Biochem. J. (1969) **113**, 773
Printed in Great Britain

Effects of Light and Darkness on Polyphenol Distribution in the Tea Plant (*Camellia sinensis* L.)

By G. I. FORREST*
Department of Biochemistry, University of Cambridge

(*Received* 17 *January* 1969)

1. Flavonoid synthesis was able to proceed in darkness in young shoots and seedlings of the tea plant, but was increased by light. 2. The initial effect of darkness was to inhibit synthesis of the A ring or its linkage to the phenylpropane moiety of the flavonoid, but later the hydroxylation state of the flavanols was affected, leading to smaller proportions of gallocatechins and of complex leucoanthocyanins. 3. The esterification of catechins with gallic acid was less affected, so that the ratio of catechin gallates to simple catechins also increased. 4. The flavylogen content of darkened stems, especially in seedlings, was much less decreased than that of leaves; however, a short subsequent light-treatment caused an increase in polymerization.

The polyphenol distribution within the tea plant has been described (Forrest & Bendall, 1969*a*,*b*), and initial experiments on the effect of environmental and nutritional modifications on the course of polyphenol synthesis have been carried out with tissue cultures derived from intact plants as a relatively simple system (Forrest, 1969). The present study was concerned with extending the work with the system *in vitro* to intact plants by means of simple physiological experiments. Perhaps the most profound effects on the polyphenol content of cultured tissue were obtained by altering the light–darkness regime, and it is this environmental parameter that has been made the basis of these studies on parts of intact plants and on seedlings.

MATERIALS AND METHODS

Plant material. Tea seeds and plants derived from cuttings or seed were obtained from Malawi as described by Forrest & Bendall (1969*a*), and were grown in a greenhouse in Cambridge Botanic Gardens. Seeds were planted in peat in 6in. pots, two or three per pot; dark-grown seedlings were obtained by keeping the pots in a large wooden light-proof box, so that the plants germinated and grew in darkness apart from a few seconds each day when it was necessary to remove the lid of the box for watering and thus to expose the seedlings to dim light. Light-grown seedlings were grown in normal daylight.

Apical shoots and leaves of mature plants were kept in complete darkness by covering them with at least two thicknesses of black light-proof cloth secured by string. In some cases the cloth was held in position by clamps to prevent its weight from bearing on delicate structures such as apical buds. In all cases light-grown control shoots were analysed from the same plant.

Analytical methods. Tissue extraction and techniques of polyphenol separation by t.l.c. and determination were as described by Forrest & Bendall (1969*a*). Flavylogens were fractionated into different molecular sizes by gel-filtration on Sephadex columns (Forrest & Bendall, 1969*b*); the column used in the present work was 12·7cm. in length, and had a capacity of 2·82ml./cm.; it was packed with Sephadex G-25 (fine grade), and the flow rate was about 58ml./hr.

RESULTS

Shoots of mature plants kept in the dark

Short-term experiment. A preliminary experiment was performed by keeping the apices of several shoots of a bush from the clone Chisunga 5/E4 in the dark for 15 days, after which they were immediately taken for analysis, together with untreated shoots as light-grown controls. Two dark-grown first leaves, which had separated from the apical bud before the dark-treatment, were extracted, and their polyphenol composition was compared with that of two normal first leaves (Fig. 1). The catechin concentrations were differentially decreased by darkness; epigallocatechin showed the greatest decrease, by about 60%, and catechin and epicatechin gallate were decreased the least, by about 30%. The catechin gallates in fact were decreased the least, and the gallocatechins the most, epigallocatechin gallate being intermediate. The flavylogen content was decreased to less than one-quarter of the normal value in the light. Most notable was that gallic acid and the depsides were not significantly

* Present address: The Nature Conservancy, Moor House Field Station, Garrigill, Alston, Cumberland.

Table 1. *Effect of 12 weeks' darkness on catechin content of shoot apex of clone Chisunga 5/E4*

Catechin contents are given as percentages of light-grown control values. Leaves and internodes are numbered from the apex downwards.

