in this report that *C. nocturnum* is both LD and LSD in its responses and that the previously reported critical day length is for the LD responses while the LSD response has a critical day length of between 12 and 16 hours.

This more clearly defining of the 2 responses in *C. nocturnum* brings into focus the previously suggested questions as to whether a single hormone can be synthesized as a result of more than 1 stimulus, and if a plant can respond to more than 1 hormone (2). One of the approaches that may give further information concerning the nature of the responses is through the study of hybrids of *C. nocturnum* with closely related species. Such an investigation is in progress.

Summary

Photoperiods were investigated in *Cestrum diurnum* L. and *Cestrum nocturnum* L. *C. nocturnum* responded to long-short day, short-long day, and long day treatment. The critical day length in the long-short day response was between 12 and 16 hours, while the critical day length in the short-long day and long day response was between 8 and 12 hours. The short-long day response occurred at a younger age than the long day response. *C. diurnum* plants with 3-month-old shoot systems responded to long-short day treatment with a critical day length between 12 and 16 hours. Plants with 6-month-old shoot systems grown on constant light produced floral primordia when transferred to a 16- or 18-hour photoperiod. Plants maintained on an 8-hour photoperiod started to bloom in from 30 to 50 weeks and continued for the duration of the experiment. These data suggest that *C. nocturnum* is both a long day and long-short day plant and *C. diurnum* is a short day plant, with both species producing floral primordia in younger plants when grown on a noninductive photoperiod and transferred to an inductive photoperiod.

Literature Cited

1. FRANCEY, P. 1935. Monographie du genere *Cestrum* L. Candollea 6: 46–398.
2. GRIESEL, W. O. 1963. Photoperiodic responses of 2 *Cestrum* species and noninterchangeability of their flowering hormones. Plant Physiol. 38: 479–82.
3. LANG, A. 1952. Physiology of flowering. Ann. Rev. Plant Physiol. 3: 265–306.
4. SACHS, R. M. 1956. Floral initiation in *Cestrum nocturnum*. I. A long-short day plant. Plant Physiol. 31: 185–92.
5. ZEEVAART, J. A. D. 1962. Physiology of flowering. Science 137: 723–31.

Some Effects of Photoperiod on the Biosynthesis of Phenylpropane Derivatives in Xanthium[1]

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The biosynthesis of many phenylpropane derivatives in plants is often promoted by or dependent upon light (1). This light requirement varies according to the compounds and plant materials studied (8, 17), but in many cases appears to be related in some manner to photosensitive systems controlling major developmental processes. The biosynthesis of anthocyanins in *Kalanchoe blossfeldiana* is regulated by the same photoperiodic conditions which control the flowering of this species (12), while high chlorogenic acid levels associated with the flowering of tobacco (2) and potato (14) are more likely to be a result of an increase in incident light energies. Possible control of photoinduced growth and developmental changes by these compounds is a matter of some speculation (3). It seems important to investigate the influence of photoperiod on the endogenous levels of these compounds in other sensitive systems.

This paper reports an investigation of the influences of a range of photoperiodic treatments, each of 26 days duration, on the endogenous levels of phenolic phenylpropane derivatives in the aerial parts of *Xanthium* plants. Studies were also made of the influence of short-term photoperiodic treatments on the biosynthesis of these derivatives in a single leaf, as judged by their relative incorporations of C^{14} from a range of tracers.

Materials and Methods

Plant Material and Growing Techniques. Two forms of *Xanthium pensylvanicum* (Wall) were used in this work. Seed of the normal experimental form which can be induced to flower by a single long dark

[1] Received July 10, 1964.

period, was kindly donated by Dr. J. Bonner, California Institute of Technology. This form was used in the majority of investigations, and in the text, reference is to this form unless specifically stated otherwise. Seed of a Mexican form which requires from 1 to 4 weeks of short-day treatment to induce flowers (Dr. B. Carpenter, personal communication) was donated by Dr. B. Carpenter, Long Beach State College.

