Quantitative Investigations of Leaf Pigments From Their Inception in Buds Through Autumn Coloration to Decomposition in Falling Leaves

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QUANTITATIVE INVESTIGATIONS OF LEAF PIGMENTS FROM THEIR INCEPTION IN BUDS THROUGH AUTUMN COLORATION TO DECOMPOSITION IN FALLING LEAVES

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Abstract. Pigments in hazel (Corylus americana), aspen (Populus tremuloides), and pin oak (Quercus ellipsoidalis) leaves were measured from their inception in buds to development of a summer maximum, and through the autumn coloration period to decomposition in dry falling leaves. Leaves contained generally high but varying concentrations of chlorophyll and carotenoid pigments throughout the summer months. The summer pigment variations among the three species are discussed in the light of the usefulness of chlorophyll content as an index of net primary productivity. During the autumn coloration period, preceding leaf desiccation and fall, chlorophyll decays rapidly, producing low levels of pheophytin with only occasional faint traces of pheophorbide and chlorophyllide during the period of most rapid chlorophyll breakdown. The levels of carotenoids begin declining at the same time as chlorophyll, but at a much slower rate. Violasaxanthin disappears most rapidly, followed closely by neoxanthin. Lutein and β-carotene are the most stable carotenoids. Falling oak leaves, whether dropped to the ground in autumn or held on the tree throughout the winter, in spring still contain measurable amounts of lutein and β-carotene and low concentrations of pheophytin a. Concurrent with the autumn degradation of plastid pigments is an abrupt and substantial rise in anthocyanin of oak and hazel. At leaf-fall aspen and hazel leaves are devoid of all pigments.

INTRODUCTION

Each year vast quantities of plastid pigments are produced in plant bodies during spring and early summer. Throughout summer the intense green of the chlorophyll masks the yellow and orange colors of the carotenoids. In autumn preferential destruction of the chlorophylls allows the more slowly disappearing carotenoids to color the countryside with a variety of orange and yellow hues. In many plants the coloration period is made even more brilliant by the appearance of the bronze and red colors of newly formed anthocyanins. The fate of pigments following autumn coloration and subsequent leaf fall remains essentially uninvestigated.

The subject of autumn pigment degradation has been of particular scientific interest since the work of Willstätter and Stoll (1918), who reported lower chlorophyll values in yellow leaves than in green leaves. They did not, however, follow closely the loss of either chlorophyll or carotenoids. It quickly became evident to early investigators that carotenoids as well as chlorophylls underwent changes during the autumn period. Details of many of the early investigations, with special emphasis on their errors and inconsistencies, are discussed by Goodwin (1958). He notes particularly the work of Tswett (1911), who believed carotenoids to be almost entirely epiphytic in autumn leaves, but with the major fraction being separable from β-carotene. Tswett termed these pigments “autumn xanthophylls.” Also discussed is the work of Kuhn and Brockmann (1932), who claimed autumn pigments were not caroten in nature but “esterified xanthophylls” behaving like carotenones in the partition test. Karrier and Walker (1934) took the view that the autumn pigments were degradation products of lutein and that the carotenones and xanthophylls both disappeared in autumn, carotenes going relatively more quickly than xanthophylls. Schertz (1929) studied the seasonal changes of the chloroplast pigments of several plants on the Mall in Washington, D.C., but made no attempt to separate the individual chlorophylls and carotenoids, and his techniques in general were quite crude. Wolf (1956) traced the changes in chlorophylls a and b in autumn leaves, but he made no attempt to identify carotenoids. Goodwin (1958) took a more careful look at the seasonal changes in plant pigments, using modern techniques to deal with carotenoids, chlorophylls, and anthocyanins. His studies, however, did not include measurement of pigments from their inception in leaf buds, and they were not carried far enough into late autumn and winter to ascertain the eventual fate of the pigments that are destined to become part of the organic fractions of forest soils, peats, or lake sediments.

The present study was undertaken for several reasons. First, the data have an intrinsic interest because no studies are available tracing pigments from their initiation in leaf buds to their decomposition beyond the period of leaf coloration and desiccation in late autumn. Second, several ecological studies have supported the use of chlorophyll content per plant and per unit landscape area as a productivity index (Odum 1959, Brougham 1960, Bray 1960, 1962, Whittaker and Garfine 1962, Ovington and

1 Received September 30, 1969; accepted July 2, 1970.
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Lawrence 1967, Sanger, unpublished data). Data concerning changes in pigment concentrations of selected components of a plant community throughout the growing season are necessary to establish the reliability and comparability of this index. Third, Goodwin's (1958) observations show that no simple generalizations can be made for the fate of carotenoids in autumn leaves because of the great differences among the three trees he examined. Fourth, before any accurate assessment of the significance of pigment levels in soils, peats, and pond muds can be made, it is necessary to know the types and amounts of pigments which are likely to reach the soils from various terrestrial sources. Last, nothing is known of the effect of varying autumn climates on pigment decomposition. The relatively short, dry autumns of Minnesota may produce conditions far different for pigment decay than those observed by Goodwin during the relatively long, moist autumns of England.