	Amounts of catechins (% of control values)					
	Apical bud	First leaf	Second leaf	Third leaf	First and second internodes	Third internode
EGC	—	—	22·5	11·6	12·4	54·1
EGCG	5·3	—	28·7	36·9	20·2	41·5
ECG	100·0	50·2	58·4	70·8	84·0	86·2
GC	—	—	35·4	18·5	17·7	37·2
EC	34·4	—	25·8	18·2	80·7	90·2
C	40·7	—	47·8	29·6	32·2	34·3
Total	18·2	7·6	29·9	29·0	30·6	53·8

	Concentrations of catechins (% of control values)					
	Apical bud	First leaf	Second leaf	Third leaf	First and second internodes	Third internode
EGC	—	—	21·6	23·1	10·5	53·5
EGCG	9·5	—	27·6	73·5	17·0	41·0
ECG	178	57·2	56·2	141	170	85·0
GC	—	—	34·1	36·8	14·9	36·7
EC	61·2	—	24·8	36·2	91·8	89·0
C	71·5	—	46·0	58·6	50·5	33·9
Total	32·4	8·7	28·7	57·8	39·9	53·1

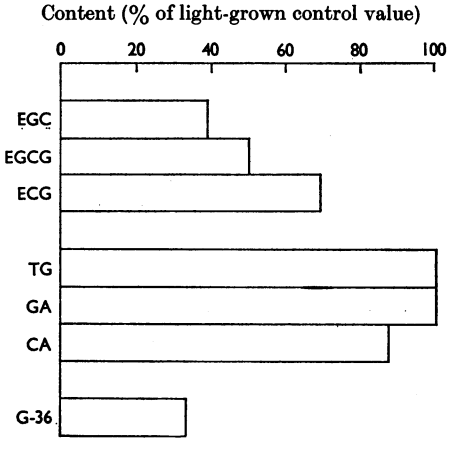

Fig. 1.* Effect of 15 days' darkness on the polyphenol content of the first leaf of apical shoots of Chisunga 5/E4.

* Abbreviations used in figures and tables: C, (+)-catechin; CA, chlorogenic acids; EC, (−)-epicatechin; ECG, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; F-29 and F-30, unidentified flavones (see Forrest & Bendall, 1969*a*); G-36, unidentified derivative of gallic acid; GA, gallic acid; GC, (+)-gallocatechin; O, origin; QA, *p*-coumarylquinic acids; TG, theogallin (galloylquinic acid).

decreased; however, the gallate G-36 suffered a heavy decrease.

These results indicate that over a short time the synthesis of the phenylpropane unit, found in chlorogenic acids and *p*-coumarylquinic acids for example, is not affected by darkness, but synthesis of the flavonoid skeleton is reduced. Accordingly, the biosynthetic stages accelerated by light must be either synthesis of the flavonoid A ring, or its linkage to the phenylpropane unit.

Long-term experiments. (*a*) Shoot apex kept in the dark. The exclusion of light from parts of young shoots for 12 weeks produced different degrees of etiolation and dwarfing. The apices of three shoots of Chisunga 5/E4 that had been kept in the dark for 12 weeks were analysed: they were moderately etiolated and pale greenish yellow. The portion kept in the dark extended from the apex to the third leaf.

The values for catechins in Table 1 show clearly that the two gallocatechins suffered the most severe decreases, whereas epicatechin gallate and catechin were the least affected; as in the short-term experiment, epigallocatechin gallate was intermediate. The gallocatechins were not detectable in the most apical region where development would have taken place in darkness, and in fact the first leaf was deficient in all catechins except epicatechin gallate. The differential decrease in the

Table 2. *Effect of* 12 *weeks' darkness on content of polyphenols of shoot apex of clone Chisunga* 5/*E*4

Polyphenol contents are given as percentages of light-grown control values. Leaves and internodes are numbered from the apex downwards.

	Amounts (% of control values)					
	Apical bud	First leaf	Second leaf	Third leaf	First and second internodes	Third internode
GA	—	—	43·0	29·4	—	—
TG	11·3	11·5	21·9	22·2	44·5	17·2
CA	—	21·9	35·4	27·1	98·8	17·1
QA	—	—	27·3	24·7	65·0	39·3
Compound G-36	—	—	—	—	—	—
Flavylogens	29·8	37·3	63·0	42·2	81·0	68·1
Total phenols	39·3	36·6	27·1	29·5	89·0	51·2

