Relationships between carotenoid composition and growth habit in British plant species

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ABSTRACT

The pigment composition of leaves from a number of different plant species collected from field sites in the region of Sheffield, UK, have been compared using high-performance liquid chromatography. Expression of pigment content per unit leaf area was dominated by variation in the total leaf chlorophyll. Neither chlorophyll per unit area nor the chlorophyll a/b ratio were found to be correlated with the habitat from which the plants originated. When the amounts of different carotenoids were expressed relative to the total carotenoid pool, it was found that whilst neither total carotene (α- + β-carotene) nor neoxanthin correlated with ability to grow in shade, the leaf content of both lutein and the total xanthophyll cycle carotenoids (zeaxanthin, antheroxanthin and violaxanthin) did, with lutein content being high in shade species and xanthophyll cycle intermediates low. There was a strong negative correlation between the relative amounts of each of these groups of carotenoids. The ratio of lutein to xanthophyll cycle carotenoids was strongly correlated to an index of shade tolerance.

Key-words: carotenoids, chlorophyll, lutein, xanthophyll cycle, shade tolerance.

Abbreviations: LHCII, light harvesting complex of photosystem II as defined by Peter and Thornber (1991); PSI, photosystem I; PSII, photosystem II.

INTRODUCTION

Carotenoids function in the photosynthetic tissues of higher plants in two major ways (Cogdell 1988; Young 1992). They act as accessory pigments, harvesting light for photosynthesis, and as photoprotective agents limiting the damaging effects of high irradiance. The absorption spectra of carotenoids are distinct from those of chlorophylls, enabling plants to harvest light over a wider wavelength range. The ability of carotenoids to interact with and quench both triplet excited chlorophyll and the highly oxidizing singlet oxygen enable them to limit the occurrence of these species and so prevent damage to the photosynthetic apparatus. Singlet oxygen is formed in the leaf following bi-molecular collision reactions between molecular O2 and the relatively long-lived triplet chlorophyll. Carotenoids primarily act by quenching triplet formation and so preventing the formation of singlet oxygen.

Recently, a large amount of work has focused on a possible additional role for one carotenoid, zeaxanthin, in another mechanism of photoprotection; the non-photochemical quenching of chlorophyll fluorescence (Demmig-Adams 1990). This model has been discussed in an accompanying paper (Johnson et al. 1993). Briefly, it is proposed that zeaxanthin is involved in the de-excitation of singlet excited chlorophyll, thereby reducing both the rate of damage to the PSII reaction centres and decreasing the rate of formation of triplet chlorophyll.

Carotenoids are generally divided into two classes: those that contain oxygen (xanthophylls); and those that do not (carotenes). The carotenoids of leaves are highly conserved, forming major components of the photosynthetic apparatus. They include β-carotene, α-carotene, lutein, neoxanthin and the xanthophyll cycle carotenoids, violaxanthin, antheroxanthin and zeaxanthin. β-carotene and α-carotene are found in association with the photosynthetic reaction centre core whilst the xanthophylls, which make up the bulk of total leaf carotenoids, are found in the light-harvesting complexes.

Although different plant species and plants at different developmental stages are known to vary quantitatively, and to a lesser extent qualitatively, in their pigment composition, little is known about the physiological significance of such variation. In particular, very few studies have been conducted that relate the pigment composition of plants to their habitat. Two studies that have recently investigated the relationship between carotenoid composition and sun-shade tolerance are

In the paper accompanying this, we have introduced an index that quantitates the shade tolerance of different plant species (Johnson et al. 1993). In the present paper, we use this same parameter to relate the pigment composition of 19 species to their ability to grow in or out of woodland.