The three species chosen for study were pin oak (Quercus ellipsoidalis), American hazel (Corylus americana), and quaking aspen (Populus tremuloides). The first two were selected because they are the chief components of the tree and shrub layers in the oak forest where soil humus layers were studied for pigment composition (Sanger 1971). In addition, both species exhibit development of anthocyanin during autumn coloration. This was considered important because falling leaves are often reddish in color, yet no trace of anthocyanin has been found by the author or reported by others in soils, peats, or pond muds. Aspen was chosen because it is found commonly throughout the area in scattered clumps and along the edges of the oak woodlands. In addition, anthocyanin does not develop in aspen leaves during the autumn, enabling comparisons to be made with pigment degradation in the species that do develop anthocyanin. It is also important that pin oak leaves tend to overwinter on the tree while hazel and aspen leaves do not.

**Methods and Materials**

A series of small branches totaling approximately 100 leaves (buds in spring), of which only a portion was actually used, was picked at random throughout the canopies of designated trees. No particular plants were designated for hazel. Instead, branches were picked at random from the area near the designated oak tree. The leaves (blade + petiole) were harvested from the branches, sealed in airtight polyethylene bags, and taken to the laboratory to be processed immediately or stored for less than 1 day at 2°C. Prior to extraction 10–15 leaves were chosen at random from the initial 100 and their areas calculated. Five to seven leaves were taken at random for drying, and a similar number for extraction of pigments. Dry, weighed leaves were ashed in a muffle furnace at 550°C for 4 hr to enable calculation of pigments per gram organic matter.

Pigments were extracted in a Waring blender with 90% acetone and 1 g MgCO₃. The solution was filtered with successive portions of 90% acetone through a Buchner funnel with a fluted disk of coarse porosity until all pigment was removed, and the final volume was brought up to 100 ml.

A 25-ml portion of the acetone extract was saponified by gentle shaking with an equal volume of 20% methanolic KOH for 2 hr in a flask covered with a black polyethylene bag. Transfer of carotenoids to petroleum ether (30°–60°) was carried out in a separatory funnel in a partially darkened room. The solution of petroleum ether and carotenoid was washed with distilled water, dried with granular, anhydrous Na₂SO₄, and shaken with 90% methanol in a separatory funnel. The hypaphasic component (xanthophyll) was drawn off and its volume brought up to 50 ml. The epaphasic component (β-carotene) was washed with an additional 100 ml of distilled water, dried with Na₂SO₄, and its volume brought up to 50 ml. Measurements of absorbance were made on a Beckman DU spectrophotometer at 445 μm for total xanthophyll and at 451 μm for total carotene.

An additional 25-ml portion of the original acetone extract was diluted to 50 ml in a volumetric flask, and a carefully measured spectrum was recorded with a Beckman DU spectrophotometer from 640 μm to 700 μm to measure total chlorophyll.

The remaining 50 ml of the original acetone extract were transferred to diethyl ether. The solution of pigment and ether was concentrated to approximately 5 ml by rapid evaporation with continued swirling in a fume hood. Care was taken not to allow the pigment to go to dryness. Squares of Whatman No. 1 filter paper, 12 by 12 cm, were spotted in the lower left-hand corner, placed in chromatography jars with ground glass lids, covered by black, light-proof polyethylene film, and chromatographed in the first direction with a mixture of 10 parts hexane (practical grade from Eastman Kodak), 2.5 parts petroleum ether (60°–70°), and 2 parts acetone (reagent grade). The papers were run in a second direction in a similar manner with 10 parts hexane (practical grade), 2.5 parts petroleum ether (60°–70°), 1 part acetone, and 0.25 part methanol (technical grade). A two-dimensional chromatogram of oak-leaf pigments is illustrated in Fig. 1. Identification of the separate spots was made from spectral analysis. The separated pigment spots were cut out of the paper, eluted with suitable solvents, and the eluates made up to constant volumes. Beta-carotene was eluted with petroleum ether; lutein, violaxanthin, and neoxanthin with absolute ethanol; chlorophyll
TABLE 1. Specific absorption coefficients used to calculate pigment concentrations

