AN INVESTIGATION OF SOME FACTORS THAT MAY INFLUENCE THE DEVELOPMENT OF FALL FOLIAGE COLOR IN SUGAR MAPLE

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Abstract

Fall foliage development is an important part of Vermont's economy and culture. However, few data exist on the exact nature of the timing and brilliance of fall color. In this exploratory study, leaf tissue from ten sugar maples was collected periodically during the period of September 4 through October 8, 1998 and analyzed for nutrient concentration, moisture content, carbohydrate concentration, and the extent of color development. Data were analyzed to identify significant relationships between chemical parameters and color data. Several parameters were related to the extent of fall color development, including aluminum, iron, and nitrogen concentrations and moisture content. Emphasis will be placed on these variables in a subsequent, more rigorous analysis of fall foliage development.

Background

The display of fall foliage in Vermont is valued highly by both residents and non-residents. This yearly event is responsible for generating much of the region's fall tourism revenue. For example, between September and October of 1996, total revenue from taxable rooms, meals, and alcohol in Vermont was nearly $140 million (Ramaswamy, VT Tourism Data Center, pers. com.). Despite its many benefits, few substantiated data exist regarding the exact nature 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 a procedure for manipulating fall color development on selected trees. Other than data regarding anthocyanin pigments (i.e., Harborne 1965), much of the information available on the nature of fall foliage development is only anecdotal in nature. In the following literature survey, I will present the available information, and attempt to integrate this information in order to provide sufficient background for my study, and to justify the selection of my study variables.

Most of the basic physiological processes involved in fall foliage development are known. The chlorophyll molecule begins to breakdown in response to lower temperatures and shorter daylengths associated with the approaching winter

months (Kozlowski and Pallardy 1997). As chlorophyll breaks down, the yellow carotenoid pigments are revealed. These pigments are present in leaves during the entire growing season, as they aid chlorophyll in light absorption for photosynthesis. What is unclear is the cause for the formation of red anthocyanin pigments. This pigment yields the highly valued mosaic of colors in species such as sugar maple. This part of the process of fall foliage development will be the focus of my study.

Literature and anecdotal information are available on the following factors that may influence the development of fall foliage coloration: pH, stress, light, carbohydrate concentration, and environmental conditions.

pH

Early biochemical studies of anthocyanin pigments suggest the possibility that pH plays a role in the color these pigments produce. Swain (as cited in Harborne 1965) showed that many anthocyanin pigments *in vitro* would turn into related anhydro bases at pH values above 7.0. These forms of anthocyanins are much bluer in shade than are the normal, red structures observed at pH values similar to those found in plants (5.5). Scott-Moncrief (1936) noted differences in flower color of *Primula sinensis*, *P. acaule*, and *Papaver rhoeas* with only a 0.5- to 1.0-unit change in flower sap pH values. Also, it has been suggested, anecdotally, that the reason for the different shades of red in ash (purple) and maple (red) involve differences in the pH of cell sap (Murakami, USDA Forest Service Northeastern Experiment Station, pers. com.).

Stress

Other information points to the involvement of stress in the development of fall color. Tomato seedlings exposed to stressful conditions such as high light intensity, low nitrogen availability, and extremely low temperatures, accumulated high levels of red anthocyanin pigments (Hussey 1963). Also, Harborne (1965) suggests that fungal and viral infections can also induce the

formation of anthocyanin pigments, but provides no experimental data to support this claim. Anecdotal information provided on a Vermont tourism website (www.vtweb.com) also suggests that "trees that begin to turn very early are usually diseased or stressed... ". This site did not provide any information to substantiate that claim.

Light

A few sources cite specific light conditions necessary for the development of fall color. High light intensity produced an accumulation of anthocyanin pigments in tomato seedlings (Hussey 1963). A wildflower webpage (www.ncnatural.com/wildflower/fall/fallfact.html) asserts that bright sunlight is imperative for the development of brilliant red anthocyanin pigments. This source illustrates this hypothesis by suggesting that if a black mask is placed over part of a leaf before fall color develops, the part of the leaf under the mask will turn yellow, while the exposed part will turn red. However, no actual data were presented. Harborne (1965) provides more convincing evidence of the role of light in anthocyanin development. He presents, as an example, the rose variety "Masquerade", which is orange-yellow when young, but turns a deep red just before senescing. He asserts that there is delayed anthocyanin production that creates the deep red. The underside of some petals, which do not receive direct sunlight, often remain yellow in color.

