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The Effect of Light Intensity on the Soluble Carbohydrate Level and Macronutrient Composition of *Ilex opaca* Ait.[1]

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Abstract. Soluble leaf carbohydrates, macronutrient elements and growth responses to 3 levels of light were studied on field grown plants of *Ilex opaca* Ait. cv. Miss Helen . Soluble D-fructose, α-D-glucose, B-D-glucose and sucrose reached a maximum concentration expressed as a percentage of the dry weight of leaf tissue during the winter sampling periods, followed by a decline as bud-expansion approached. The maximum concentration of soluble D-galactose was found in newly matured leaf tissue. Soluble D-fructose, α-D-glucose, B-D-glucose and sucrose levels were not effected by the shade environments. The level of D-galactose increased under the shaded environment as compared to full sun plants.

P and K levels in the leaf tissue were at high concentrations in newly matured leaf tissues, while Ca and Mg were at low levels. Both K and Mg levels were observed to be higher in leaf tissue from plants grown under the 92% shade conditions compared to full sun plants.

Stem diameter was significantly reduced under the 92% shade conditions, while leaf size of plants grown under both 50% and 92% shade was significantly increased. Flower production was significantly reduced in plants grown under the 92% shade conditions.

American Holly, *Ilex opaca* Ait., a native broadleaf evergreen tree of the Eastern United States has been a plant traditionally used for decorative effects at the Christmas season. At one time, annual sales of cut American holly exceeded one-half million dollars (7, 8). However, American holly sales have decreased considerably, due to several factors, including a decline in the supply of native holly, the superior quality of western grown English holly, and poor conservation practices; moreover, a primary cause has been the irregularity with which these trees flower and produce fruit in the native state (3). This research was initiated to investigate the influence of reduced light upon various growth responses and physiological conditions in an attempt to determine reasons for the irregularity in flowering and subsequent fruiting.

Materials and Methods

Nine, 6 year old female trees of *Ilex opaca* Ait. cv. 'Miss Helen' were planted in the fall of 1966 at the University of Delaware Substation Farm at Georgetown, Delaware, on a Plummer sandy loam soil. Three plants were allowed to receive full sunlight while the remaining 6 plants were enclosed in 5 X 5 X 5 ft structures and covered with saran cloth. One-half of these were covered with saran cloth designed to reduce the light intensity by 50% while the remaining plants were covered with similar material capable of reducing the light intensity by 92%. The plants were not fertilized during the course of the experiment, and were entirely dependent on native soil fertility for sustaining growth.

Leaf samples were collected monthly from the trees in July, August, and September of 1968. Bi-monthly collection of leaves was necessary from September 1968 until the completion of the study because of the limited number of leaves produced on those plants grown under the severe shade conditions. These leaf samples consisted of a random selection of approximately 25 leaves from the current season's growth. All leaves collected in the experimental period were produced in the growth flush of May, 1968. Each tree was treated as a single replicate for carbohydrate and mineral analysis.

Collected leaf tissues were dried in a forced air oven at 80°C for 3 to 5 days in order to insure uniform dryness, ground in a Wiley mill to pass a 20 mesh sieve, and stored in stoppered bottles. Soluble carbohydrates were determined by the method of Fretz, Dunham, and Woodmansee (6). Calcium, K, P and Mg were determined spectrophotometrically with a Technicon Auto Analyzer, after perchloric acid digestion[4]. Nitrogen was determined by routine Kjeldahl analysis as described by Chapman and Platt (4).

Leaf size was determined by selecting 5 leaves from each replication, tracing their outlines on paper, and determining the area with a K & E planimeter. Stem diameter measurements were obtained by driving a small brass nail into the base of each tree approximately four inches above the soil line on August 15, 1967, and measuring the diameters with a K & E steel diameter tape on several subsequent dates. The experiment was designed and statistically analyzed as a random block design with 3 replications. Statistical analyses were performed by an IBM

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[4]Fretz, T. A. 1970. The seasonal variations in the soluble carbohydrates in leaf tissue of *Ilex opaca* Ait. cv. 'Miss Helen' as affected by various light levels with possible relationships to flowering. Ph.D. Thesis. University of Delaware, Newark, Delaware.