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Absorption coefficient</th>
<th>Wavelength of maximum absorption (m(\lambda))</th>
<th>Solvent</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll (a) and Chlorophyllide (a)</td>
<td>90.8</td>
<td>665</td>
<td>80% acetone</td>
<td>Vernon (1960)</td>
</tr>
<tr>
<td>Chlorophyll (b)</td>
<td>52.5</td>
<td>648--649</td>
<td>80% acetone</td>
<td>Vernon (1960)</td>
</tr>
<tr>
<td>Pheophytin (a) and Pheophorbide (a)</td>
<td>55.2</td>
<td>667</td>
<td>80% acetone</td>
<td>Vernon (1960)</td>
</tr>
<tr>
<td>Pheophytin (b) and Pheophorbide (b)</td>
<td>34.8</td>
<td>655</td>
<td>80% acetone</td>
<td>Vernon (1960)</td>
</tr>
<tr>
<td>(\beta)-carotene</td>
<td>258.0</td>
<td>451</td>
<td>Petroleum ether</td>
<td>Zscheile (1934)</td>
</tr>
<tr>
<td>Total xanthophyll</td>
<td>255.0</td>
<td>445</td>
<td>90% methanol</td>
<td>Sanger</td>
</tr>
<tr>
<td>Lutein</td>
<td>255.0</td>
<td>445</td>
<td>Abs. ethanol</td>
<td>Strain (1938)</td>
</tr>
<tr>
<td>Lutein tail (assumed same as lutein)</td>
<td>255.0</td>
<td>445</td>
<td>Abs. ethanol</td>
<td>Strain (1938)</td>
</tr>
<tr>
<td>Violaxanthin</td>
<td>255.0</td>
<td>440</td>
<td>Abs. ethanol</td>
<td>Karrer and Jucker (1950)</td>
</tr>
<tr>
<td>Neoxanthin</td>
<td>227.0</td>
<td>438</td>
<td>Abs. ethanol</td>
<td>Strain (1938)</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>70.0</td>
<td>520</td>
<td>1% acidic methanol</td>
<td>Geissman (1955)</td>
</tr>
</tbody>
</table>

*Experiments by Vernon (1960) and by the author reveal no significant differences in the specific absorbivity of pheophytin \(a\) and \(b\), pheophorbide \(a\) and \(b\), and chlorophyllide \(a\) between 80\% and 90\% acetone.

a and \(b\), pheophytin \(a\) and \(b\), pheophorbide \(a\) and \(b\), and chlorophyllide \(a\) with 90\% acetone. (Experiments by the author reveal no significant difference in the specific absorbivity of pheophytin \(a\) and \(b\), pheophorbide \(a\) and \(b\), and chlorophyllide \(a\) between solutions of 80\% and 90\% acetone. Although 90\% acetone was used throughout, some water is always present in the plant tissue so that solutions are actually between 80\% and 90\% acetone.)

Absorption measurements were made with a Beckman DU spectrophotometer with 10-mm absorption cells at the blue maximum for carotenoids and the red maximum for the chlorophylls. Pigment values were calculated as milligrams per 100 g organic matter with the specific absorption coefficients listed in Table 1. Accuracy of the calculations depends upon the accuracy of measurement of a given volume of pigment extract after it is concentrated, applied, chromatographed, and eluted from the paper chromatograms. Because volumes of pigment extract applied to chromatograms are so small, it is very difficult to make these measurements accurately enough for reliable calculation of pigment concentrations. For this reason carotenoids per unit organic matter were first ascertained by the partition technique and chlorophylls by direct measurements of the original acetone extract. Individual pigments were then calculated from the chromatograms (see Sanger 1968).

In general there is close agreement between the pigment concentrations calculated in this study and published values. Pigments were identified by their visible absorption spectra, and by their relative positions on two-dimensional chromatograms employing two or more different solvent systems (Strain 1938, Goodwin 1952, Watson 1953, Holt and Jacobs 1954, Smith and Benitez 1955, Holden 1962, Colman and Vishniac 1964, Michel-Wolwertz and Sironval 1965, Strain et al. 1965, Strain and Svec 1966).

Anthocyanin was extracted and chromatographed according to the technique of Harborne (1965). The anthocyanin was concentrated by evaporation in a rotary vacuum flash-evaporator, and the final volume was carefully measured. To remove the last traces of plastid pigments, circular chromatograms were run on 27-cm Whatman No. 1 filter paper. The chromatogram was spotted with 0.1 ml of anthocyanin solution and developed with 1-butanol-acetic acid-water (4:1:5); the top layer was used as irrigation fluid. The anthocyanin was eluted from the paper with 1% acidic methanol, and measurements of absorption were made with a Beckman DU spectrophotometer. The anthocyanin in the leaves studied had a spectrum very similar to that of cyanidin, and amounts were calculated from its specific absorption coefficient, which is listed in Table 1.

RESULTS AND DISCUSSION

Oak leaves

It is first helpful to consider changes in leaf area and in the dry, ash-free weight per leaf and per unit area of leaf tissue throughout the sampling period. These data are presented for oak in Fig. 2 and 3.
Buds break in late May and leafing takes place quickly. By mid-July leaves reach their average maximum size and weight. The seasonal changes in absolute leaf organic matter parallel the gradual build-up of organic matter per unit leaf area as the mesophyll tissue develops. There is a rise from 4.5 mg/cm² in immature leaves to an average summer value, beginning in early July, of about 7.8 mg/cm². At the initiation of autumn coloration, organic matter values drop somewhat, both per unit area and on an absolute basis, and considerable variation is observed as the leaves are drying and the pigments are decomposing. In late autumn and throughout winter, when leaves are dry and brittle on the tree, the individual values become less erratic and do not decline appreciably by the end of the sampling period in April from the average autumn value. In leaves decaying on trees, pigments are broken down at a rate far greater than that for total organic matter. This is an important comparison to make with leaves decaying on the forest floor (Sanger 1971). Changes in pigment concentrations throughout the season are expressed as milligrams per 100 g organic matter to make the data more comparable with existing data of pigments in soil humus layers and lake sediments.