Carbohydrates

Many sources suggest that carbohydrates play a major role in the development of autumnal coloration. Harborne (1965) cites, as an example, the reddish flush of color sometimes noted in young leaves that have just broken bud. He believes this red color may come from anthocyanins produced from the excess starch stored in the young bud before leaf development. He also asserts that autumnal coloration is related to the liberation of sugar (starch degradation), but concedes that this fact has not been quantified. In the aforementioned tomato seedling experiment (Hussey 1963), tomatoes whose "carbohydrate sinks" were removed accumulated large levels of anthocyanin pigments. This suggests that anthocyanin production is related to excess carbohydrate accumulation resulting from a lack of "sinks" for this excess.

Kozlowski and Pallardy (1997), suggest that fall foliage development is related to the ratio of soluble to insoluble carbohydrates, but provide no quantitative data to support this. Dr. C.R. Bell (www.ncnatural.com/fall-color/bell.html) asserts that, since anthocyanins are byproducts of carbohydrates, the amount of red color observed is dependent on the amount of carbohydrates left in the leaf when the abscission layer develops just before senescence. Another website (www.waterw.com/~science/october.html) suggests that excess glucose in the leaf is turned to red pigments when photosynthesis ceases. This same assertion was also presented in an article by Klein in the Stowe, Vermont Reporter (1971).

Environmental Conditions

Several sources cite specific environmental conditions necessary for the development of brilliant fall color. These conditions seem to be most related to the belief that excess carbohydrates are necessary for anthocyanin development. Most sources (www.ncnatural.com/fall-color/bell.html, Klein 1971, www.vtweb.com, Kozlowski and Pallardy 1997) suggest that warm, sunny days followed by cool, but not freezing, nights are the best conditions for the most brilliant autumn color. This combination, the sources suggest, would cause the leaves to produce excess amounts of carbohydrates. But, during the chilly nights plants would be unable to transport carbohydrates from the leaves to other carbohydrate sinks. The large amount of sugar would then be trapped in the leaf as the abscission layer formed and these sugars would subsequently be turned into anthocyanins. It is essential not to neglect that other factors must play a role in this scenario. For example, an unhealthy tree may not be able to produce high levels of carbohydrates, even under high light and temperature conditions. Based on information provided from an interview with Dr. C. R. Bell, Franklin (1998) suggests that dry or cloudy weather causes sugar maple to cease carbohydrate production well before the abscission layer forms and decreases anthocyanin production.

Other Notable Factors

Several other factors have been implicated, at least indirectly, in the development of fall color. In addition to the possible effects nutrient stress may have on fall

color development, studies have indicated a role of metals in the nature of anthocyanin pigments. It was noted that some flowers with the same chemical anthocyanin content produced different colors. Harborne (1963) showed that metal chelation to anthocyanins, especially by iron and aluminum, changed the observed color in flowers. Harborne (1963) also showed that copigmentation, especially with flavones, flavonols, and flavans, changed the absorption spectra of the anthocyanins, resulting in a bluer shade of flower color.

To date, no study I am aware of has directly linked any factor, climatic or physiological, with the timing and brilliance of autumn foliage in sugar maple. The objective of my project was to test some of the reported theories by examining several variables in an attempt to identify factors that are related to the timing and/or intensity of fall foliage development in sugar maple. Based on the information available, I selected the following variables: 1) foliar carbohydrate content; 2) foliar moisture; and 3) foliar macro and micronutrients. Moisture was chosen to examine as it might be indicative of a tree's stress level. These variables were quantified and compared to quantified foliar color.