Seedlings were potted into a 3 to 1 pumice and *Sphagnum* peat mixture and supplied with a modified Hoagland's nutrient solution (22) every 3 days. Plants were grown in temperature controlled glasshouses under continuous illumination (natural daylight plus 400 ft-c incandescent supplementary) till the commencement of any treatment. All experimental treatments were given in growth cabinets at 21 ± 1°. Light in these cabinets was supplied by Philips color 33 white fluorescent tubes (1250 w total) and Philips Comptalux incandescent lamps (1800 w total) producing an intensity of approximately 2500 ft-c around the plants. Relative humidity was only controlled in investigations involving the use of labeled amino acids where it was kept at 96 % to 98 % to facilitate their uptake.

Labeled Precursors. Uniformly labeled L-phenylalanine-C^{14} (60 μc/mg) and L-tyrosine-C^{14} (76 μc/mg) were purchased from the Radiochemical Centre, Amersham. Leaves of plants used in radiochemical investigations were first immersed in 0.05 % Tween 80. After drying, labeled amino acids (20 μc) in a minimum of 2 % ethanol, were applied to the leaf lamina as well spaced drops with a microsyringe. Leaves were washed before harvest to determine the percentage incorporated. Penetration was always better than 90 %. Investigations on the pattern of uptake of $C^{14}O_2$ were done on single plants in enclosed display jars. Barium carbonate (98 mg of specific activity 7 μc/mg) was treated with 0.7 ml of concentrated H_2SO_4 to produce an initial atmosphere of 0.13 % CO_2.

Preparation and Extraction of Material. At the completion of photoperiodic treatment, harvest plant material was immediately frozen and lyophilized. Dried "whole plant" material from the 26-day photoperiodic treatment investigations was ground to a fine powder in a Wiley Mill and stored below −10°. Portions of this material (8 g) were extracted in Soxhlets with successive portions of ether (700 ml), and absolute ethanol (700 ml). After extraction for 12 hours both solvents were removed under vacuo. Ether extractives were taken up in boiling water and filtered to remove most of the chlorophyll, then cooled and extracted into 500 ml ethyl acetate to produce extract A. Ethanol extractives were taken up in 250 ml water containing 8 g $NaHCO_3$, and shaken with 500 ml of ethyl acetate. This ethyl acetate fraction contained most of the ethanol extracted chlorophyll with only traces of phenolics and was discarded. The aqueous layer was then acidified by the addition of 50 ml of 5 N HCl, and extracted with 600 ml ethyl acetate to produce extract B. Extracts A and B were concentrated under vacuo for chromatography.

The dried leaves and terminal stem portions (ca. 2.5 cm) from radiochemical investigations were cut into fragments and ground in a mortar and pestle. Leaf material was extracted for 12 hours with successive portions of ether and absolute ethanol. Extracts A and B were then prepared as previously described, but with the quantity of all reagents halved. Stem material was extracted with ethanol (250 ml) only, and the extractives purified in a similar manner to leaf extract B.

Chromatography. Extracts from the "whole plant" investigations were applied as wide streaks (A on 1 sheet, and B on 4 sheets) to the top of 46- × 57-cm sheets of Whatman No. 1 filter paper and developed by the descending technique. Extract