Changes in the chlorophyll a and b content of oak leaves from spring buds through the autumn coloration period to leaf desiccation and drop the following spring are presented in Fig. 4. In early spring, before the leaf buds open, chlorophylls are low. As soon as buds open, and at the time of rapid increases in leaf area and leaf weight per unit area, there is an abrupt and substantial rise in both chlorophyll a and b. Although total chlorophyll concentrations (Fig. 5) are consistently high throughout the summer period, considerable fluctuation is apparent, as was noted earlier by Schertz (1929) and Goodwin (1958).

For buds in early spring the ratio of chlorophyll a to chlorophyll b (Fig. 6) is relatively low. After leaf-blade expansion begins, the ratio increases rapidly to a high level, which persists throughout the entire summer. The high summer levels (approximately 2.6) correspond to the average values for green tissue of higher land plants given by Smith and Benitez (1955).
At the onset of autumn coloration in October total chlorophyll concentrations (Fig. 5) drop rapidly while leaf weight per unit area drops only slightly (Fig. 2). The ultimate fate of the chlorophylls destroyed in drying autumn leaves is as yet essentially unknown. Because Seybold (1943) could not demonstrate products of high molecular weight in autumn leaves he concluded that extensive cleavage of the chlorophyll molecule into colorless fragments must occur. During the period of most rapid chlorophyll decline, traces of pheophorbide $a$ and chlorophyllide $a$ can occasionally be detected, but one cannot be certain that these are not artifacts of the extraction or separation procedure. Pheophytin $a$, however, can be detected in small but measurable quantities during rapid chlorophyll degradation, as well as throughout the entire winter in dry leaves on the tree and in leaves overwintering on the forest floor (Fig. 4 and 5). It appears that a small fraction of chlorophyll $a$ gets converted to pheophytin $a$ and then is stable enough to be present for long periods in dry leaves and in upland soil organic matter layers (Gorham 1959, Gorham and Sanger 1964, 1967). Only faint traces of pheophytin $b$ are encountered during the period of most rapid chlorophyll degradation.

Willstätter and Stoll (1913) demonstrated that colloidal solutions of chlorophyll in water could be decomposed by CO$_2$ and that chlorophyll $a$ was more rapidly converted to pheophytin $a$ than chlorophyll $b$ to pheophytin $b$. This apparent greater ease in conversion of chlorophyll $a$ to pheophytin $a$ is suggested here in oak leaves, because only pheophytin $a$ is found in measurable amounts in dry leaves on trees and in forest organic matter layers. Possibly the slowness of reaction of chlorophyll $b$ to pheophytin $b$ allows all the chlorophyll $b$ to be destroyed before pheophytinization is able to preserve any of it. However, because the preserved levels of pheophytin $a$ are so very low in dried leaves compared to the original chlorophyll concentration, it is also possible that levels of pheophytin $b$, even if preserved in equal proportion to pheophytin $a$, would be too low for detection of more than trace amounts by the methods employed.

Studies involving the extraction and measurement of chlorophyll under a number of experimental situations have revealed the presence of isomers of chlorophyll $a$ and $b$ ($a'$ and $b'$) (Strain and Manning 1942, Michel-Wolwertz and Sironval 1965, Bacon and Holden 1967). It is possible that chlorophyll isomers appear during the most rapid breakdown of pigment. Their chromatographic and spectral properties, however, differ very slightly from those of the parent compounds, and the techniques employed in this study may not have been sufficiently sensitive to detect such subtle differences. Strain and Manning (1942) indicated that chlorophyll $a'$ is more blue-green than the parent chlorophyll $a$, and $b'$ more yellow-green than chlorophyll $b$. At no time was there any indication of a dual nature of the chlorophyll spot, nor was any color change apparent in the chlorophyll $a$ and $b$ spots during any of the separations.

When autumn coloration begins, the ratio of chlorophyll $a$ to chlorophyll $b$ again becomes low (Fig. 6). Studies of Willstätter and Stoll (1918) revealed no change in the ratio of chlorophyll $a$ to chlorophyll $b$ in autumn leaves. The more rapid breakdown of chlorophyll $a$ as compared to chlorophyll $b$ under a variety of circumstances involving the decay of green plant tissue has since been demonstrated, however (Rudolf 1934, Nagel 1940, Seybold 1943, Egle 1944, Jeffrey and Griffith 1947, Strain 1949, Wolf and Wolf 1955, Wolf 1956, Goodwin 1958), so it appears that the earlier views of Willstätter and Stoll were wrong. Egle (1944), from his studies on silage, concluded that acids arising during fermentation constituted an important factor in chlorophyll destruction, with chlorophyll $a$ less resistant than $b$. Similar observations on drying plant material suggested to him that acids normally present in leaves are very important in the destruction of chlorophyll. Goodwin (1958) noted in autumn leaves, and the present study of oak leaves confirms, that only when the chlorophylls had almost disappeared was there a tendency for chlorophyll $a$ to be destroyed more quickly than chlorophyll $b$. The rate of destruction of chlorophyll $a$ relative to
b may reflect the rapidity of leaf desiccation of different species in autumn. Oak leaves in this study tended to retain moisture much longer than those of aspen and hazel, and as a result the rapid decline of chlorophyll a relative to chlorophyll b was more apparent in oak.