METHODS

Study Location

Sampling was conducted at the University of Vermont Proctor Maple Research Center in Underhill, Vermont. Trees were located at an elevation of approximately 2000 feet on the slope of Mt. Mansfield. Soils at the Proctor Center are considered spodosols, which are typical of the Green Mountains. Sites where sugar maple predominates can be summarized as being moderately well-drained, gravely to fine sandy loam, usually in the Lyman or Marlow soil series, which are rocky, acidic, and of low natural fertility. The area typically receives about 100 centimeters of precipitation per year (Tim Wilmot, Proctor Maple Research Center, pers. com.).

Field Methods

Ten sugar maple trees, without seed, were selected for study. Five were forest-grown trees for which longterm phenological records have been maintained by

the Vermont Department of Forests, Parks, and Recreation. The remaining five were on sites at the edge of the forest and have no longterm records. Each tree was between 50 and 90 feet tall. Beginning September 4, 1998, samples were collected weekly. Three leaves were collected from a single branch selected from each cardinal direction in the lower crown of each tree. Branch samples were obtained using a shotgun loaded with steel shot. At the time of collection, each leaf was rinsed with distilled water to remove particulate matter. One leaf was retained for moisture analysis (these leaves were blotted dry to avoid affecting the moisture analysis). Tissue was removed with a hole-punch from the remaining two leaves to be used for carbohydrate analysis. The remaining tissue from these two leaves was used for all subsequent chemical and color analysis. Sample collection continued until October 9, 1998, at which time a rainstorm removed all remaining leaves from the crowns of sample trees.

Laboratory Methods

Immediately following field collection, samples were taken to the Aiken Center at the University of Vermont. There, leaves were scanned into the computer at a resolution of 250 dots per inch. NIH*Image*, an image processing program, was used to quantify the amount of green, yellow, and red/orange in each of the images.

After scanning was complete, the leaves were dried in an oven. This dried tissue was subsequently ground as an aqueous sample in order to prevent sample loss. Two types of analyses were performed on the ground tissue: 1) macro and micronutrients; and 2) nitrogen analysis. Nutrient analysis was completed with ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy). Nitrogen analysis was completed using combustion analysis.

Carbohydrate analysis was completed at the USDA Forest Service Northeastern Experiment Station using HPLC (High Pressure Liquid Chromatography) techniques.

Moisture analysis was conducted by recording the fresh and dry weight of leaves.

Data from the following three sampling dates were examined: the first, middle, and last week (September 4, 25, and October 9) of the study. Data were blocked by location, forest- or open-grown. Within these blocks, regressions were performed between each parameter and color data to determine if moisture, nutrient and/or carbohydrate concentrations were correlated with the amount of leaf color. The acceptable level of significance was considered 0.05.

RESULTS AND DISCUSSION

Leaves of forest grown trees were generally much greener than open grown trees. No red pigment was detected in any forest-grown samples over the entire study period. In contrast, many samples from open-grown trees were very physiologically advanced, close to senescence, and exhibited large amounts of red fall coloration.

Statistical tests were performed to see if correlations existed within each location, parameter, and percent of color. Significant parameters and their r-values are listed in Table 1. In addition, some parameters which did not show significant differences but did show patterns when levels of parameters where examined between the two locations were also examined. These parameters were manganese, calcium and potassium.

Nitrogen

The percentage of nitrogen was positively correlated with the amount of green in leaves of open-grown trees; conversely, this parameter was negatively correlated with the amount of yellow in forest-grown trees, and the amounts of yellow and red in leaves of open-grown trees (Table 1).

Forest Grown

	Green			Yellow			Red		
	r	Prob > F	n	r	Prob > F	n	r	Prob > F	n
Parameter									
Starch	0.643	0.0177	13						
Xylose	0.694	0.0085	13	-0.819	0.0006	13			
Stachiose									
Sucrose									
Glucose									
Fructose				0.681	0.0104	13			
Raffinose									
% Moisture				-0.562	0.0292	15			
% Nitrogen				-0.939	<0.0001	13			
Calcium				0.627	0.0124	15			
Phosphorous				-0.737	0.0017	15			
Potassium				-0.538	0.0386	15			
Magnesium									
Aluminum	-0.564	0.0284	15	0.926	<0.0001	15			
Iron				0.688	0.0046	15			
Zinc									