1620 computer.

Results

Phosphorous. Mature leaves produced in the growth flush of May 1968 and first sampled on July 1, contained the highest level of P, with subsequent samples from this same growth flush exhibiting decreasing levels of P. There was no consistent difference in the amount of phosphorous in the various shade levels (Fig. 1a).

Potassium. Leaf K exhibited a similar seasonal trend to that observed for P. Leaf tissue from plants grown under both the 50% shade and the full sun conditions decreased in K from July 1 through March 1, 1969, followed by an increase in K as bud-expansion approached. Similar plants grown under the 92% shade conditions exhibited a decrease in K after the initial high in July, followed by an increase in K at the November sampling. Following this slight rise in the K levels in November, a decline was observed through March 1969. As bud-break approached in the late spring, those plants grown under the 92% shade responded with an increase in the amount of leaf K (Fig. 1b).

Calcium. All leaves sampled on July 1, regardless of the light level, exhibited a low level of Ca. As the season progressed, the

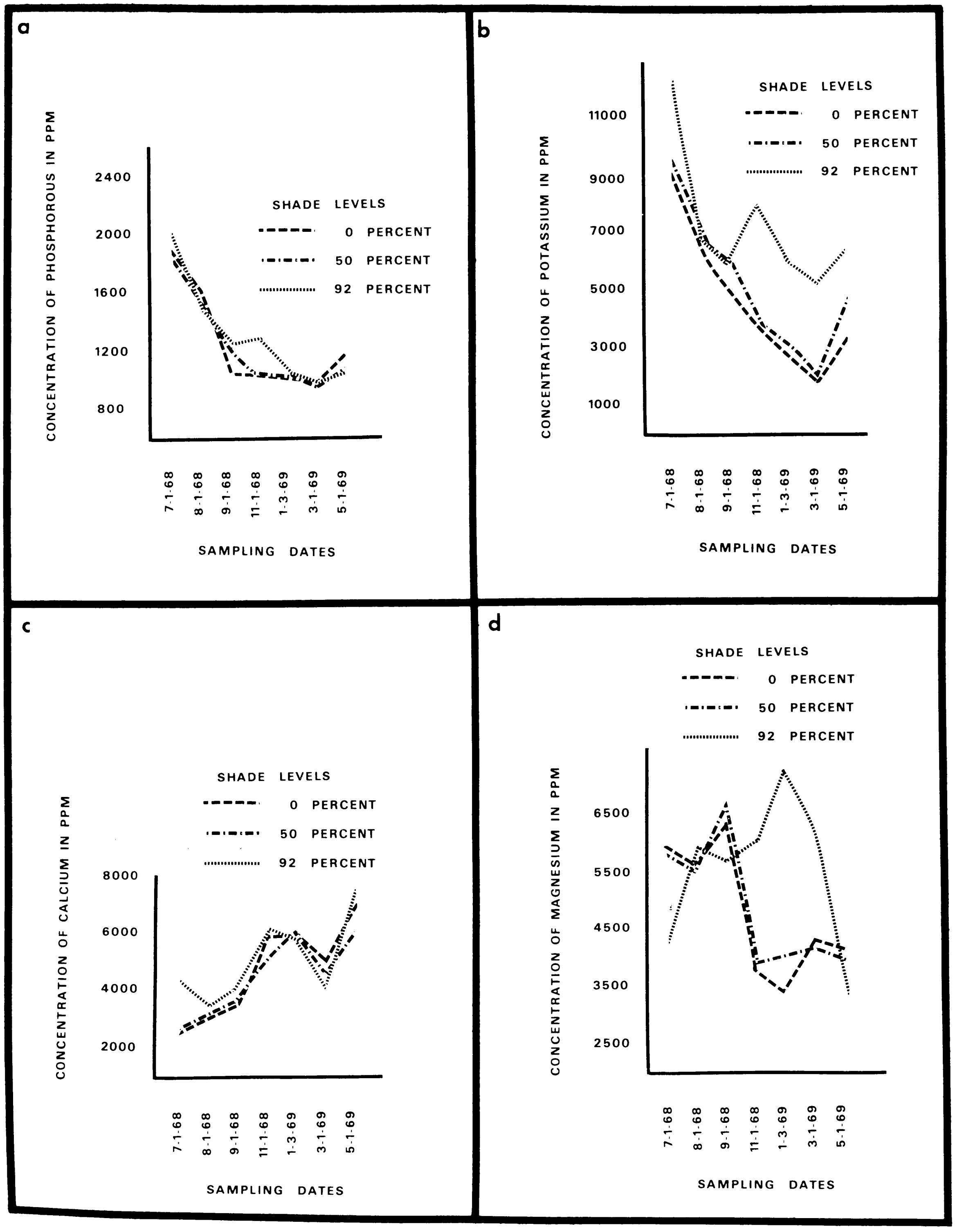

Fig. 1. Seasonal fluctuations in the concentration of various macronutrients in *Ilex opaca* leaf tissue as influenced by 3 light levels. (a) P; (b) K; (c) C; and (d) Mg.

Ca increased and reached a maximum in December. Calcium levels decreased after the mid-winter high only to show a second increase at bud-expansion. There was a trend toward a higher level of Ca in those plants which were grown under the 92% shade conditions, when compared to similar plants grown under either the 50% shade or full sun conditions (Fig. 1c).