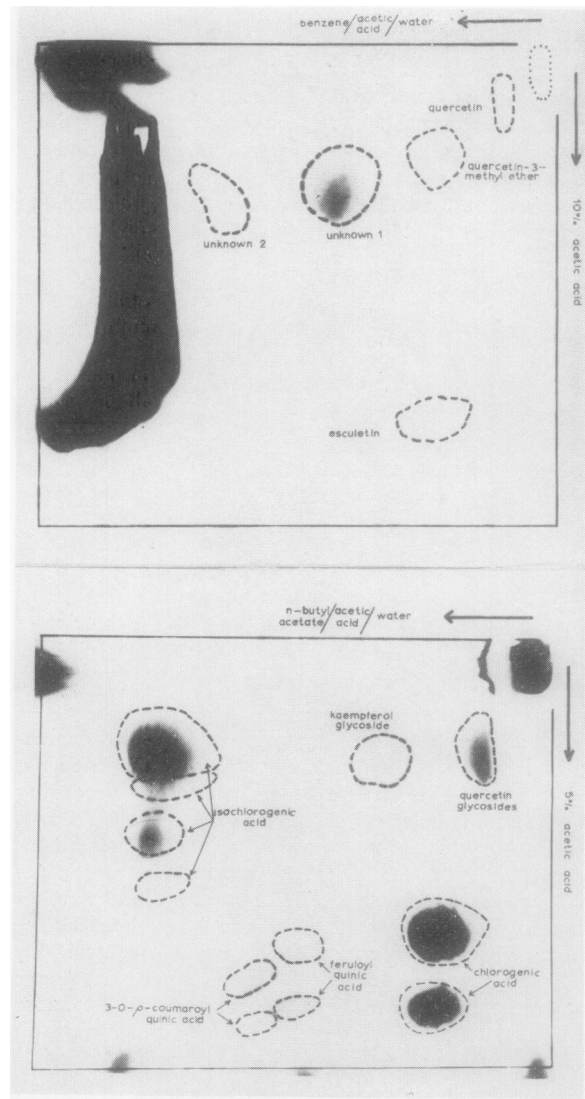

FIG. 1. Radiochromatograms of $C^{14}O_2$ labeled phenylpropane derivatives in extracts A and B of *Xanthium pensylvanicum* leaves. (Extract A, above; Extract B, below).

A was chromatographed first in benezene-acetic acid-water, and extract B in *n*-butyl acetate-acetic acid-water (see table I). Following development, chromatograms were dried in a forced draft and viewed under UV light with and without fuming ammonia. Fluorescent bands were cut from the paper as strips, held vertically in chromatogram tanks and eluted with 75 % methanol for 2 days (4). Elution efficiencies of 90 to 95 % for the cinnamic acid esters, and 80 to 90 % for the flavonoid glycosides, were routinely obtained when compounds were removed from the chromatograms as soon as the paper had become dry enough to handle without tearing. Difficulty was only experienced with the elution of heavily hydroxylated flavonol aglycones, viz quercetin. Eluates of the various phenylpropane derivatives were further purified by chromatography in aqueous solutions of acetic acid (5–30 % v/v) on prewashed paper.

Leaf and stem extracts from radiochemical investigations were chromatographed in a 2-dimensional manner in the solvent systems described, to produce patterns shown in figure 1. Chromatograms were viewed under UV light ($\pm NH_3$), then autoradiographed with Kodak Royal Blue X-ray film to determine the proximity of any other labeled compounds to the phenolic components.

Assay Procedure. Final eluates of the various phenylpropane derivatives from the "whole plant" investigations were made up to a suitable standard volume with 75 % methanol. The OD of these solutions were measured at the λ max of the compounds concerned (table I), before and immediately after

Table I. *Cinnamic Acid Esters and Flavonoids Extracted from* Xanthium pensylvanicum

	Extract A			
	Quercetin	Quercetin-3-methyl ether	Unknown 1	Unknown 2
R_F Values in				
Benzene-acetic acid-water (125:72:3) (23)	0.10	0.27	0.44	0.76
15 % Aqueous acetic acid	0.07	0.10	0.17	0.19
Colour reactions after				
UV long wavelength (366 mμ)	Yellow	Purple	Purple	Purple
UV + ammonia vapour	Gold	Yellow	Brown yellow	Brown
Diazotised sulfanilic acid (18)				
Absorption maxima in				
75 % Methanol	372	360	357	348
75 % Methanol + NaOH	...	410	411	400