Open Grown

	Green			Yellow			Red		
	r	Prob > F	n	r	Prob > F	n	r	Prob > F	n
Parameter									
Starch	0.692	0.0042	15	-0.601	0.0179	15			
Xylose	0.949	<0.0001	15	-0.718	0.0026	15	-0.682	0.0051	15
Stachiose									
Sucrose	0.597	0.0187	15						
Glucose									
Fructose	-0.605	0.0168	15						
Raffinose	0.783	0.0005	15	-0.696	0.004	15			
% Moisture	0.832	0.0001	15	-0.717	0.0026	15			
% Nitrogen	0.945	<0.0001	15	-0.687	0.0045	15	-0.735	0.0018	15
Calcium									
Phosphorous	0.675	0.0057	15				-0.585	0.022	15
Potassium							-0.6561	0.0079	15
Magnesium									
Aluminum	-0.746	0.0014	15				0.616	0.0145	15
Iron	-0.762	0.001	15	0.595	0.0194	15	0.627	0.0123	15
Zinc				0.592	0.0202	15			

Table 1. Regression coefficients and sample sizes for each of the parameters with each color. Data are listed as leaves of forest-grown trees and leaves of open-grown trees. Data significant at the 0.10 level are listed in Appendix A.

Although these statistical relationships are strong, it seems that nothing can be deduced from them as data were not gathered during a full growing season. Consequently, these data may reflect an effect, rather than a cause, of fall coloration. As nitrogen is an important constituent of the chlorophyll molecule and not of the carotenoid or anthocyanin pigments, it seems intuitive that trees with greener leaves would contain more chlorophyll and thus more nitrogen. Although nothing can be deduced about nitrogen's relationship to the causes of differential fall foliage development, it seems to be sufficiently important to warrant further study.

Moisture

Statistical analysis yielded positive correlations between moisture content and the amount of green in leaves of open-grown trees, negative correlations between moisture content and the amounts of yellow in leaves of both open- and forest-grown trees, and negative correlations between moisture content and the amount of red in leaves of open-grown trees (Table 1). This parameter, like nitrogen, may be more of an effect of fall foliage development rather than a cause. The closer to senescence a leaf is, the less moisture it will contain. Thus, one would expect the more physiologically advanced, red or yellow leaves, to contain less moisture than would those with more green. However, moisture may prove to be an interesting variable to examine, if an entire season's data are examined.

Xylose

In both forest- and open-grown trees, the amount of xylose was strongly positively correlated with the amount of green, and negatively correlated with the amounts of yellow and red (Table 1). This may be related to the fact that xylose is an important constituent of pectinaceous compounds that hold cells together. Since the leaves of open-grown trees were closer to senescence, they may be more likely to have broken down more physiologically and thus contain less pectin. However, it is uncertain what exact mechanism drives the statistical relationship observed between the amount of xylose and fall coloration.

Aluminum

In both open- and forest-grown trees, the amount of aluminum was negatively correlated with the amount of green. In contrast, aluminum concentrations were positively correlated with the amount of red in leaves of open-grown trees and the amount of yellow in leaves of forest-grown trees (Table 1). This relationship supports the hypothesis outlined earlier that larger amounts of aluminum would be associated with more reds, oranges, and yellows due to the requirement of metal chelation with aluminum and iron by anthocyanins in order to produce more vivid color (Harborne 1965). Metal chelation in anthocyanins, however, was observed *in vitro* only. Although these data seem to support this observation, a more specific, empirical analysis would be required before any firm conclusions could be reached.

Iron

Iron levels were positively correlated with the amount of red in leaves from open-grown trees, and the amount of yellow in leaves of trees from both locations (Table 1). Iron was negatively correlated with the amount of green in leaves of open-grown trees (Table 1). These observations may also support the hypothesis that metal chelation by aluminum and iron play a role in the expression of color in anthocyanin pigments.

Starch

Starch levels were positively correlated with the amount of green in leaves of trees from both locations, and negatively correlated with the amount of yellow and red in leaves of trees from open-grown trees (Table 1). The cause of this correlation is uncertain.