	Concentrations (% of control values)					
	Apical bud	First leaf	Second leaf	Third leaf	First and second internodes	Third internode
GA	—	—	41·2	58·9	—	—
TG	20·2	13·1	21·0	42·5	19·0	17·1
CA	—	25·0	34·0	54·2	41·9	16·8
QA	—	—	26·2	48·5	27·3	38·8
Compound G-36	—	—	—	—	—	—
Flavylogens	53·3	42·6	60·5	84·1	34·0	67·5
Total phenols	69·8	41·6	26·0	58·9	37·3	50·7

apex caused an alteration in the normal distribution of the catechins, for the gallates increased down the shoot. The three typical stem catechins, not being gallocatechins, suffered much less decrease than the gallocatechins in the stem, as well as in the leaves; epicatechin in particular was much less decreased in the stem than in leaves. The inhibition of growth caused by darkness actually brought about increases in concentration of epicatechin gallate above the normal values in the light, especially in the most apical region.

Flavylogens were decreased less than the catechins in the most apical parts of the shoot (Table 2), but in the lower leaves and stem the decreases were more comparable. Chromatograms showed a differential decrease in certain monomers, for in the dark-grown stem compounds LA-11, -42, -45, -46 and -47 (Forrest & Bendall, 1969*b*), which were present in the youngest parts of the control stem grown in the light, were either absent or present only in traces. Conversely, compounds LA-43, -49 and -50 were present in equal amounts in the dark-grown stem and in the control, and compounds LA-13, -41 and -44 were present in greater amount. Thus there was a relative increase in the simplest monomers in the dark (see Forrest & Bendall, 1969*b*), elaboration on the flavandiol structure being inhibited. In the leaves, darkness caused a decrease in the concentrations of compounds LA-43 and -44, and there appeared to be a small amount of oligomeric material present.

Gallic acid and the depsides were decreased maximally in the youngest leaves (Table 2), and in the young stem the etiolation caused by darkness produced relatively small losses in weight but much greater decreases in concentration. The gallate G-36 was not detectable throughout the dark-grown shoot.

Overall, total phenols were more decreased in leaves than in stem; the classes of polyphenols to suffer the least decrease were the simplest catechins and the flavylogens.

(*b*) Mature stem and leaf kept in the dark. To investigate the effects of darkness on the polyphenols of a mature leaf and its internodes, the third leaf and the two adjacent internodes of a shoot of a bush of the clone R/52 were kept in the dark, the rest of the shoot, including the apex, being left exposed to the light. Then 9 weeks later the shoot had grown so that the darkened leaf was the eighth from the apex; brown bark had developed on the stem up to the level of the darkened leaf, and in addition internode 7, immediately above the leaf, was partly brown and partly yellow; upwards from internode 6 the stem was green. The seventh and ninth leaves, wholly dark-green and large, and the

Table 3. *Effect of 9 weeks' darkness on catechin content of mature leaf and stem of clone R/52*

Leaves and internodes are numbered from the apex downwards.

	Amounts of catechins (mg.)						
Treatment	Leaf 7 Light	Leaf 8 Dark	Leaf 9 Light	Internode 6 Light	Internode 7 Dark	Internode 8 Dark	Internode 9 Light
EGC	60·4	24·7	58·6	—	—	—	—
EGCG	45·7	27·6	36·8	—	—	—	—
ECG	6·73	4·38	5·59	0·79	0·76	1·07	1·58
GC	7·80	2·51	6·40	—	—	—	—
EC	11·4	4·56	9·58	0·94	1·29	2·07	2·17
C	0·97	0·40	0·67	0·18	0·22	0·49	0·41
Total	133·0	64·2	117·6	1·91	2·27	3·63	4·16

	Concentrations of catechins (% of fresh wt.)						
Treatment	Leaf 7 Light	Leaf 8 Dark	Leaf 9 Light	Internode 6 Light	Internode 7 Dark	Internode 8 Dark	Internode 9 Light
EGC	1·93	1·36	1·66	—	—	—	—
EGCG	1·46	1·52	1·04	—	—	—	—
ECG	0·22	0·24	0·16	0·11	0·06	0·10	0·11
GC	0·25	0·14	0·18	—	—	—	—
EC	0·36	0·25	0·27	0·13	0·10	0·19	0·14
C	0·03	0·02	0·02	0·03	0·02	0·05	0·03
Total	4·25	3·53	3·33	0·26	0·18	0·33	0·28

Table 4. *Effect of 9 weeks' darkness on content of polyphenols of mature leaf and stem of clone R/52*

Values for flavones are given in arbitrary units; values for flavylogens are given as E_{550}; values for total phenols are given as equivalent mg. of pyrogallol. Concentrations are given per g. fresh wt. Leaves and internodes are numbered from the apex downwards.

