventually causes more anthocyanin precursors to be present during senescence; the opposite appears to be true for trees experiencing low levels of stress.

In relation to the timing of fall coloration, stress has been shown to expedite the onset of senescence in many woody plants (Addicott, 1982; Kozlowski and Pallardy, 1997). Thus, in this context, levels of leaf constituents associated with high stress (i.e., low moisture, low nitrogen and high starch) would be expected to indicate expedited fall senescence. Levels of constituents associated with low stress (i.e., adequate moisture, adequate nitrogen and high xylose) would be expected to indicate the opposite (Figure 1).

This hypothesis was developed from the identification of leaf constituents found to be significantly predictive of the timing and quality of fall coloration. In our study, leaf

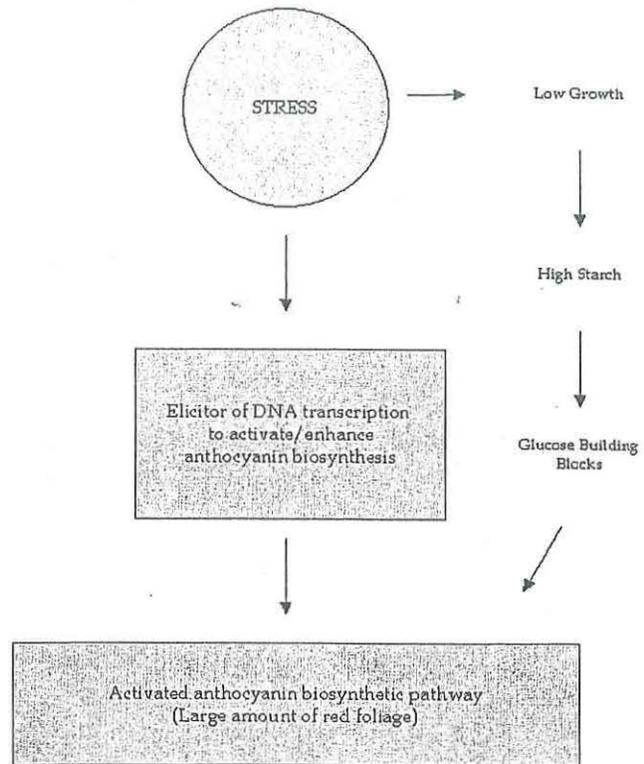


Figure 2. Proposed scheme of the connection between physiological stress, an activated anthocyanin pathway and starch as its indicator.

constituents that retard stress i.e., nitrogen and moisture, were positively correlated with the time to color and negatively correlated with the quality of color. Starch, a possible indicator of high stress, was negatively correlated with the time to color and positively correlated with the quality of fall coloration. The opposite was true for xylose, a possible indicator of lower levels of stress.

There may be an even larger, overlying process occurring. Anthocyanins are thought to protect plants from stressors such as photooxidative damage from UV light and tropospheric ozone (Takahashi *et al.*, 1991; Foot *et al.*, 1996). Stress, then, may act as an elicitor of DNA transcription that either activates or enhances the biosynthetic pathway of anthocyanin formation. Thus, high levels of starch may indicate enhanced activity of this pathway, while high levels of xylose may indicate reduced activity. Figure 2 illustrates the hypothetical connection between stress and anthocyanin formation.

## Conclusion

Data from our study appear to indicate that nitrogen, xylose, moisture and starch are useful in predicting the timing and quality of fall foliage coloration in sugar maple. These data are also consistent with the hypothesis that stress may influence fall coloration via activation of the biosynthetic pathway of anthocyanins. While we have probably identified leaf constituents useful for predict-

ing the timing and quality of fall coloration, further experimentation is necessary to elucidate the larger picture of the process. Previous research has shown that light stress was effective in inducing the accumulation of phenylalanine ammonia lyase and chalcone synthase messenger RNA's in germinating *Arabidopsis thaliana* seedlings that exhibited anthocyanin pigments (Kubasek *et al.*, 1992). These are two of the enzymes involved in the anthocyanin biosynthetic pathway. We hypothesize that activity of the messenger RNA of chalcone synthase, the enzyme that catalyzes the formation of the first dedicated intermediate in anthocyanin formation, is enhanced in trees experiencing physiological stress. Confirmation of this hypothesis requires further experimentation:

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