Fructose

Fructose levels were positively correlated with the amount of yellow in leaves of trees from both locations, red in leaves of open-grown trees, and negatively correlated with green in leaves of open-grown trees (Table 1). This could also be related to the role of fructose in pectinaceous compounds. Pectinaceous compounds breakdown during leaf senescence. Thus, leaves that are physiologically closer to senescence, such as those that contain more color, should contain fewer pectins and consequently less fructose.

Other interesting observations were made with some parameters. These parameters did not show statistically significant relationships with the amount of leaf color. Instead, they showed interesting patterns when examined as mean levels of that parameter over time and between locations. I will briefly outline these observed patterns.

Manganese

While levels of manganese increased over time in leaves of trees from both locations (Figure 1), leaves of forest grown trees had consistently higher levels of this element.

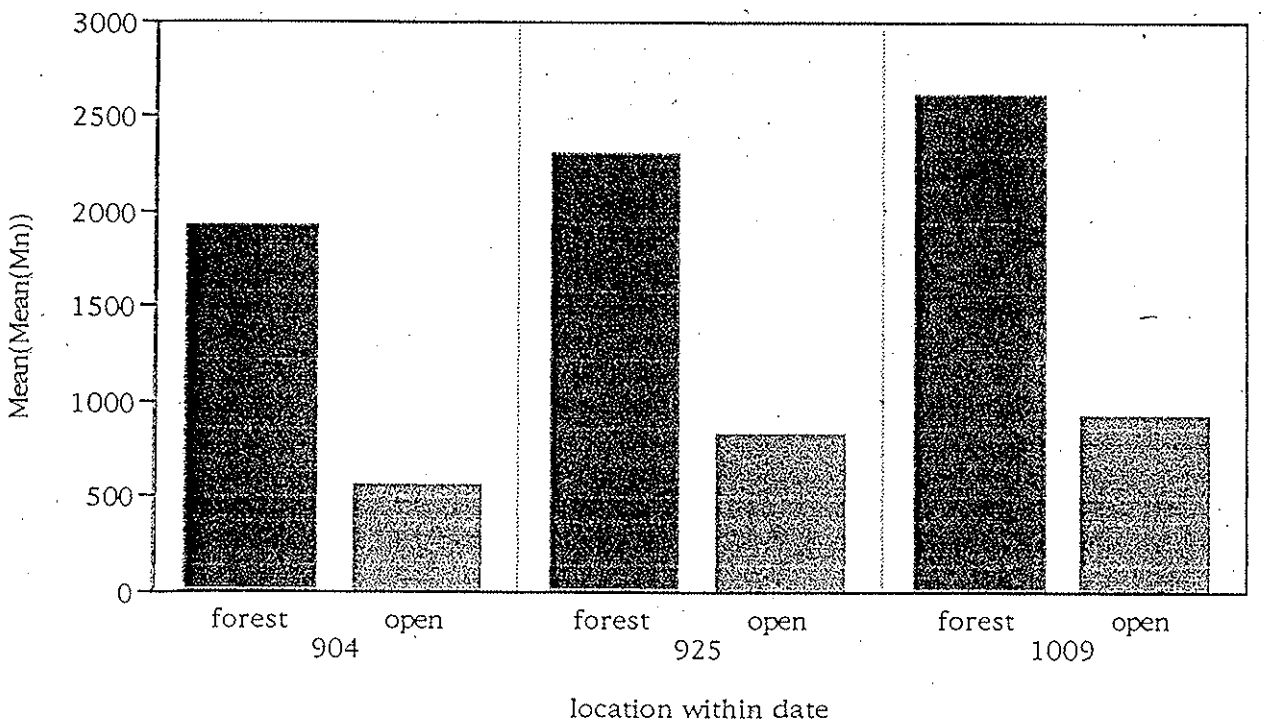

Figure 1. Mean manganese levels in leaves of forest- and open-grown trees over time. Data are in parts per million and x-axis numbers indicate the date.

Manganese is an important activator of many plant enzymes (Salisbury and Ross 1997). Levels were consistently higher in open-grown trees, and a consistent increase over time was observed in leaves of trees from both locations. However, no explanation for these observations can be deduced at this time.

Calcium

Mean calcium levels increased with time in leaves of trees from both locations (Figure 2) and were consistently higher in open grown trees.