Like the chlorophylls, the major carotenoid components are all present in spring leaf buds, but in relatively small amounts (Fig. 5, 7, and 8). After a sharp rise as buds open, total carotenoid concentrations (Fig. 5) increase gradually to summer maxima at the end of July. Neoxanthin reaches maximum concentration in early summer followed slightly later by violaxanthin (Fig. 7). Beta-carotene and lutein, the two components most stable in autumn, do not reach maximal concentrations until midsummer (Fig. 7 and 8).

The ratio of β-carotene (the only carotene observed in the tree leaves studied) to total xanthophyll reveals a rather sudden increase from 0.38 in buds to 0.49 in early July (Fig. 9). Average values of 0.45 are recorded for summer up until autumn coloration.

Although variations in summer pigment levels are apparent, concentrations remain high until the initiation of autumn coloration, when the level of carotenoids begins declining at the same time as the chlorophylls. As indicated previously, the generally accepted theory has been that the chlorophylls disappear first, unmasking the carotenoids, which disappear only gradually in late autumn. The curves for total chlorophyll and total carotenoid (Fig. 5) show, however, that carotenoids, although retained a few days longer in oak leaves than are chlorophylls, decline at the same rate. The major difference is in the ultimate amount of the decline, with very small concentrations of chlorophyll being preserved as pheophytin a throughout late autumn and winter compared to much higher concentrations of the carotenoids lutein and β-carotene during the same period.

Violaxanthin and neoxanthin are the least stable of the carotenoids (Fig. 7), with concentrations dropping in autumn at about the same time as the chlorophylls decline (see Fig. 4). Violaxanthin is the least stable, disappearing 1–2 weeks before neoxanthin. In Goodwin’s study the level of violaxanthin remained in oak and plum leaves with comparative constancy until the end of his sampling period in late November. Thus broad generalizations cannot be drawn for the fate of xanthophylls in autumn leaves since differences between species and between climates may have considerable influence on pigment decomposition.

During the period of sharp decline in total carotenoid, an additional pigment spot was noted tailing closely behind lutein, although distinctly separate from it (Fig. 7). The pigment could be detected in relatively small amounts for about 2 months during autumn, but disappeared shortly after neoxanthin. Positive identification was not made because of its spectral peculiarities and inconsistencies. Further study was limited by the very low concentrations of
this pigment. The most plausible explanation is that the lutein tail was a mixture of esters of violaxanthin and lutein. The pigments within the spot were epiphatic initially, but on saponification became for the most part hypophatic. These pigments are most likely those noted by Willstätter in 1918, the autumn carotenones of Tsweet (1911), and autumn xanthophylls of Kuhn and Brockmann (1932) who, according to Goodwin (1958), correctly identified them as xanthophyll esters. No pigments with spectra resembling lutein-5:6-epoxide, which Goodwin detected in small quantities in autumn leaves of oak, plum, and sycamore, could be detected in any leaves from this study with techniques similar to those of Goodwin.

Beta-carotene and lutein are the most stable carotenoids of those examined in this study (Fig. 7 and 8). This was to be expected, because these are the two components found commonly in the organic layers of woodland soils (Sanger 1971). Both pigments decline rapidly during the autumn coloration period, but once oak leaves dry on the tree the rate of pigment loss becomes considerably less. Concentrations of β-carotene drop to much lower levels than lutein in autumn, but nevertheless β-carotene remains preserved in measurable amounts throughout the entire winter in leaves dry on the tree, declining gradually to quite low levels by spring. Slightly higher concentrations of β-carotene are noted in the spring for leaves that overwintered on the soil surface beneath the winter snows, which that year accumulated 3–4 ft. Lutein, the dominant carotenoid preserved in dried leaves, declines slowly throughout the winter in dry leaves on the trees, but remains on the average about 7 times as abundant as β-carotene. Like β-carotene, lutein concentrations are much greater in leaves overwintering on the soil surface. The important point to note is that when oak leaves drop from the trees in the desiccated condition, whether in spring or in autumn, they contain small but measurable amounts of lutein, β-carotene, and pheophytin a. Leaves dropping in autumn and becoming incorporated in the forest litter, frozen and covered with snow, contain pigment levels in spring only slightly lower than dry leaves on trees in late autumn. This is probably a reflection of protection from light and temperature extremes throughout the winter. The higher pigment levels of oak leaves falling in autumn undoubtedly makes them greater contributors to pigments in terrestrial soils or aquatic sediments than leaves falling in spring.