	Amounts						
Treatment	Leaf 7 Light	Leaf 8 Dark	Leaf 9 Light	Internode 6 Light	Internode 7 Dark	Internode 8 Dark	Internode 9 Light
F-29	8·3	5·8	8·5	—	—	—	—
F-30	7·2	6·3	9·4	—	—	—	—
Flavylogens	23·7	10·4	20·2	3·7	8·8	5·5	10·4
Total phenols	58·2	30·2	51·6	4·2	3·6	2·8	5·6

	Concentrations						
Treatment	Leaf 7 Light	Leaf 8 Dark	Leaf 9 Light	Internode 6 Light	Internode 7 Dark	Internode 8 Dark	Internode 9 Light
F-29	2·7	3·2	2·4	—	—	—	—
F-30	2·3	3·4	2·7	—	—	—	—
Flavylogens	7·6	5·7	5·7	5·0	7·1	5·0	6·9
Total phenols	18·6	16·6	14·6	5·6	2·9	2·6	3·8

sixth and ninth internodes, were taken as controls. The dark-grown leaf was a lighter green, more flexible and smaller than these adjacent control leaves.

The results of polyphenol analysis (Tables 3 and 4) show that decrease in growth of the darkened leaf caused a corresponding fall in total phenol content, but the phenol concentration was unaffected, so synthesis had been decreased to the same extent as growth. The reverse held in the stem, where a little etiolation took place, decreasing the phenol concentration while the total weight of phenols was less

depressed. There was no significant change in the flavylogen or total catechin concentration either of leaf or of stem, although these of course showed a decrease in total weight in the leaf. However, the relative proportions of the different catechins in the leaf were significantly altered (Table 3). By weight, the gallocatechins were decreased the most, by about 65%, and epicatechin gallate the least, about 30%. In fact there was again an increase in the proportion of catechin gallates, so that epigallocatechin gallate was the predominant component in the dark-grown leaf instead of epigallocatechin. The relative concentrations of epicatechin and its gallate show that there was even an increase in the proportion of gallates to simple catechins when the leaf was kept in the dark; clearly, esterification is one of the least light-dependent stages in the elaboration of the catechin molecule. The stem catechins showed no decrease either in weight or in concentration. An estimation of the two major flavones of the leaves showed that keeping the leaves in the dark significantly increased their concentration, although the total weight present was decreased.

(*c*) Extreme etiolation. In some cases keeping the apex of a shoot in the dark led to very marked dwarfing of the leaves and to a great decrease in stem elongation. In a specimen of clone Chisunga 5/E4 the total phenols were decreased further than in the shoot referred to in section (*a*) above, to about 15–20% of the control value, but owing to the dwarfing the concentrations in both stem and leaf were higher. There was little net phenol synthesis beyond the first leaf and internode. Leucoanthocyanins also showed a great decrease in weight, but nevertheless increased steadily down the shoot; as in normal plants, they were characteristic of stems rather than of leaves.

The apex of a darkened shoot of the clone R/21 after 12 weeks had swollen considerably, owing to failure of the youngest leaves to separate from it. Here again the phenol concentrations were high, owing to inhibition of growth, and total amounts were small, but the leucoanthocyanins were decreased in weight to a smaller extent than were the catechins, for although little flavylogen synthesis occurred in the leaves, the weight in the stem was normal at the apex and decreased only to about 50% of the light-grown control value in the older stem; the concentration as usual was maximal in the youngest stem. In the leaves, epigallocatechin was most decreased in weight (Fig. 2*c*), by about 92% in the second leaf and 97% in the third leaf, and epicatechin gallate the least, the corresponding values being 20% and 55%. In Fig. 2 these decreases are compared with those in the darkened apex (Fig. 2*a*) and mature leaf (Fig. 2*b*) of the less dwarfed shoots described in sections (*a*) and (*b*) above; the differential decrease of gallocatechins compared with simple catechins and their gallates becomes increasingly evident.