Magnesium. Plants grown under either the 50% shade or the full sun conditions exhibited similar trends for leaf Mg content. July through September 1968 produced first a slight decrease and then a slight increase. Between September and November 1968, the leaf samples declined sharply in Mg, for the remainder of the experimental period. Similar plants which were exposed to the 92% shade conditions exhibited an increase in the amount of Mg between the July and August 1968 analyses, followed by a rather constant level of Mg through November. Leaf Mg increased greatly in the plants exposed to the 92% shade conditions during the winter period, followed by a rapid decrease in the spring (Fig. 1d).

Nitrogen. The N in leaves of plants grown under either the full sun or 50% shade conditions exhibited a marked increase between September and November 1968. The level of N in leaves of plants grown under the 50% shade conditions then decreased through March 1969, while the full sun leaves remained unchanged until the end of the experimental period. The 92% shade leaves showed fluctuations in N content with 2 maximums occurring, one in September of 1968 and a second in March 1969 (Fig. 2).

There was no consistent trend for leaf N content and light intensity.

Soluble D-fructose. From the initial July sampling through fall, there was a trend of decreasing amounts of soluble D-fructose in the leaves of holly measured as percent of leaf dry weight. This was especially clear in the leaves of plants exposed to full sunlight (Fig. 3a). This trend was reversed in late fall when all plants, regardless of the light conditions imposed, began to increase in D-fructose content and reached a maximum in January, 1969. A decrease followed this maximum and continued through May 1969, corresponding to the redistribution at bud-expansion.