The ratio of β-carotene to xanthophyll (Fig. 9) begins falling at the initiation of autumn coloration from the average summer value of 0.45 to a late-autumn value of about 0.15. It remains about the same throughout the winter in leaves on trees and leaves on the forest floor. The autumn change dem-

![Graphs](https://example.com/graphs.png)

Fig. 10. Temporal changes in the percentage of total carotenoid components of oak leaves.
main fairly constant from spring buds until the initiation of the autumn coloration period, when violaxanthin and neoxanthin disappear, leaving only β-carotene and lutein. The presence of the pigments in the lutein tail can be noted during the peak of autumn coloration, at which time they may make up as much as 13% of the total carotenoid. Eventually in late autumn lutein and β-carotene are the only remaining carotenoids, with β-carotene averaging about 15% and lutein the remaining 85% throughout much of the winter and spring.

At the initiation of autumn coloration, as soon as chlorophyll begins to decompose, anthocyanin forms (Fig. 11). At the time of maximum anthocyanin concentration, levels of chlorophyll have dropped to 20% of the average summer value. The concentration of anthocyanin rises very abruptly to a maximum and thereafter throughout the winter declines at a slow rate. Only trace amounts remain in April when the leaves fall from the trees. Unlike lutein and β-carotene, anthocyanin is not present to any great extent in the spring in either dry leaves on the tree or in dry leaves overwintering on the forest floor. Leaves on the trees remain relatively high in anthocyanin during much of the winter, probably because they are kept continuously cold and dry. It is likely that in spring, when temperatures increase and when periods of wetting and drying become frequent, the water-soluble anthocyanins are even more vulnerable to destruction than the plastid pigments, especially more so than lutein. The lack of anthocyanin in dry leaves in spring is not entirely unexpected because, as previously indicated, no trace of the pigment has been found in soils, lake muds, or peats.

**Hazel leaves**

Changes in leaf area and in the dry, ash-free weight per leaf and per unit area of hazel leaf tissue throughout the sampling period are presented in Fig. 12 and 13. Leaf weight per unit area remains essentially unchanged from young leaves through leaf desiccation and fall in autumn despite the fact that leaf area and organic matter per leaf do not reach high levels until mid-July. This is contrary to the case in oak leaves, where leaf weight per unit area increased from low levels in buds to high summer levels by mid-July, paralleling the seasonal changes in absolute leaf organic matter and leaf area.

Concentrations of total chlorophylls and carotenoids increase gradually, not reaching summer maxima until the period from mid-June to mid-July (Fig. 14). As in oak leaves, all the plastid pigments inves-
tigated were present in leaf buds, but in relatively small amounts. Total pigments do not remain at consistently high concentrations throughout most of the summer as in oak, but are high for only a short time during midsummer. Chlorophylls $a$ and $b$ rise slowly, reaching highest summer concentrations in mid-June (Fig. 15), about 1 month before maximum leaf area is attained (Fig. 12). Concentrations then begin to fall at a nearly uniform rate until the total disappearance of all pigments in dry autumn leaves.

Violaxanthin, unlike the other carotenoids, rises abruptly in late May, and for a short time exceeds the concentration of lutein (Fig. 16). It then begins a continual slow decline which is essentially unbroken until total decomposition in mid-October. Beta-carotene, lutein, and neoxanthin display rates of increase and decrease similar to the chlorophylls and reach maximum summer concentrations in mid-July (Fig. 16 and 17).

Instead of dropping sharply during autumn coloration, pigment concentrations begin to decline gradually in August. The chlorophylls and carotenoids decline at essentially equal rates, reaching zero by the end of October (Fig. 14). In hazel leaves, lutein is preserved only slightly longer than violaxanthin and neoxanthin, which are lost about 10 days before the last traces of lutein disappear (Fig. 16). Only faint traces of pheophytin $a$ are detectable during the period of most rapid chlorophyll decline, and no pigments are found after the leaves dry. Hazel leaves become much more thin and brittle when dry than do oak leaves, and all leaves fall from the bushes in autumn free from pigment.

A comparison of Fig. 14 with Fig. 5 reveals that hazel leaves are about twice as rich in both carotenoids and chlorophylls as oak leaves. This may be an adaptation for photosynthesis under shade con-
Fig. 20. Temporal changes in the percentage of total carotenoid components of hazel leaves.

ditions, since hazel is almost always in shady situations and locally is the dominant component of the shrub synusia of the oak forest. For comparable periods, however, the ratio of \( \beta \)-carotene to xanthophyll (Fig. 18) and the summer ratio of chlorophyll \( a \) to chlorophyll \( b \) (Fig. 19) are much the same in the two species. In autumn the ratio of chlorophyll \( a \) to chlorophyll \( b \) does not show evidence of a decline as in oak, so that chlorophyll \( b \) in hazel is not preserved relative to chlorophyll \( a \). This may be a consequence of the gradual autumn decline of chlorophyll, as opposed to the very rapid decline in oak.

The carotenoid components of hazel leaves expressed as percentages of total carotenoid are shown in Fig. 20. The summer values are almost identical with the values for oak, with lutein representing about 35\% of the total summer carotenoid, \( \beta \)-carotene from 25\% to 35\%, violaxanthin from about 15\% to 20\%, and neoxanthin from 10\% to nearly 20\%. Percentages of violaxanthin in hazel are somewhat higher at the beginning of the season, reflecting the rapid development of this pigment in hazel leaves (Fig. 16).