Growth of seedlings in darkness

Dark-grown seedlings were etiolated, with white or pale-yellow stems and leaves; the lower leaves were dwarfed but the upper ones attained a larger, but still subnormal, size; they exhibited normal veining and serration. The seedlings grew in height continuously, without passing through dormant banjhi periods.

Four such seedlings were analysed (Table 5), and were found to be capable of synthesizing all the main polyphenols apart from flavones or flavonols (which in any case were very low in amount in light-grown seedlings), and the gallate G-36, the synthesis of which was shown above to be very light-dependent. Chlorogenic acids and *p*-coumarylquinic acids were very low in amount, and not always detectable. The catechin contents are summarized in Tables 5 and 6, which should be compared with Tables 8 and 9 in Forrest & Bendall (1969*a*) for corresponding data for light-grown seedlings. Owing to the low leaf weights, the amounts of catechin synthesized were far below normal, although the concentrations were of the

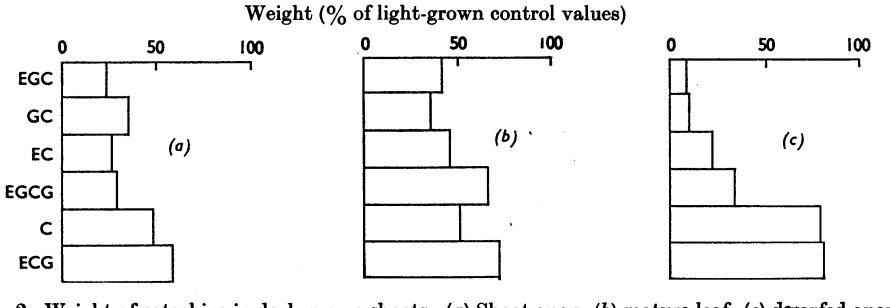

Fig. 2. Weight of catechins in dark-grown shoots. (*a*) Shoot apex; (*b*) mature leaf; (*c*) dwarfed apex.

Table 5. *Dark-grown seedlings and their leaf catechin concentrations*

All seedlings were of clone MT7.

	Concentration (% of fresh wt.)			
Seedling no.	SD-1	SD-2	SD-3	SD-4
Fresh wt. of shoot (g.)	0·25	0·39	0·47	0·56
Height of shoot (cm.)	5·5	15·0	19·8	24·3
Approx. length of root (cm.) ...	8·5	3·5	2	6
Larger apical leaves				
EGC			—	0·80
EGCG			—	1·69
ECG			0·73	1·65
Total catechins			1·05	4·93
Upper leaves				
EGC		0·80	—	0·81
EGCG		2·46	—	1·52
ECG		0·95	0·70	1·37
Total catechins		4·54	1·13	4·44
Small scale leaves				
EGC	0·62	0·43	Trace	
EGCG	1·95	1·72	0·62	
ECG	1·40	0·82	0·98	
Total catechins	4·54	3·38	1·94	

Table 6. *Catechins in dark-grown seedling shoots*

For details of the seedlings see Table 5.

	Weight (mg.) of total catechins throughout shoot			
Seedling no.	SD-1	SD-2	SD-3	SD-4
Apical bud	0·39	0·45	0·11	0·73
Leaves	0·42	1·46	0·50	1·64
Scale leaves		0·42	0·33	
Stem	0·38	0·2		
Total	1·19	2·53		
% of fresh wt. of shoot	0·47	0·62		

	Individual catechins in shoots							
Seedling no.	SD-1		SD-2		SD-3		SD-4	
Part	Shoot		Leaves and stem apex		Leaves		Leaves	
	Wt. (mg.)	% of total	Wt. (mg.)	% of total	Wt. (mg.)	% of total	Wt. (mg.)	% of total
EGC	0·19	16	0·36	15	—	—	0·42	18
EGCG	0·48	41	1·34	55	0·11	11	0·81	34
ECG	0·30	26	0·53	22	0·55	59	0·75	32
GC	0·07	6	0·04	2	—	—	0·12	5
EC	0·11	9	0·15	6	0·28	30	0·24	10
C	0·04	3	0·01	0	—	—	0·03	1
Total	1·19		2·43		0·94		2·37	

normal order of magnitude. The seedling SD-3 was unusually deficient in catechins, as were some of the leaves of the light-grown seedlings. Epigallocatechin never formed as high a proportion of the total catechins in the dark as in light, and there was a marked decrease in the proportions both of epigallocatechin and of its gallate in the two oldest seedlings examined. These two facts correspond

Table 7. *Concentrations and amounts of flavylogens in dark-grown seedlings*

For details of the seedlings see Table 5. Concentrations are given as E_{550}/g. fresh wt. and amounts are given as total E_{550}/organ.