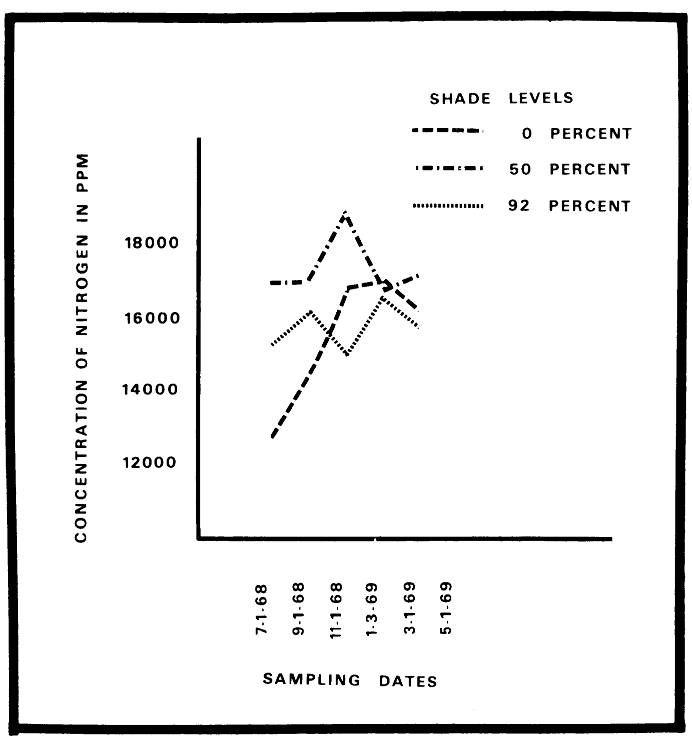

Fig. 2. Seasonal fluctuations in the concentration of N in *Ilex opaca* leaf tissue as influenced by 3 light levels.

Leaf samples from plants grown under shade conditions were sometimes higher and sometimes lower in D-fructose than samples from corresponding plants grown under full sun conditions but followed the same general trend (Fig. 3a).

Soluble D-galactose. The concentration of D-galactose was at its highest level in newly matured leaves, regardless of the shade conditions. The soluble D-galactose content in leaf tissues decreased uniformly for all 3 light conditions through November 1968. A small maximum was observed in January 1969 for all plants, regardless of the light conditions employed. Following this January maximum, all plants declined in soluble D-galactose in leaf tissue for the remainder of the experimental period (Fig. 3b).

There was a trend toward a higher level of D-galactose in the leaf tissues of plants grown under the shade conditions, when compared to plants grown under the full sun conditions (Fig. 3b).

Soluble α-D-glucose. In general, fluctuations in the level of α-D-glucose in leaf tissue was remarkably similar to that observed for soluble D-fructose. Newly matured leaves had a high level of soluble α-D-glucose in July, followed by a decline to a minimum in September 1968, in all plants, regardless of the light conditions. A mid-winter maximum in the α-D-glucose concentration was noted in January for all plants. The soluble α-D-glucose content clearly declined as the spring bud-break approached, suggesting a redistribution of carbohydrate reserves (Fig. 3c).

There appeared to be no real differences in the concentration of soluble α-D-glucose in those plants which were grown under the shade conditions when compared with comparable plants grown under full sun conditions (Fig. 3c).

Soluble B-D-glucose. The soluble B-D-glucose concentration in leaf tissue was similar to that previously observed with soluble D-fructose and α-D-glucose. New leaves sampled in July were initially high in B-D-glucose, but declined to a minimum in September. A mid-winter maximum in the B-D-glucose content occurred in January 1969, followed by a decrease in concentration as bud-expansion approached, indicative of a redistribution of carbohydrate reserves (Fig. 3a).

As with soluble α-D-glucose, there were no real differences in those plants grown under the shade conditions and full sun plants (Fig. 3d).

Soluble sucrose. New leaves were initially low in soluble sucrose. All plants exhibited an increase in soluble sucrose as the leaves aged, regardless of the shade conditions employed, to a maximum level in January 1969. Soluble sucrose level decreased rapidly from the winter maximum, corresponding to a redistribution of soluble materials as spring growth approached (Fig. 4).

There were no differences in the sucrose content of leaves between plants growing under the shade conditions and corresponding plants grown under full sun (Fig. 4).

Leaf size. The physical size of selected mature leaves as affected by the light sources was measured July 1, 1968. Leaves from plants grown under either the 50% or 92% shade were significantly larger than leaves of a corresponding age, grown under the full sun conditions (Table 1).