**MATERIALS AND METHODS**

Plants of 19 species were collected from field sites in the region of Sheffield, UK, and dark adapted overnight, as described in Johnson et al. (1993). Leaf discs (approximately 4 cm²) from each of these species were either illuminated at 2000 μmol m⁻² s⁻¹ for 30 min in a laboratory-built attached-leaf chamber or were left darkened prior to rapid freezing in liquid nitrogen. Samples were then stored at −70°C until subsequent extraction of pigments in redistilled ethanol. Analysis of pigments was performed using reversed-phase high performance liquid chromatography (Barry et al. 1990). The total pigment extract was dissolved in a known volume of ethyl acetate/acetonitrile/water (10:9:1) and injected onto a 5 μm Spherisorb ODS 2 column (250 x 4.6 mm). A solvent gradient of 0–100% ethyl acetate in acetonitrile/water (9:1) over 25 min at 1.0 cm³ min⁻¹ was used to elute the carotenoids and chlorophylls. This system resulted in the clean separation of lutein and zeaxanthin (Fig. 1), α- and β-carotene could not always be effectively resolved on this gradient and so in all data these pigments were not distinguished. Pigments were detected using a Hewlett-Packard 1040 A diode-array detector, monitoring at 447 nm. Compounds were integrated at their individual λmax. All data shown represent the mean of four to six replicates, unless indicated otherwise. For all data where there was found to be no light-dependent change in pigment concentration (all pigments except the xanthophyll cycle carotenoids), results from both light-treated and dark-adapted samples are averaged. Where error bars are shown these represent the standard error of the mean. Lines that have been fitted to data represent, in all cases, first order regression lines fitted to the means of data. The pigment content of the species measured are listed in Table 1.

Where estimates have been made of chlorophyll and carotenoid per unit area, these data are based on spectrophotometric measurements of 80% acetone extracted leaf samples, using the method described by Lichtenthaler & Welburn (1983). Absorbances were measured using a Cecil spectrophotometer.

Each plant species was assigned an index of shade tolerance, which was calculated according to the tendency for the plant to grow in woodland (Grime et al. 1988). This index has a range of zero (a full sun plant) to infinity (an obligate shade species). A full description is given in the preceding paper (Johnson et al. 1993).

**RESULTS**

Figure 2 shows the relationship between the content of total carotenoid and total chlorophyll, each expressed on a unit area basis for the plant species measured. These parameters were found to correlate very strongly. Such an observation is not surprising, since both will depend upon the total amount of pigment-protein complexes in the leaf. When the content of individual carotenoids were expressed in a similar way strong correlations were seen in all cases (data not shown). If expressed on the basis of unit area, any variation in the relative amounts of different pigments will be masked by changes in the total leaf pigment content. It is for this reason that in most cases data have not been expressed on a unit area basis.
Table 1. Pigment composition of leaves from different plant species. Data for individual carotenoids are expressed as a percentage of total carotenoids. Data for C. album are shown for two successive years.