	Concentrations				Total amounts			
Seedling no.	SD-1	SD-2	SD-3	SD-4	SD-1	SD-2	SD-3	SD-4
Apical bud	57·5	34·5	30·3	29·7	0·48	0·43	0·32	0·44
Leaves		25·3	12·7	29·0		0·81	0·56	1·07
Dwarf leaves		49·0	35·6			0·61	0·61	
Total in leaves	66·0				0·61	1·42	1·48	1·51
Stem sections	71·7	85·0	10·7	10·5	3·46	0·75	0·63	0·85
(from apex downwards)	14·5	22·1	10·4	9·7	1·81	1·19	0·86	0·89
		24·1	11·3	10·9		1·33	0·90	1·18
		19·5	12·4	11·3		0·97	0·94	0·90
		22·3		8·2		0·97		0·73
		18·1				1·41		
Epicotyl	35·6	40·5	16·5	12·4	2·20	1·80	1·73	0·75
Total in stem					7·47	8·41	5·06	5·30
Total in shoot					8·56	10·26	6·54	6·81
Root	10·6	10·4		8·4	1·98	0·95		0·99
Root apices	11·7				0·29			
Total in plant					10·83	11·21		7·80

with the results obtained with darkened shoots of mature plants. Theogallin, the main depside present, soon became maximal in the shoot apex; gallic acid was present always in very low concentration.

The flavylogen concentrations at the stem apex (Table 7) were significantly higher than those of normal seedlings, which is remarkable since the dark stems were elongating more rapidly than the light-grown stems, allowing less chance of an apical accumulation. Within the main length of the stems, however, the concentrations were as normal, and gradually declined as the seedling aged. Total flavylogen per plant was not decreased to any great extent, indicating that flavylogen synthesis is predominantly an activity of the stem, and that it is not dependent on light for its continuation. The flavylogen concentrations in the dark-grown leaves were considerably above normal, although the total weight per leaf was more comparable with that of light-grown leaves.

The main flavylogens present in the dark-grown stems were, as usual, LA-13, -41, -42, -49 and -50. There was evidence of a considerable amount of polymerized flavylogen from chromatograms of dark-grown stem extracts, which was confirmed when the flavylogen fractions were separated on a Sephadex G-25 column (Fig. 3). Variation in the relative amounts of polymeric, oligomeric and monomeric material seemed to occur (see Fig. 4), but this may have depended upon the age of the seedling, since the stems analysed in Fig. 4 were three times as high as those taken for Fig. 3. Nevertheless, large proportions of oligomer and polymer were present, so that polymerization evidently was not light-dependent.

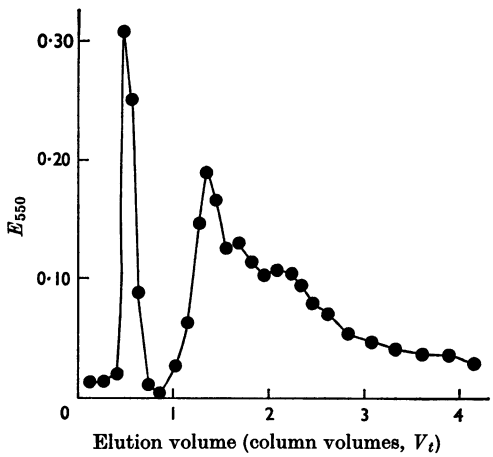

Fig. 3. Fractionation on a Sephadex G-25 column of flavylogens from an extract of stems of five similar dark-grown seedlings of clone MT7 (height 10cm.). The load was 680mg. fresh wt.