	Extract B					
	Quercetin glycosides	Kaempferol glycoside	Chlorogenic acid (3-caffeoyl-quinic acid)	Feruloyl-quinic acid	3-*o*-*p*-Coumaroyl-quinic acid	Isochlorogenic acids*
R_F Values in						
n-Butyl acetate-acetic acid-water (4:1:5) (2)	0.11	0.28	0.21	0.41	0.47	0.68
15 % Aqueous acetic acid	0.52	0.56	0.59 0.77	0.57 0.73	0.62 0.76	0.15 0.23 0.35 0.46
		2% acetic acid				
Colour reactions after						
UV long wavelength (366 mμ)	Purple	Purple	White blue	Dull blue	Faint blue	White blue
UV + ammonia vapour	Yellow	Yellow	Bright yellow green	Yellow green	Blue	Bright yellow green
Diazotised sulfanilic acid (18)			Light brown	Purple → fades	Red → fades	Light brown
Absorption maxima in						
75 % Methanol	360	358	329	321	312	330
75 % Methanol + NaOH	406	400	380	371	361	376

* Numerous spots exhibited by the isochlorogenic acid fraction in 2 % acetic acid may indicate the presence of more than one dicaffeoylquinic ester (16) in these extracts.

the addition of 2 drops of 2 N NaOH to the spectrophotometer cuvettes. The concentration of the compounds was calculated by assuming an extinction coefficient of 20,000 for the major λ max of the phenolic moiety of the cinnamic acid esters (11), and for band II (at 340–370 mμ) of the flavonoids (7).

In radiochemical investigations compounds were not eluted from the paper for counting, because the activity of major components was relatively high and the spots discrete. Areas containing the various derivatives were subdivided into 2 × 2 cm squares, and these squares counted with a Phillips 18506 end wall GM tube.

Experimental Results

Identification of Phenylpropane Derivatives. Recent investigations on the inhibition or activation of in vitro enzyme systems have emphasized the contrasting action of phenylpropane derivatives differing only in their hydroxylation patterns (20). Accordingly, an attempt was made to determine the hydroxylation pattern of at least the phenolic moiety of any ester. Reference to table I shows the range of compounds found.

Compounds were tentatively identified by their chromatographic behavior, UV spectra, UV color reactions and colors after spraying with diazotized sulfanilic acid. More complete evidence was obtained by counterspotting with synthetic standards, and by anthocyanase hydrolyses of the esters (11). Synthetic feruloylquinic acids were unobtainable, so the isomeric configuration of the *Xanthium* derivative could not be determined. Rechromatography of the quercetin glycoside fraction indicated at least 2 components. These have been partially identified, but in this paper an average attachment of two 6-carbon sugars for each quercetin moiety is assumed for convenience of molecular weight calculations. Information from NMR and UV spectroscopy suggests that ether soluble unknown 1 is a tetrahydroxy dimethoxy flavonol, with 1 methoxy group in the 3-position and a free ortho-dihydroxyl grouping in the B-ring. Unknown 2 appears to be structurally similar to unknown 1.

Investigations Involving 26 Days of Photoperiodic Treatment. The influence of prolonged photoperiodic treatments on the concentration of specific phenylpropane derivatives, and on bulk phenol levels was investigated as a first step. This work was done on powders of the whole aerial parts of plants to overcome the difficulty of attempting to compare their concentrations in tissues which by this time had been profoundly changed by the photoperiodic treatments.

Groups of 40 seedlings were transferred to growth cabinets for 26-day treatments immediately after potting out. Some groups were given light breaks (LB) of 2 minutes' duration from the normal cabinet light rig to permit the comparison of flowering and nonflowering plants receiving similar periods for photosynthesis.

These treatments were as follows: 20 hours light–4 hours dark, nonflowering; 16 hours light–8 hours dark, nonflowering; 15 hours light–9 hours dark + LB, nonflowering; 15 hours light–9 hours dark, macroscopic flowers visible; 8 hours light–16 hours dark + LB, nonflowering; 8 hours light–16 hours dark, macroscopic flowers visible.

Thirty uniform plants from each group were harvested by cutting the stem just above the cotyledons. This material was then fractionated as described. The influence of the above photoperiodic treatments on the endogenous levels of major phenylpropane derivatives is shown in table II.

The length of the light period is seen to have the predominant effect on the concentration of the major phenolic components. Caffeoylquinic acids are affected least, showing a decrease of 40 % between treatments giving 20 hours of light to those giving 8 hours of light. The concentration of the quercetin glycosides decreases slightly more under the same

Table II. *Influence of 26 Days of a Range of Photoperiodic Treatments on the Concentration of Major Phenolic Phenylpropane Derivatives in "Whole Plant" Extracts of* Xanthium pensylvanicum *(Normal Form)*

The concentrations are given as mg/100 g of dry wt.