Growth rate. The overall growth of the *Ilex opaca* cv. Miss Helen trees, as measured by the increases in stem diameter in inches was significantly smaller for those plants grown under the 92% shade conditions, than corresponding plants grown under the other conditions. No differences in stem diameter were observed between the 50% shade or the full sun plants (Table 2).

Flower production. Plants grown under the 50% shade or the 92% shade conditions exhibited a significant reduction in the number of flowers when compared to corresponding full sun plants in both 1968 and 1969 (Table 3).

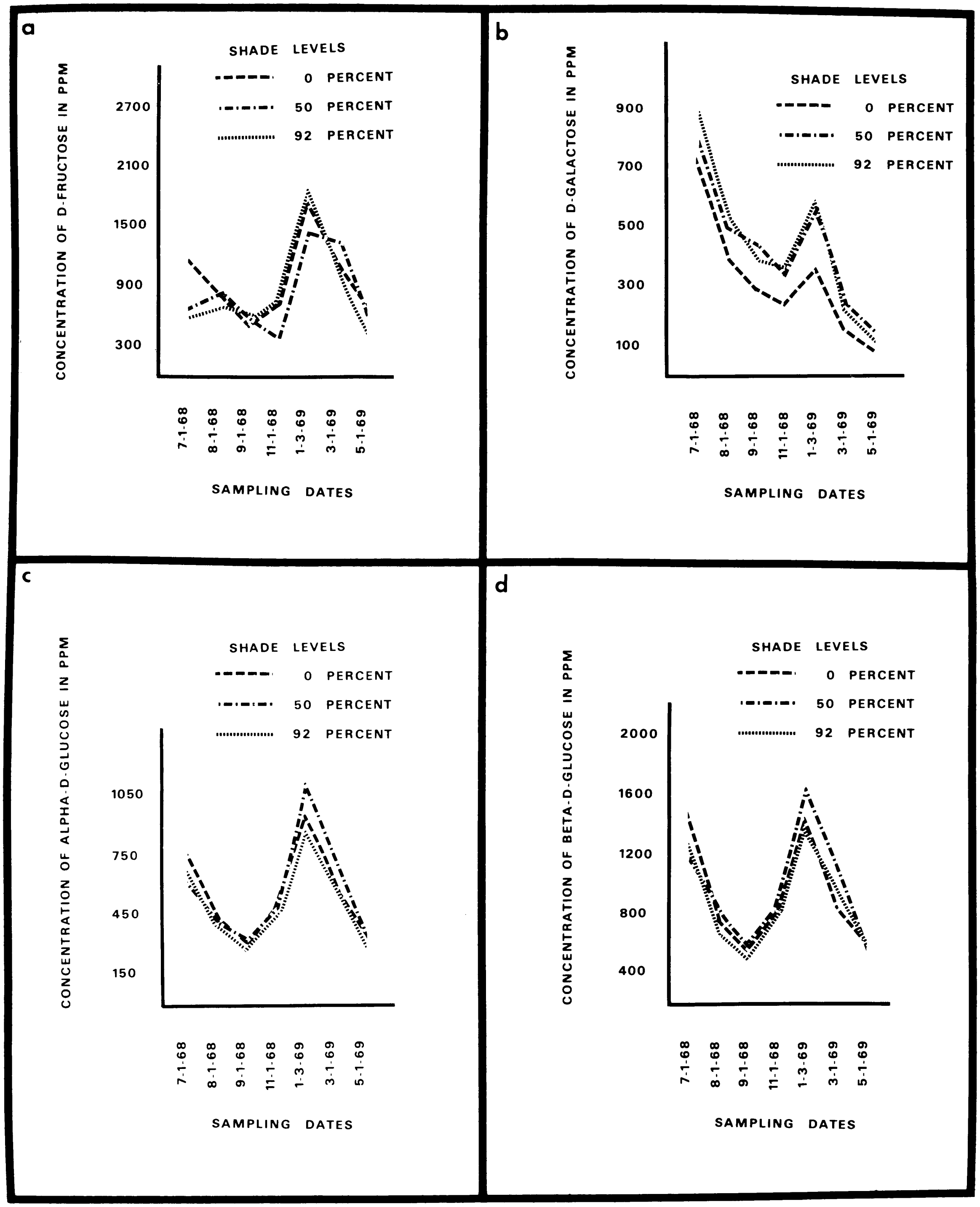

Fig. 3. Seasonal fluctuations in the concentration of the soluble monosaccharides in *Ilex opaca* leaf tissue as influenced by 3 levels of light. (a) D-Fructose. (b) D-Galactose. (c) α-D-Glucose, and (d) B-D-Glucose.

Discussion

Both P and K were at their highest levels in newly matured leaves but declined for the remainder of the experimental period. Data collected on the fluctuations of K agrees with that observed by Davidson for a number of woody plants (5); however, he noted that P remained unchanged throughout the sampling period. Several researchers (1, 2, 22), noted that P and K content of leaves was high at leaf expansion, but decreased, and maintained a constant level for the remaindei of the season.