To determine the effect of a short light-treatment on the flavylogen pattern of the stems of dark-grown seedlings, four such stems, each 30cm. high, belonging to the clone MT7 were chosen, being almost identical in appearance and leaf size. Two were

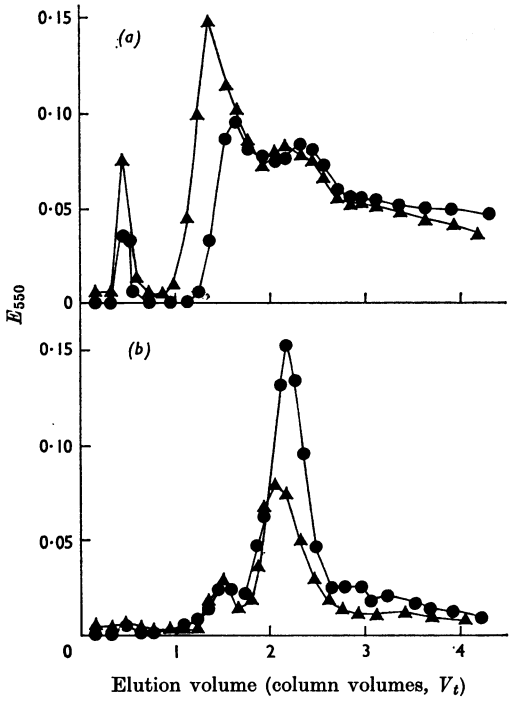

Fig. 4. Fractionations on a Sephadex G-25 column of flavylogens from extracts of (*a*) stems (loads 720mg. fresh wt.) and (*b*) leaves (loads 480mg. fresh wt.) of dark-grown seedlings (height 30cm.) of clone MT7 (●), and of extracts of similar material after a 3 day light-treatment (▲).

subjected to normal daylight for 3 days before being harvested, while the other two were kept as normal in darkness. After the light-treatment the two seedlings showed no differences from the dark-grown controls apart from a slight greening of the apical buds. There had been no further increase in height, and those leaves already unfolded showed no greening or any other change. The results of Sephadex separations of the stem and leaf flavylogens are shown in Fig. 4. In the stems there was an overall increase in flavylogen concentration, due primarily to a very large increase in the oligomer fraction and to a smaller increase in the polymers; at the same time there was a slight decrease in the trailing monomer fraction, although much of the monomer part of the curve was identical in the two cases. Clearly, 3 days' light effected a marked stimulation of polymerization in the stems. In contrast, the effect in the leaves was to decrease the sole monomer peak to about one-half of the dark-grown value, and no polymers appeared; the small peak in the oligomer position was due to catechins, which, being concentrated in leaves, reacted with the acid–butanol reagent to produce a brown colour, which affected slightly the extinction at 550nm.

This result suggested the possibility of flavylogen translocation from leaves to stem, which is also relevant when the distribution of flavylogens within light-grown seedlings is considered: generally they are found at maximal concentrations in the shoot apex but accumulate near the stem base. Since the leaves are the chief site of synthesis of most other

Table 8. *Effect of defoliation on flavylogens of seedling stems*

Concentrations are given as E_{550}/g. fresh wt. and amounts are given as total E_{550}/organ. The control plant was a Rajghur hybrid that was not defoliated.

(*a*) Seedling characteristics

Seedling	MT7	Rajghur hybrid	Control
Leaves removed initially	Three scale leaves	Three scale leaves and one normal leaf	—
No. of successive young leaves removed	6	3	—
Period of defoliation (days)	29	14	—
Final height of shoot (cm.)	12·5	10·0	13·5

(*b*) Flavylogen content

	Concentrations			Total amounts		
Seedling	MT7	Rajghur hybrid	Control	MT7	Rajghur hybrid	Control
Stem lengths						
1. Upper	10·2	30·3	12·4	0·55	0·93	0·58
2. Middle	11·6	15·1	12·0	0·77	0·46	0·58
3. Middle	16·4	23·0	18·4	1·54	0·82	1·11
4. Lower	15·0	24·7	19·0	2·40	1·64	2·10
Epicotyl	33·0	31·7	28·6	1·14	2·16	2·15
Total in stem				6·42	6·00	6·52
Root	6·9	13·8	9·5	1·74	1·33	1·14
Total in axis				8·15	7·33	7·67

classes of polyphenols, the possibility was considered that leucoanthocyanins also might be formed in leaves initially, and then be translocated to the stem. To test whether the stem itself was capable of synthesizing its entire flavylogen content, seedlings were grown in the light without leaves, by defoliating them with a scalpel as soon as each successive leaf separated from the apical bud. One seedling of clone MT 7 and one of a Rajghur-hybrid clone were grown defoliated, while a second Rajghur-hybrid seedling at a similar developmental stage acted as a control. At the close of the experiment, each stem was cut into four equal lengths, which were then analysed for flavylogens, the apical buds being discarded. The results (Table 8) indicated that, although there was a slight decrease in the total weight of flavylogen in the Rajghur-hybrid seedling, the concentrations in this plant actually increased significantly, perhaps due in part to the slight retardation in stem elongation caused by defoliation; the concentrations and weights of flavylogens in the clone MT 7 seedling were very similar to those of the control. It is thus apparent that the leaves do not contribute significantly to the stem flavylogen content; analysis for total phenols gave a similar result.