Compounds	Photoperiodic treatments					
	20 Light 4 Dark	16 Light 8 Dark	15 Light 9 Dark + LB*	15 Light 9 Dark	8 Light 16 Dark + LB*	8 Light 16 Dark
Flavonoid Aglycone Unknown 1	6.5	8.0	6.9	5.9	1.4	0.6
Quercetin Glycosides	11	7.1	6.4	5.3	4.9	4.0
Chlorogenic Acid	76	63	61	55	45	41
Isochlorogenic Acids	174	159	151	137	106	91
Bulk phenol** levels	40	36	35	34	23	21

* Light breaks.

** Determined by methods of Swain and Hillis (21) and shown in mg equivalents of ferulic acid/g dry weight.

treatments, while the level of the flavonoid aglycones decreases to approximately twice this extent under the lowest light regimes.

A much smaller change was evident between plants given long dark periods with or without a light break. It seems that photoperiodic flower induction causes no major long-term change in the dry weight levels of these compounds in *Xanthium*.

Spectroscopic estimation of the minor phenolic components showed maximum concentrations of 2.1 mg quercetin-3-methylether, 1.3 mg kaempferolglycoside, 3.6 mg ferulylquinic acid and 1.8 mg 3-*o*-*p*-coumaroylquinic acid on a 100 g dry weight basis. Photoinduced changes in the concentration of these compounds closely followed those exhibited by related major components seen in table II: viz, derivatives within the broad classes of cinnamic acid ester, flavonoid glycoside and flavonoid aglycone responded as a group. There was no evidence of any change in the balance of mono- and ortho-dihydroxy substituted derivatives under the photoperiodic treatments given.

Short-term Radiochemical Investigations. Radiochemical techniques were used to investigate changes during a single long dark period to overcome difficulty in the previous experiments of attempting to interpret small changes in the level of phenylpropane derivatives in plants of such different developmental condition. Leaves are the site of perception of most photoperiodic stimuli in mature plants, and dried powders of one-half to three-fourths expanded *Xanthium* leaves also showed 3 to 4 times higher levels of phenylpropane derivatives than "whole plant" powders. Tracers were applied to the leaves therefore, and sufficient time allowed for some of the labeled products to be transported to the terminal stem portions before harvest.

Plant material was kept in the glasshouse under continuous illumination for 18 days after potting out (i.e. until two-thirds expansion of fifth leaf). Uniform plants were then transferred to the growth cabinets for treatment, and semi-defoliated by removing 4 leaves below and 1 leaf above the fifth leaf. A single plant was used for each treatment. Photoperiodic treatments consisted of a single 16-hour dark period with and without a 2 minute light break, or with 4 long-night (16 hours) pretreatment. These treatments are abbreviated to 0, 1 and 5 respectively in tables III, IV, and V. Phenylalanine-C^{14} was applied to the leaves 20 minutes before, and $C^{14}O_2$ evolved 40 minutes before the start of the single or last dark period. After the dark period plants were given 8 hours of light before harvest. Leaf material used for extraction was composed of the lamina only, while the stem portion was that above the point of inception of the petiole of the fifth leaf (ca. 2.5 cm). Chromatographic separations of phenylpropane derivatives extracted from these tissues are shown in figure 1, and are typical of those routinely obtained.

Activity incorporated into the major flavonoid aglycones present in leaf extracts of the 2 forms of *Xanthium* is shown in table III. The main point of interest is the 7- and 13-fold increase in the total uptake of activity from phenylalanine-C^{14} and $C^{14}O_2$ into these aglycones by the Mexican form as compared to the normal form. Most of this enhanced labeling appears in unknown 1 and to a lesser extent in unknown 2, while the percentage incorporated into 3-methoxy quercetin drops markedly. The amount of activity incorporated into the flavonoid aglycones of the normal form is low, but the values suggest no major changes in relative incorporations under the various photoperiodic treatments.