<table>
<thead>
<tr>
<th>Species</th>
<th>Chl:Car</th>
<th>Chl a:b</th>
<th>Neoxanthin</th>
<th>Violaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>A. elatius</td>
<td>3.93</td>
<td>0.072</td>
<td>2.87</td>
<td>0.068</td>
</tr>
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<td>A. sylvetris</td>
<td>3.40</td>
<td>0.120</td>
<td>2.17</td>
<td>0.050</td>
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<tr>
<td>C. album</td>
<td>2.76</td>
<td>0.087</td>
<td>3.11</td>
<td>0.096</td>
</tr>
<tr>
<td>C. album</td>
<td>2.49</td>
<td>0.174</td>
<td>2.99</td>
<td>0.158</td>
</tr>
<tr>
<td>D. glomerata</td>
<td>3.58</td>
<td>0.204</td>
<td>2.76</td>
<td>0.093</td>
</tr>
<tr>
<td>D. purpurea</td>
<td>4.28</td>
<td>0.298</td>
<td>2.61</td>
<td>0.115</td>
</tr>
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<td>F. gigantea</td>
<td>3.94</td>
<td>0.070</td>
<td>2.81</td>
<td>0.079</td>
</tr>
<tr>
<td>F. vesca</td>
<td>3.74</td>
<td>0.100</td>
<td>2.63</td>
<td>0.090</td>
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<tr>
<td>H. helix</td>
<td>5.30</td>
<td>0.343</td>
<td>2.12</td>
<td>0.065</td>
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<td>H. mollis</td>
<td>3.86</td>
<td>0.085</td>
<td>2.75</td>
<td>0.040</td>
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<td>H. pilosella</td>
<td>4.34</td>
<td>0.342</td>
<td>2.73</td>
<td>0.041</td>
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<td>L. communis</td>
<td>3.65</td>
<td>0.104</td>
<td>2.44</td>
<td>0.263</td>
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<tr>
<td>M. perennis</td>
<td>4.76</td>
<td>0.119</td>
<td>2.81</td>
<td>0.040</td>
</tr>
<tr>
<td>P. trivialis</td>
<td>3.61</td>
<td>0.168</td>
<td>3.26</td>
<td>0.318</td>
</tr>
<tr>
<td>R. fruticosa</td>
<td>3.42</td>
<td>0.106</td>
<td>2.13</td>
<td>0.109</td>
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<td>S. holostea</td>
<td>3.93</td>
<td>0.163</td>
<td>2.83</td>
<td>0.259</td>
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<tr>
<td>S. sylvaticus</td>
<td>3.92</td>
<td>0.248</td>
<td>2.89</td>
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<tr>
<td>T. repens</td>
<td>4.39</td>
<td>0.093</td>
<td>2.35</td>
<td>0.046</td>
</tr>
<tr>
<td>U. dioica</td>
<td>5.00</td>
<td>0.213</td>
<td>3.29</td>
<td>0.119</td>
</tr>
</tbody>
</table>

Figure 3 shows the chlorophyll content per unit area (Fig. 3a) and the chlorophyll a/b ratio (Fig. 3b) of different species, expressed in relation to their shade tolerance. There was no obvious correlation between chlorophyll content and light habitat. The amount of chlorophyll per unit area varies considerably both between species and between plants of individual species grown under different conditions (Boardman 1977). However, this variation has not been shown to correlate with plant habitat. Thayer & Bjorkman (1990) found that the mean chlorophyll content of a group of shade species did not differ significantly from that of a group of sun species. Hence the lack of correlation in Fig. 3a is consistent with previous observations.

It has previously been observed that plants native to shaded habitats have lower chlorophyll a/b ratios than
Figure 3. (a) Chlorophyll content per unit area and (b) chlorophyll $a/b$ ratio of leaves of different species expressed relative to their shade tolerance. Data were determined spectrophotometrically using the method of Lichtenthaler & Welburn (1983).

Figure 4 shows the relative content of four carotenoid groups, expressed in relation to shade tolerance. Carotenoids are divided into total carotene ($\alpha$- and $\beta$-carotene), neoxanthin, lutein and the xanthophyll cycle intermediates (zeaxanthin, antheraxanthin and violaxanthin). All data were expressed as a percentage of total carotenoid, which gives a sensitive indication of the relative levels of different carotenoids. Such differences would be masked if the data were expressed on a chlorophyll basis because of the variation in the chlorophyll/carotenoid ratio. Total carotene and neoxanthin, although varying between species, do not correlate with shade tolerance. However, there was a significant correlation between shade tolerance and the content of both lutein and the xanthophyll cycle pool. These correlations were complementary; as lutein content rose with increasing shade tolerance so the xanthophyll cycle pool decreased. This inverse relationship is emphasized in Fig. 5a, where lutein content is plotted against total xanthophyll cycle carotenoids. In this plot, there is a highly significant negative correlation between these two parameters.