Calcium in *Ilex* leaf tissue increased with increasing leaf age. Data of several researchers (2, 5, 16, 18) is in agreement, although Smith and Reuther (20), noted that once Ca reached its maximum it remained at a constant level. In the data presented here, the Ca continued to increase even in nearly 1 year old leaves.

Magnesium in *Ilex opaca* leaf tissue was different from that reported for other woody plants. The Mg content increased during the summer but decreased drastically in late fall, early winter. In the leaves of the plants grown in the 92% shade plots the pattern was similar but delayed by several months. Davidson (5) noted that Mg increased with time, while McVikar (16) and Simpson (18) observed that it remained constant throughout the growing season. Smith and Reuther (20) noted that the Mg level in 'Valencia' orange leaves rose from a low level in newly

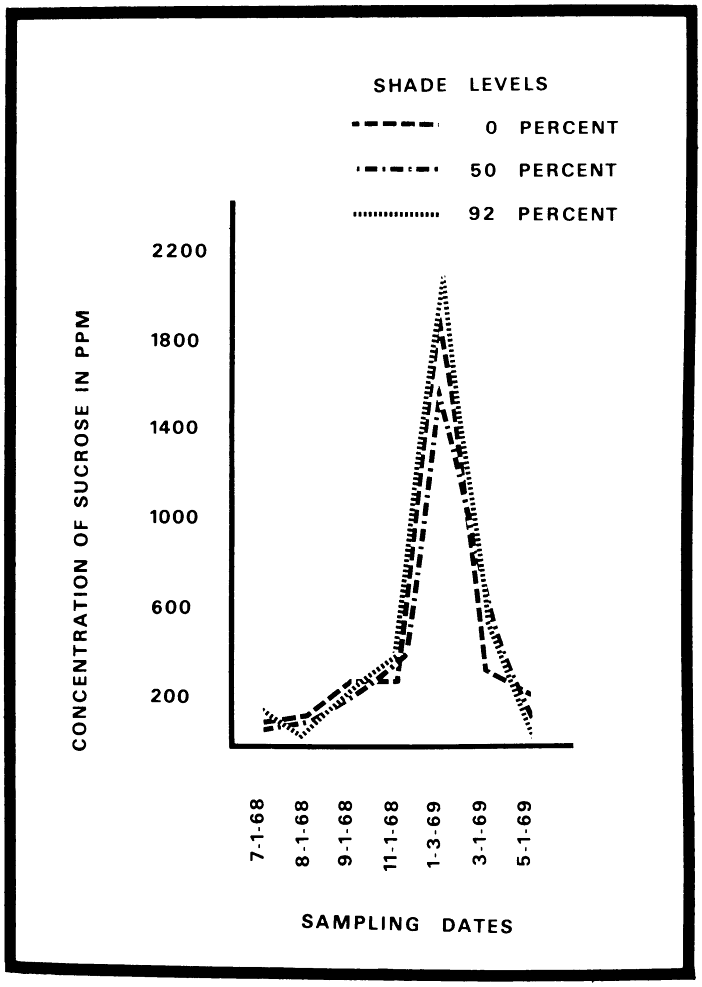

Fig. 4. Seasonal fluctuations in the concentration of soluble sucrose in *Ilex opaca* leaf tissue as influenced by 3 levels of light.