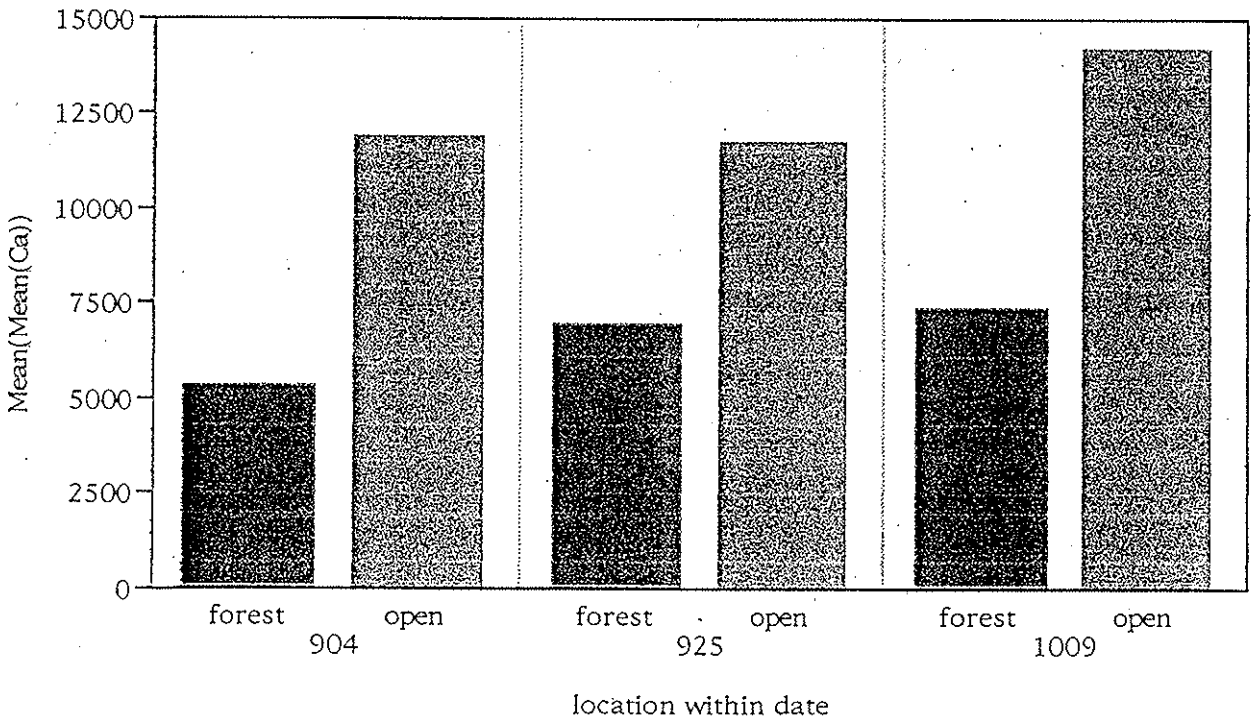

Figure 2. Mean calcium levels in leaves of forest- and open-grown trees over time. Data are in parts per million and x-axis numbers indicate the date.

Most of calcium's functions, such as cell wall structure, would lead one to think the opposite: more physiologically active tissue should contain more calcium. This was not observed. In addition, calcium was only positively correlated with the amount of yellow in forest-grown trees (Table 1). The reasons for these findings are unknown.

Potassium

Mean levels of potassium were consistently lower in leaves of open grown trees than in leaves of forest grown trees (Figure 3).