A negative correlation between the xanthophyll cycle pool and lutein, expressed as a percentage of total carotenoid, might be produced if one of these were to vary relative to total chlorophyll and the other remain constant. If this were the case, however, a negative relationship between the carotenoid that varied and all other carotenoids would be predicted. No such relationships were observed. That the relative levels of lutein and the xanthophyll cycle pools were indeed inversely related is evident from Fig. 5b, which shows the relationship between these carotenoids expressed on a unit chlorophyll basis. In this figure, a positive correlation is seen, showing that both the xanthophyll cycle...
Figure 5. Relationship between leaf lutein and total xanthophyll cycle pigments each expressed as a percentage of (a) total carotenoid or (b) per unit chlorophyll. Data were determined by HPLC analysis.

The data in this paper show clear differences in the carotenoid composition of plant species that are correlated with their ecology. That such relationships have been exposed is perhaps somewhat surprising since the measurements were made on leaves collected from field-grown material and it is well known that the pigment content of leaves varies in response to environmental conditions. It is likely that the process of collection of plant material reflected the environmental ‘preference’ of each species, so that, for example, a ‘shade species’ was collected from a shade environment. Also, it is concluded that not all species have the capability to show the complete range of carotenoid profiles and that there is a genetic basis for the differences in carotenoid composition observed.

The most striking observation made in this paper is the inverse relationship between the relative amounts of lutein and the xanthophyll cycle pool. The correlation between these parameters is highly significant and is suggestive of a tight control process in the synthesis of these carotenoids. Furthermore, the strong relationship between the ratio of these carotenoids and the shade tolerance index (Fig. 6) suggests that this ratio is of adaptive importance. Lutein (β,ε-carotene-2,2-diol) is derived from α-carotene (β,ε-carotene), whereas the xanthophyll cycle carotenoids, including zeaxanthin (β,β-carotene-2,2-diol) are derived from β-carotene (β,β-carotene). It has previously been observed (Thayer & Bjorkman 1990) that leaves of shade plants often contain significant amounts of α-carotene. It was suggested that light was exerting control on the synthesis of carotenoids at the level of the cyclisation of lycopene to form either α-carotene or β-carotene. It seems likely that the formation of α-carotene and the high levels of lutein in shade plants reflect the same control process. An association between a high level of lutein and low content of xanthophyll cycle pigments has previously been commented on by Demmig-Adams & Adams (1992), although a strict inverse relationship was not shown.

The significance of the variation in lutein and xanthophyll cycle pool levels is not obvious. Both of these groups of carotenoids are known to be bound to light-harvesting complexes of PSII and PSI (Peter & Thornber 1991; Thayer & Bjorkman 1992). It has been shown that for the light-harvesting complexes of PSII (LHCII) there are significant differences in the ratio of lutein/xanthophyll cycle carotenoids bound to each component complex (Peter & Thornber 1991; Bassi et al. 1993). The differences in the levels of these carote-
noid groups therefore may reflect difference in the contents of the LHCII components. Alternatively, it is possible that the carotenoid content of a given complex can vary. In either case, it can be assumed that the differences observed between species will be associated with differences in the function of the light harvesting system.

The role of the xanthophyll cycle has not been established although there is abundant evidence that it is involved in the photoprotective dissipation of excitation energy in the thylakoid membrane (see ‘Discussion’ in Johnson et al. 1993). However, the lutein:xanthophyll cycle carotenoid ratio was found not to correlate with the capacity for energy dissipation in the species studied (data not shown). Until it is known exactly how zeaxanthin is involved in energy dissipation and why the xanthophyll composition of the individual LHCII complexes differ, it would be dangerous to over-interpret this negative finding. Indeed the association between the content of the xanthophyll cycle carotenoids and growth in open habitats is strong evidence for a role in preventing light-related stress.

In summary, we have observed a strict, highly significant, negative correlation between the levels of lutein and of the xanthophyll cycle carotenoids when comparing plant species. The ratio of lutein/xanthophyll cycle carotenoids was found to be related to the shade tolerance of the species concerned. We suggest that this ratio is likely to reflect an important feature in the biochemical adaptation of plants to different habitats.

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REFERENCES


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