Activity incorporated into caffeoylquinic esters and quercetin glycosides in the leaf and stem under the various photoperiodic treatments is shown in tables IV and V respectively. It should be noted however, that no change in the concentration (mg/g dry wt) of the major phenolic components could be

Table III. *Distribution of Activity into Major Flavonoid Aglycones in Single Leaves of* Xanthium pensylvanicum (*Extract A*)

Values reported are averages of 3 replicates. Activities greater than 1000 cpm are reproducible with ± 4 %. Activities of 100 cpm show variation within ± 15 %.

Tracer and plant type	Number of inducive treatments	Percentage of activity in 3-Methoxy quercetin	Unknown 1	Unknown 2	Total* cpm
Normal					
PA-C^{14}**	0	14.4	60.6	25.0	188
PA-C^{14}	1	14.0	62.8	23.2	164
PA-C^{14}	5	13.3	61.7	25	180
$C^{14}O_2$	0	15.7	60.0	24.3	70
$C^{14}O_2$	1	19.0	58.4	22.6	95
$C^{14}O_2$	5	18.2	51.3	30.5	236
Mexican					
PA-C^{14}	1	4.4	84.2	11.4	1102
$C^{14}O_2$	1	2.7	79.5	17.9	1288

* Total activity taken up by naturally occurring flavonoid aglycones.
** Phenylalanine-C^{14}.

Table IV. *Distribution of Activity into Major Phenolic Phenylpropane Derivatives in Single Leaves of* Xanthium pensylvanicum *(Extract B)*

The number of replicates, reproducibility and abbreviations as in table III.

Tracer and plant type	Number* of inducive treatments	Percentage of activity in Quercetin glycosides	Chlorogenic acid	Isochlorogenic acid	IC × 100** C	Total cpm × 10^3
Normal						
PA-C^{14}	0	25.4	45.2	25.2	56.7	75.0
PA-C^{14}	1	23.9	39.3	33.1	84.2	74.6
PA-C^{14}	5	17.3	42.4	36.7	86.6	50.2
$C^{14}O_2$	0	38.4	45.4	11.9	26.3	36.4
$C^{14}O_2$	1	26.4	55.6	14.6	26.4	38.9
$C^{14}O_2$	5	6.4	59.2	31.8	53.6	146.0
Mexican						
PA-C^{14}	1	26.4	41.5	28.1	67.7	62.8
$C^{14}O_2$	1	15.3	55.1	24.1	43.8	68.2

* These treatments were not sufficient to cause a spectroscopically detectable change in the concentration of these compounds.

** Percentage of activity in isochlorogenic acids (IC) compared to chlorogenic acid (C).

detected after these short-term radiochemical investigations by spectrophotometric assay following their elution from the chromatograms. The proportion of activity incorporated into the flavonoid glycosides falls during uninterrupted long dark periods, the extent of this drop being dependent on the number of such cycles, type of tracer used, and the tissue involved. Activity incorporated into the caffeolylquinic acids from phenylalanine-C^{14} did not, however, show any significant photoperiodically induced variation. In $C^{14}O_2$ investigations this pattern is complicated by enhanced CO_2 uptake induced by several long-dark periods (10). Enhanced labeling of cinnamic acid esters under these conditions occurs only in the leaf however, and there was no evidence of any increase in the activity of these compounds in the upper stem portions. The degree of labeling of minor components was rather low for accurate assay, but results indicated that as in the long-term photoperiodic investigations, changes closely followed those exhibited by related major components.

The proportion of activity incorporated into the 2 chlorogenic acid isomers (IC × 100/C) is seen (tables IV and V) to differ in leaf and stem tip extracts of the same treatment, and to vary markedly under different photoperiodic treatments and using different radiochemical tracers. A general observation is that the specific activity of chlorogenic acid in the leaves is 2 to 6 times higher than that of isochlorogenic acid, but that this difference is reduced after transport of the label to the stems. This observation and consideration of some preliminary work on the reintroduction of labeled caffeoylquinic esters into plants, suggest that isochlorogenic is formed relatively slowly from chlorogenic acid and is more metabolically inert than the mono-caffeoyl ester. Some indication of a differential incorporation of activity into caffeoylquinic acid isomers from C^{14}

Table V. *Distribution of Activity into Major Phenolic Phenylpropane Derivatives in Stem Portions of* Xanthium pensylvanicum

The terminal 2.5 cm of stem with attached young leaves was analyzed.