No traces of "esterified xanthophylls" or other autumn pigments could be found in hazel. The "lutein tail" noted for oak could not be detected, and no evidence was found for the presence of lutein-5:6-epoxide as noted by Goodwin (1958). Its absence may be associated with the more gradual decline of the hazel pigments in autumn, with their subsequent complete disappearance when the leaves dry and turn brown.

As in the case of oak, hazel leaves do develop anthocyanin pigment in relatively large quantities for a short period in autumn (Fig. 21). Anthocyanin reaches maximum concentration after chlorophylls and carotenoids decline to less than 40\% of their summer maxima. Anthocyanin disappears about the same time as lutein, a few days later than the other plastid pigments. Its spectrum is identical to that recorded for the anthocyanin of oak, which in turn is very similar to that of cyanidin.

**Aspen leaves**

The time required for development of maximum aspen leaf area, and the values of organic matter per leaf and per unit leaf area, are presented in Fig. 22 and 23. Both leaf area and leaf weight increase rapidly after buds break in mid-May to relatively high levels by late May. As in hazel, leaf weight per unit
area remains consistently high throughout the entire growth period, and it drops only slightly during autumn desiccation. Oak and aspen are similar in leaf weight per unit area, with average summer values of about 7.0 mg/cm², whereas hazel shows much lower values (near 3.0 mg/cm²).

Although the sampling intervals are less frequent for aspen, the pigment concentrations and curves show similarities to both oak and hazel. All pigments investigated were present in low concentrations in leaf buds (Fig. 24). Total chlorophylls and carotenoids rise very sharply after the buds open, reaching high concentrations in late May when leaves have nearly attained their maximum area. Chlorophyll a and b remain consistently high throughout the summer (Fig. 25). The carotenoids reach maximal concentrations in mid- to late May, and total carotenoid shows a slight decline thereafter through the summer. Violaxanthin, as in hazel, rises very sharply in spring and exceeds the concentration of lutein during much of the early part of summer (Fig. 26). Violaxanthin and neoxanthin concentrations decline slowly from late May to early September. Beta-carotene and lutein do not show this tendency clearly (Fig. 26 and 27).

The average summer ratio of chlorophyll a to chlorophyll b is about 1.9 (Fig. 28) compared to values greater than 2.5 for oak and hazel. The general character of the curve is similar to oak. As in oak the ratios are relatively low in buds, rising to a summer level that is maintained more uniformly than in oak.

The ratio of β-carotene to xanthophyll does not resemble closely either that of hazel or oak (Fig. 29). Average summer values are about 0.38 in aspen, compared to values around 0.45 for oak and hazel. Values for aspen are at the highest recorded level in buds and drop substantially when the young leaves
the trees. The sharp drop in the level of lutein takes place about a month later than the sharp drop in chlorophylls \( a \) and \( b \), violaxanthin, and neoxanthin. Although \( \beta \)-carotene begins a slight decline in late September, it is maintained in relatively high concentrations until late October when it falls rapidly along with lutein. Faint traces of pheophytin \( a \) are occasionally detectable during the period of most rapid chlorophyll decline. No traces of the lutein tail found in oak leaves were noted, and no evidence was found for lutein-5:6-epoxide. The absence of autumn xanthophylls may result, as in hazel, from anatomical or chemical leaf conditions less favorable to pigment preservation than those of oak.

The ratio of chlorophyll \( a \) to chlorophyll \( b \) falls from the summer values during late autumn when chlorophyll concentrations drop to low levels (Fig. 28). In late autumn there is a slightly greater preservation of chlorophyll \( b \) than of chlorophyll \( a \), a tendency not noted at all in hazel but very evident in oak.

The ratio of \( \beta \)-carotene to xanthophyll does not decline in autumn (Fig. 29) as in hazel and oak. This may be because of the late, sudden and extreme drop of both \( \beta \)-carotene and lutein.

The carotenoid pigment components disappear from aspen leaves at roughly the same time in late autumn as they do in hazel (Fig. 30). Summer values of about 30–35% for lutein, 25% for \( \beta \)-carotene, 25–35% for violaxanthin, and 10–12% for neoxanthin are similar to those for both oak and hazel.