If, then, translocation of flavylogens from leaves to stem is excluded, the decrease in leaf monomers due to the light-treatment noted above must have been due either to degradation or to conversion into other compounds.

DISCUSSION

These experiments have shown that, as in tissue cultures derived from tea, polyphenol synthesis in the intact plant is able to proceed in darkness, though at a decreased rate. A blockage of synthesis of the flavonoid A ring, or of its linkage to the rest of the molecule, occurred, as found by Hillis & Swain (1959) and Swain (1960). The differential decreases in gallocatechins show that the hydroxylation of the catechin B ring was stimulated by light; similar effects have been observed for flavonol glycosides (Bottomley, Smith & Galston, 1965) and for anthocyanidins (Stafford, 1965; Miller, Miller & Deal, 1965); there was also an increase in the ratio of simpler to more complex leucoanthocyanin monomers in the dark. The decreased hydroxylation may have been partly due to the inhibition of cellular development; it was suggested by Forrest (1969) that gallocatechin synthesis might be intimately connected with vacuolation in shoot meristems. The different effects of darkness on stems and on leaves (the stem polyphenols being relatively unaffected) may then have been due partly to the small concentrations of gallocatechins normally present in stems, partly to the preponderance of leucoanthocyanins that were relatively unaffected by darkness, and partly to the etiolation-induced cellular enlargement being promoted in contrast with the dwarfing induced in leaves.

It is difficult to reconcile the stimulation of leucoanthocyanin polymerization in seedling stems by a short light-treatment with the great differential increase in monomer synthesis observed in tissue cultures (Forrest, 1969), although the different modes of growth and responses to light of the two systems may be involved. That there is a basic difference in this respect is suggested also by similar results obtained for other species: inhibition of tannin synthesis by light has been shown for juniper tissue cultures by Constabel (1963), whereas the leucoanthocyanins in the leaves of intact plum trees were stimulated to change into higher-molecular-weight material by light (Hillis & Swain, 1959), so that Swain (1960) suggested that light is involved in the polymerization of the monomers.

Light therefore appears to stimulate flavonoid synthesis in several ways, apart from the photosynthetic production of precursors. Synthesis or linkage of the A ring and hydroxylation of the B ring are promoted, and polymerization of flavanols is increased. The greater decrease of leucoanthocyanins than of catechins observed in the experiment with a short period of darkness and the greater stimulation of monomeric-leucoanthocyanin synthesis than of catechin synthesis obtained by subjecting tissue cultures to light (Forrest, 1969) suggest that hydroxylation of the heterocyclic ring is also stimulated by light.

I thank Dr D. S. Bendall for his interest in this work, and the Science Research Council for financial support.

REFERENCES

Bottomley, W., Smith, H. & Galston, A. W. (1965). *Nature, Lond.*, **207**, 1311.

Constabel, F. (1963). *Proc. int. Conf. Plant Tissue Culture, Pennsylvania State University*, p. 183.

Forrest, G. I. (1969). *Biochem. J.* **113**, 765.

Forrest, G. I. & Bendall, D. S. (1969*a*). *Biochem. J.* **113**, 741.

Forrest, G. I. & Bendall, D. S. (1969*b*). *Biochem. J.* **113**, 757.

Hillis, W. E. & Swain, T. (1959). *J. Sci. Fd Agric.* **10**, 135.

Miller, J. H., Miller, P. M. & Deal, R. H. (1965). *Pl. Physiol.* **40**, Suppl. lxix.

Stafford, H. A. (1965). *Pl. Physiol.* **40**, 130.

Swain, T. (1960). In *Phenolics in Plants in Health and Disease*, p. 45. Ed. by Pridham, J. B. London: Pergamon Press Ltd.