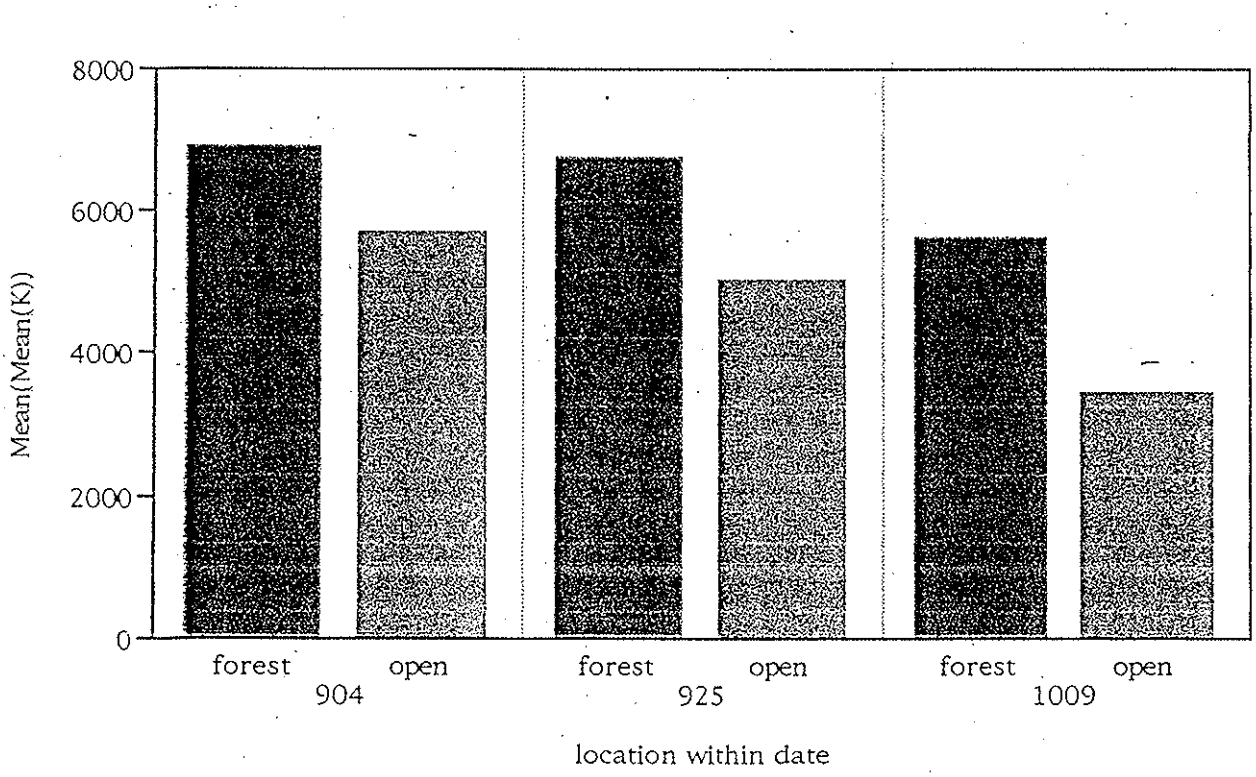

Figure 3. Mean potassium levels in leaves of forest- and open-grown trees over time. Data are in parts per million and x-axis numbers indicate the date.

In addition, levels decreased over time in leaves of trees from both locations. An explanation for these observations is unavailable using these data. These patterns do, however, warrant further study.

CONCLUSIONS

Definitive relationships between parameters and differential development of fall color are difficult to state using these data. Although interesting relationships were identified, without data from a full growing season, these data are incomplete and present only a very small fraction of the entire picture.

Further studies will include data from at least one entire growing season. These studies will focus on the most promising constituents identified in my study, especially nitrogen, moisture, aluminum, and iron. However, in order to be thorough, a reexamination of all the parameters in this study may be necessary. In addition, further studies will include greenhouse experiments to expand on the simply observational nature of my study. These experiments will manipulate variables, such as pH and light, that cannot be examined in an observational study. Imperative to the success of further studies is the inclusion of data from a full growing season. Hopefully, with the addition of a more nearly complete data set and greenhouse experiments, the causes of differential fall foliage color development can be further elucidated.

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http://ncnatural.com/fall-color/bell.html. This URL contains an interview with botanist Dr. C.R. Bell about the science of fall color. It was last updated in 1997.

http://www.waterw.com/~science/october.html. This URL contains information on fall foliage.

http://www.vtweb.com. This URL contains information on Vermont and is maintained by the Vermont Department of Tourism. As it is updated regularly, information on fall foliage is found there only seasonally.

http://ncnatural.com//wildflowr/fall/fallfact.html. This URL contains fall foliage information.

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Glucose									
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Manganese									
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