Phytochemistry, 1966, Vol. 5, pp. 1345 to 1348. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

# ASSIMILATORY PIGMENTS IN COTYLEDONS OF FOUR SPECIES OF PINE SEEDLINGS GROWN IN DARKNESS AND IN LIGHT

S. WIECKOWSKI* and T. W. GOODWIN

Department of Biochemistry and Agricultural Biochemistry,
University College of Wales, Aberystwyth

(*Received* 23 *March* 1966)

**Abstract**—A comparison was made of the concentrations of chlorophyll *a*, chlorophyll *b*, total xanthophylls, α-carotene and β-carotene in cotyledons of four species of pine seedlings (*Pinus silvestris*, *P. contorta*, *P. radiata* and *P. jeffreyi*) grown in darkness and in light. Dark-grown pine seedlings synthesized not only chlorophylls but also considerable amounts of carotenoids. The ratio of chlorophylls to carotenoids was lower in darkness than in light; this means that relatively more carotenoids are synthesized in the absence of light. No consistent differences in the ratio of xanthophylls to carotenes were observed in seedlings growing in light and darkness. The ratio of β-carotene to α-carotene is higher in dark-grown than in light-grown seedlings. The effect of light on carotenoid biosynthesis is briefly discussed.

## INTRODUCTION

SEEDLINGS of many gymnosperms are able to become green in the dark (for literature review see Egle[1]). Many experiments, for example those of Schmidt,[2] have shown that the chlorophyll which is produced in the dark is spectroscopically identical with that which is synthesized in light. The formation of chlorophyll in a pine embryo is stimulated by a factor (or factors) supplied by the megagametophyte.[2,3] In some cases glucose or sucrose are known to play a part in this kind of stimulation.[4,5]

The present investigation was undertaken to determine the nature of the carotenoids produced by pine cotyledons in the dark and to compare their synthesis in light and darkness with that of chlorophyll. Four species of pine seedlings were examined in this way.

## RESULTS AND DISCUSSION

Table 1 shows that dark-grown seedlings of pine accumulate not only chlorophylls but also relatively large amounts of carotenoids. The concentration of these pigments, however, is lower in dark-grown than in light-grown seedlings. In light there is about four times as much chlorophyll as in darkness. Our experiments also confirm the results of other authors[6]

* Present address: Laboratory of Plant Physiology, Jagellonian University, Cracow, Poland.

[1] K. EGLE, In *Encyclopaedia of Plant Physiology* (Edited by W. RUHLAND), Vol. 5, p. 323. Springer-Verlag, Berlin (1960).
[2] A. SCHMIDT, *Botan. Arch.* **5**, 260 (1924).
[3] L. BOGORAD, *Botan. Gaz.* **111**, 221 (1950).
[4] L. SCHOU, *Physiol. Plantarum* **4**, 617 (1951).
[5] K. C. ENGVILD, *Physiol. Plantarum* **17**, 866 (1964).
[6] A. SEYBOLD and K. EGLE, *Planta (Berl.)* **28**, 87 (1938).

TABLE 1. CONCENTRATION OF ASSIMILATORY PIGMENTS IN COTYLEDONS OF PINE SEEDLINGS GROWN IN DARKNESS AND IN LIGHT

| | | Concentration ($\mu$g/g fresh wt.) | | | | Ratios | | |
|---|---|---|---|---|---|---|---|---|
| | | Chlorophyll *a* | Chlorophyll *b* | Carotenes | Xanthophylls | Chlorophyll *a* / Chlorophyll *b* | Xanthophylls / Carotenes | Chlorophylls / Carotenoids |
| *P. jeffrei* | Dark | 416 | 135 | 31·3 | 61·5 | 3·09 | 1·96 | 5·93 |
| | Light | 856 | 342 | 48·5 | 87·0 | 2·50 | 1·79 | 8·84 |
| *P. silvestris* | Dark | 129 | 19 | 11·3 | 29·8 | 6·72 | 2·63 | 3·61 |
| | Light | 562 | 187 | 37·0 | 50·7 | 3·00 | 1·37 | 8·54 |
| *P. radiata* | Dark | 238 | 74 | 16·3 | 60·1 | 3·20 | 3·69 | 4·09 |
| | Light | 789 | 333 | 50·2 | 86·3 | 2·37 | 1·72 | 8·22 |
| *P. contorta* | Dark | 136 | 35 | 14·4 | 26·9 | 3·93 | 1·87 | 4·14 |
| | Light | 617 | 281 | 40·8 | 71·5 | 2·20 | 1·75 | 8·00 |

that the ratio of chlorophyll *a* to chlorophyll *b* is higher in darkness than in light. The concentration of carotenoids in dark-grown seedlings is about one-half that in light-grown seedlings. The ratio of xanthophylls to carotenes does not change to any great extent in two species, but in *P. silvestris* and *P. radiata* relatively more xanthophylls are synthesized in the dark. The ratio of chlorophylls to carotenoids also changes; in light and in darkness these values are about 8:1 and about 4:1, respectively. Relatively greater amounts of carotenoids accumulate in darkness than in light.