Table 1. The effect of 3 levels of light intensity on the leaf size in square inches of *Ilex opaca*, cv. Miss Helen.

	Light Levels			
Date	0%	50%	92%	L.S.D.[2]
7-1-68	1.79[1]	2.52	3.11	0.52

[1]Leaf size determined with a planimeter. Average of 5 leaves/replication.
[2]Significance at the 0.05% level.

expanded leaves, to a maximum where it remained constant throughout the remainder of the growing season.

Table 3. The influence of 3 levels of light on the number of flowers on *Ilex opaca* cv. Miss Helen in 1968 and 1969.

	Light levels			
Date	0%	50%	92%	L.S.D.
5-23-68	2215	995	0	129.5
5-29-69	375	460	33	418.4

Table 2. The influence of 3 levels of light on the increase in stem diameter in inches of *Ilex opaca* cv. Miss Helen.

	Light Levels			
Date	0%	50%	92%	L.S.D.[1]
11-4-68	0.20	0.24	0.10	0.14
6-6-69	0.24	0.28	0.19	N.S.

[1]Significance at the 0.05% level.

The seasonal cycles of total carbohydrate content in plants is particularly well defined for deciduous trees of the temperate zones. Generally, total carbohydrate content is observed to reach a maximum in the autumn. Total carbohydrates are also observed to decrease in late winter and early spring, indicating a redistribution of food reserves for growth and development of newly developing tissues (9, 14).

In citrus, Jones and Steinacker (10) observed that leaf carbohydrates were at their lowest levels during the summer months, while sucrose and total hexose content were at their maximum during the winter months. Sharples and Burkhart (19) recognized that at the initiation of spring growth and flowering the reserve carbohydrates were exhausted. Leaves were observed to exhibit large amounts of soluble carbohydrate material during the winter, which decreased rapidly as bud-expansion approached in the spring. Our findings on the fluctuation in the soluble carbohydrate levels in the leaves of holly are in agreement with those cited by the above investigators.

Kliewer (11) reported that the major sugars present in grape leaves were sucrose, glucose, and fructose. Galactose was observed in fruit, but never in leaf tissue, however, with holly, D-galactose was readily found in leaf tissue.

From the data of Kramer and Clark (12), Kramer and Decker (13), and others (15, 17, 21) we suspect that the shade plants did not flower because the light intensity greatly reduced the photosynthetic rate. This supposition depends directly on the fact that photosynthetic rate determined the amount of carbohydrates present in the plant tissue and that the carbohydrate level influences flowering at some stage in development.

No significant differences were regularly observed between the levels of soluble sugars in regard to the light regimes imposed, so it can be deduced that the flowering responses observed, particularly under the 92% shade, were related to factors other than the level of soluble sugar and mineral composition.

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Fertilizer and Lime Rates Influence Highbush Blueberry Growth and Foliar Elemental Content During Establishment[1]

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Abstract. Nitrogen and P fertilization increased growth of 1 and 2 year-old highbush blueberry plants (*Vaccinium corymbosum* L., cv Wolcott) at 2 locations in eastern North Carolina. The influence of K upon growth during the years of establishment was not conclusive. Dolomitic lime application prior to establishment raised soil pH from 3.7 and 3.9 to 4.5 and depressed growth.