**Chlorophyll content and site productivity**

In recent years several studies have suggested determination of chlorophyll load per unit of landscape area as an index to net primary productivity of terrestrial communities (Odum 1959, Bray 1960, 1962, Brougham 1960, Whittaker and Garfine 1962, Ovington and Lawrence 1967, Sanger, unpublished data). The present studies of pigment levels throughout the season have demonstrated two phenomena which may influence correlations between productivity and chlorophyll load. First, concentrations of chlorophyll per unit leaf area exhibit distinct fluctuations about an average high summer value. This is especially true for oak. Second, not all plants carry maximum pigment loads throughout the entire summer per unit organic matter and per unit leaf area (see also Ovington and Lawrence 1967). This is also true if pigments are expressed on an absolute basis (per leaf). Because leaf number remains essentially unchanged throughout the canopy during summer, any change in absolute pigment concentration involves a pigment change per unit area of landscape. In hazel leaves, increases in absolute total chlorophyll and carotenoid (Fig. 31) parallel the relatively slow increases in absolute values of organic matter.
leaves from before the breaking of buds in spring until leaf desiccation and fall. By late autumn hazel and aspen leaves dry and drop to the ground essentially devoid of pigment. Pin-oak leaves, of which a large percentage remain attached to the tree throughout the entire winter, retain much of their lutein (25%), a moderate percentage of their β-carotene (5%), and a small amount of chlorophyll a in the form of pheophytin a (0.1%) in leaves remaining on the trees well past the period of major autumn coloration. The same three pigments are still present in small amounts when these leaves fall in spring. Such findings indicate that pigments in the allochthonous organic matter reaching the sediments of lakes and ponds may be derived from a restricted number of plants among the upland flora. This may be an important consideration for interpreting sedimentary pigments in lakes which derive much of their organic matter from the surrounding environment, especially from falling leaves in autumn or from material that has weathered on the soil surface.

In most situations chlorophyll a is more rapidly decomposed than chlorophyll b. The ratio of chlorophyll a to b is relatively low in buds and very young leaves, remains high throughout the summer months, and drops to low values in autumn as the chlorophylls decompose. In hazel leaves, however, where the autumn decomposition of chlorophyll is relatively gradual and carotenoids and chlorophylls disappear simultaneously, there is no evidence for relatively greater autumn stability of chlorophyll b.

During the summer both carotenoids and chlorophylls differ among the three tree species in maximal concentrations as well as in the timing and duration of maximum levels per unit organic matter and per unit area. Such differences may need to be considered in the use of pigment load per unit of landscape area as a productivity index. Aspen and oak are nearly equal in pigment concentration, but hazel, growing entirely in a shaded environment, has about twice the pigment concentration of oak and

CONCLUSIONS

Comparisons have been made of the changes in pigment concentrations of oak, hazel, and aspen

Fig. 31. Temporal changes in total chlorophyll and carotenoid per leaf of hazel.

Fig. 32. Temporal changes in total chlorophyll and carotenoid per leaf of oak.

(Fig. 13) throughout spring and early summer to highest levels in mid-July. Thereafter organic matter remains relatively constant while pigments decline steadily to zero in late autumn. In oak total pigments rise steadily from low levels in mid-May to high levels in July (Fig. 32), as does organic matter (Fig. 3). Pigment concentrations then rise more slowly until autumn, declining rapidly in late September, when autumn coloration begins. Absolute values for pigments and organic matter of aspen leaves show a very rapid initial rise to high levels in late May (Fig. 33 and 23). Thereafter aspen resembles oak with a more gradual rise throughout the summer to highest concentrations just before the initiation of the autumn decline. These data suggest that chlorophyll content per unit area of landscape is likely to vary considerably, depending upon the sampling time. Therefore, before any truly meaningful studies of productivity in relation to chlorophyll content are made, the investigator should be aware of the time of year and the duration and magnitude of fluctuation of maximum chlorophyll load for the major components of the plant community. For some communities the period during which reliable maxima can be estimated may be relatively short, and this period may well differ in different communities.

CONCLUSIONS

Comparisons have been made of the changes in pigment concentrations of oak, hazel, and aspen
aspen per unit organic matter. Duration of maximum pigment level is shortest for hazel.

Although differences in absolute summer values are apparent, carotenoid components expressed as a percentage of total carotenoid are remarkably consistent in the three species. In autumn the carotenoids violaxanthin and neoxanthin are more susceptible to decomposition than \( \beta \)-carotene and especially lutein. This is most easily demonstrated in oak leaves, where apparently anatomical or chemical conditions in the leaves favor pigment preservation to a greater degree than in hazel and aspen. The changes in ratio of \( \beta \)-carotene to xanthophyll show little similarity among the species investigated. Curves for hazel and aspen leaves from spring to autumn are nearly opposite: hazel leaves show ratios high in summer and low in spring and autumn, whereas aspen leaves are low in summer and higher in spring and autumn. Lutein and \( \beta \)-carotene remain throughout the winter in dry oak leaves on the trees and in oak leaves lying on the forest floor. (This indicates the ratio \( \beta \)-carotene to xanthophyll in winter is in fact expressed as the ratio \( \beta \)-carotene to lutein with the average value of the latter remaining essentially unchanged throughout the winter.)

An “autumn xanthophyll” (the lutein tail in Fig. 7 and 10) appeared temporarily in relatively small quantities in oak leaves but was not detected during the autumn coloration of hazel or aspen leaves. This was not the lutein-5:6-epoxide detected by Goodwin (1958), which was not found during the autumn period in any of the three species examined.

Anthocyanin appears in both oak and hazel leaves during autumn. In oak, concentrations rise sharply in early October, decline slowly throughout the winter in leaves dry on the tree, and drop to only trace amounts when the leaves fall from the trees in early spring. No anthocyanin is present in the spring in leaves dropping in late autumn and overwintering on the forest floor. In hazel, anthocyanin begins forming in early September and reaches maximum concentration in early October. It declines to zero when leaves dry and fall to the ground in late October.

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