Light affects many processes in plant cells; for example, in most of the angiosperms it is necessary for the transformation of protochlorophyll (protochlorophyllide) into chlorophyll *a* (chlorophyllide *a*),[7] it stimulates the biosynthesis of protochlorophyll,[8,9] it affects the formation or activity of many compounds not directly concerned in photosynthesis[10] and it stimulates chloroplast development.[11] The transformation of protochlorophyll into chlorophyll *a* has clearly been shown to be a photochemical reaction, but at present we do not know if there is a light reaction concerned directly with the pathway of carotenoid biosynthesis. We feel that the biosynthesis of carotenoids is stimulated by light through its

TABLE 2. CONCENTRATION OF α- AND β-CAROTENE IN PINE SEEDLINGS GROWN IN DARKNESS AND IN LIGHT

| | | β-Carotene | α-Carotene | Ratio |
|---|---|---|---|---|
| | | (μg/g fresh wt.) | | β:α |
| *P. jeffrei* | Dark | 24·0 | 7·3 | 3·29 |
| | Light | 30·2 | 18·2 | 1·66 |
| *P. silvestris* | Dark | 9·2 | 2·2 | 4·19 |
| | Light | 29·2 | 7·9 | 3·70 |
| *P. radiata* | Dark | 13·7 | 2·6 | 5·27 |
| | Light | 36·8 | 13·5 | 2·73 |
| *P. contorta* | Dark | 12·2 | 2·2 | 5·55 |
| | Light | 30·0 | 10·8 | 2·78 |

Seedlings 6 days after germination. Light intensity about 300 lx, temperature 29°.

effect on the structure of the plastids. Pine seedlings appear to have an intact and active enzymic system which is responsible for the development of chloroplasts in darkness. The lamellar system in pine seedlings grown in darkness is well developed (D. von Wettstein, personal communication). Light can affect some aspects of carotenoid synthesis in other organisms, in particular, the formation of xanthophyll epoxides.[12] This again may not be a direct effect, but possibly an aspect of the mechanism of oxygen evolution in photosynthesis.

Gymnosperms contain relatively more α-carotene than other plant species do.[13] Our observations (Table 2) show that light also modifies the ratio of β-carotene to α-carotene.

[7] J. H. C. SMITH, In *Comparative Biochemistry of Photoreactive Systems* (Edited by M. B. ALLEN), p. 257. Academic Press, New York (1960).
[8] J. B. WOLFF and R. B. WITHROW, *Plant Physiol.* **32**, Suppl. ix (1957).
[9] H. I. VIRGIN, *Physiol. Plantarum* **11**, 347 (1958).
[10] J. H. C. SMITH, *V. Int. Biochem. Congress, Moscow* (1963).
[11] D. VON WETTSTEIN, In *Biochemistry of Chloroplasts* (Edited by T. W. GOODWIN), In press (1966).
[12] T. W. GOODWIN (Ed.), In *Chemistry and Biochemistry of Plant Pigments*, p. 143. Academic Press, New York (1965).
[13] L. WIERZCHOWSKI, A. LEONOWICZ, K. SAPIECHA and A. SYKUT, *Rocznik Nauk Roln.* **81B**, 87 (1962).

In darkness it is about double that in light. In dark-grown seedlings there is a relatively smaller amount of α-carotene. This pattern has been established in all four species which were examined. If we assume that in the pathways of biosynthesis of these two components the bifurcation takes place at the neurosporene level[12] then the light modifies the channelling of this metabolite into two different ways: the first through β-zeacarotene and γ-carotene to β-carotene and the second through α-zeacarotene and δ-carotene to α-carotene. Recent observations in which it is shown that α-carotene is not formed from β-carotene in carrot roots,[14] support this view.

## MATERIALS AND METHODS

Cotyledons of *Pinus silvestris, P. contorta, P. radiata* and *P. jeffrei* were used. The method and conditions for cultivation of the seedlings are described elsewhere (T. W. Goodwin and S. Wieckowski, in preparation).

### *Determination of Pigments*

The pigments were extracted from a weighed amount of fresh material by grinding in a mortar with acetone (Analar) plus a small amount of $CaCO_3$ and quartz sand. The extract was filtered (sintered glass) and the pigments were transferred into diethyl ether (Analar, dried with sodium wire and distilled over reduced iron). This solution was dried with $Na_2SO_4$ and made up to a known volume. For chlorophyll determinations the absorptivity was measured at 662 and 644 nm. The values for chlorophyll *a* and chlorophyll *b* were calculated from the formula of Smith and Benitez.[15]

The solvent was evaporated from the remainder of the ether solution *in vacuo* and the residue was dissolved in ethanol. The lipid material was saponified[16] and the carotenoids extracted into ether. The ether was evaporated and the pigments dissolved in a small amount of light petroleum (40–60° Analar, dried over sodium wire and distilled before use). The carotenes were separated from xanthophylls on a Hyflo Super-Cel column, and the carotenes separated into α- and β-carotene on MgO. For the development of the Super-Cel column light petroleum with increasing amounts of ether was used, and for the MgO column light petroleum with increasing amounts of acetone. The various fractions were taken to dryness *in vacuo*; the α- and β-carotene fractions were dissolved in light petroleum and the mixed xanthophyll fraction in ethanol. After making up to a constant volume the absorptivities were measured in the Unicam SP 500 spectrophotometer at the following wavelengths: β-carotene, 450 nm; α-carotene, 445 nm; and the mixed xanthophylls at 443 nm. The absolute values were calculated by using appropriate $E^{1\%}_{1cm}$ values.[17]

*Acknowledgement*—S. W. is indebted to the British Council for a Research Fellowship.

[14] T. W. GOODWIN and R. J. H. WILLIAMS, *Biochem. J.* **97**, 28C (1965).
[15] J. H. C. SMITH and A. BENITEZ, In *Modern Meth. Plant Anal.* **4**, 143 (1955).
[16] T. W. GOODWIN (Ed.), *Chemistry and Biochemistry of Plant Pigments*, p. 143. Academic Press, New York (1965).
[17] B. H. DAVIES, In *Chemistry and Biochemistry of Plant Pigments*, (Edited by T. W. GOODWIN), p. 489. Academic Press, New York (1965).