The influence of treatments upon the foliar contents of N, P, K, Ca, Mg, Fe, Cu, Mn, and Zn was determined. A direct relationship existed between the rate of N, P, or K and the content of these elements in the foliage. The only effects noted from lime application were an increase in P and Ca and decrease in Fe foliar level at one location. Variations in foliar elemental content, induced by treatments, were usually similar at both locations. The level of certain elements in the foliage, however, varied widely with location.

Several studies have been reported concerning the influence of nutrition upon blueberry yield under field conditions. Results of greenhouse nutritional studies with blueberries have also been reported. Recent reviews by Cain and Eck (6) and Ballinger (3) have summarized such data. Most of the data, however, were obtained from studies with controlled greenhouse conditions, or from field plantings, where plant response from the application of specific fertilizer ratios or nutrients was ascertained. In the field Beckwith (4) and Johnston (9) found N alone was not sufficient to significantly increase yield. Beckwith (5) also obtained good responses from the application of 2 different complete fertilizers. The apparent responses from the application of a complete fertilizer of various grades or amounts without systematic comparisons of rates of N, P, and K is of limited value. It does not assure that the optimum level of any element was supplied or that all elements supplied were even needed. Data relative to the influence of variations of N, P, K and lime upon plant growth during the years of establishment and ultimate production are absent. Our study was undertaken to determine the influence of 3 rates of N, P, and K, with and without lime, upon the growth and yield of blueberry bushes from the time of planting through productive years of the plantings. We report here the experimental results during the years of establishment on newly cleared land.

Materials and Methods

Two experiments were initiated in the spring of 1965 on newly cleared and drained land, one in Duplin County and the other in Pender County, North Carolina. Soils at both locations were primarily Leon fine sand (sandy siliceous aeric Haploquod) with the more poorly drained portions of the fields gradating to Lynn Haven find sand (sandy, siliceous typic Haploquod). The soil test results of samples taken prior to treatment application and establishment of the planting for both locations are presented in Table 1.

Table 1. Chemical analysis[a] of soils used in blueberry field experiments.

	Ca	K	Mg	CEC	OM	pH	P
	meq/100 gm soil				%		ppm
Duplin County	.98	.065	.39	15.4	8.2	3.7	3.0
Pender County	.72	.053	.36	11.9	7.1	3.9	2.2

[a]Analysis performed by Soil Test Division, North Carolina Department of Agriculture.

Treatments consisted of 3 levels of N, P, and K from NH_4NO_3, concentrated superphosphate, and KCl respectively, each with or without lime, in factorial combination with 2 replications. A basal rate of fertilization consisting of N-75, P-22, and K-42 lbs/acre was selected to approximate the amount normally used by growers in North Carolina (N-75, P_2O_5-50, and K_2O-50 lbs/acre). Treatments for each element included the basal rate and one higher and one lower rate. The high rate was double the basal rate, the low was 30 lbs. of N/acre and zero for P and K. Only 1/4 of the above rates were applied the first year in split applications and the amount was increased an equal increment each year until the fourth year when the full amount was applied. Sufficient dolomitic lime was applied in the lime treatment and mixed into the soil prior to establishment to raise soil pH from the original 3.7 in Duplin County and 3.9 in Pender County to pH 4.5 at both locations. All cultural practices, except fertilization, were done by the cooperating grower in accordance with the practices normally employed by the grower. Experimental plots consisted of 9 bushes spaced 4 feet apart in the row with 10 feet between rows. Plots were arranged in blocks of 18 as described by Li (10) with 2 replications and separated by a guard bush within the row. One year old 'Wolcott' rooted cuttings were transplanted in March, 1965, and the first of 2 annual fertilizer applications was made in May.

Measurements of growth were made by weighing the prunings removed each winter. The youngest mature leaves were sampled in August each year and elemental analysis determined by the North Carolina State University service laboratory as follows: N by Kjeldahl, P colormetrically by vanadate molybdate, K by flame spectroscopy, and Ca, Mg, Fe, Zn, Cu,

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