Tracer and plant type	Number* of inducive treatments	Percentage of activity in Quercetin glycosides	Chlorogenic acid	Isochlorogenic acid	IC × 100 C	Total cpm
Normal						
PA-C^{14}	0	7.5	24.4	68.1	227.8	761
PA-C^{14}	1	6.8	24.6	68.6	227.8	755
PA-C^{14}	5	4.9	25.0	70.1	227	551
$C^{14}O_2$	0	12.1	33.1	52.3	115.7	23930
$C^{14}O_2$	1	7.8	37.5	53.4	114.2	28600
$C^{14}O_2$	5	5.9	28.7	64.2	226	22104
Mexican						
PA-C^{14}	1	7.2	25.6	67.2	226	594
$C^{14}O_2$	1	8.0	28.6	62.2	221.4	10015

* Number of replicates, reproducibility and abbreviations as in table III.

labeled CO_2 and phenylalanine has also been reported in tobacco (15).

Activity from tyrosine-C^{14} was incorporated into the phenylpropane derivatives with approximately 5 % the efficiency of phenylalanine-C^{14}. Results obtained with this tracer were not, therefore, listed. It would appear that *Xanthium* does not contain an efficient tyrosine deaminase.

Discussion

Literature concerned with the influence of light on the biosynthesis of phenolic compounds is complicated, in some instances, by difficulty in differentiating between the possible effects of bulk incident light energies and truly photoperiodic responses. Garay and Sagi (5) report levels of 32 and 26.5 mg equivalents of ferulic acid in dried leaves (per g) of the facultative long-day plant *Lupinus albus* after long and short days respectively. These authors, and Hare (6) have interpreted this work from a purely photoperiodic standpoint. Similar photoperiodic treatments given to *Xanthium* result in bulk phenol levels of 36 and 21 mg of equivalent units in powders of the aerial plant parts. Light-break controls indicate that most of this change is related to the length of exposure of the plants to high light energies. The concentration of individual phenylpropane derivatives under these same light treatments show a similar pattern of change to that seen in bulk phenol levels. It is difficult therefore, to imagine these compounds as having any control in photomorphogenic responses triggered by low light energies. A change of approximately 40 % in the concentration of the chlorogenic acids observed between the various light treatments was however, attributable to a bulk light effect. The possibility that these esters could be involved in some high energy developmental light response cannot therefore be disregarded.

Investigations suggesting a possible relationship between photoinduced growth and development and photoinduced synthesis of compounds of this type, have been based almost entirely on the IAA oxidase system. Chlorogenic acids are powerful inhibitors of this enzyme, yet they make up 93 % of the soluble phenylpropane derivatives of *Xanthium* with no evidence of any substantial light-break dependent change in their biosynthesis, or of a major change in the relative concentrations of any compounds of this general type. Endogenous concentrations of these compounds also appear very high for control of an enzyme of the IAA oxidase type, especially as the concentration of phenylpropane derivatives and bulk phenol levels generally, are 3 to 5 times higher in young leaves and terminal stem regions as in whole plant powders. It may be that some proportion of these phenols is functionally inactive in intact systems, and that critical concentrations in in vitro systems bear no resemblance to those in vivo. In this connection there are reports (13) of the possible localization of chlorogenic acid in special plastids, chlorogenoplasts, near the nucleus. This question at least must be clarified before a more dogmatic line can be taken, as there are continuing reports of at least a temporal relationship between the level of phenolic IAA oxidase inhibitors and flower induction in *Pharbitis nil* (9) and *Nicotiana* sp. (24).

It would be interesting to know how closely photoinduced CO_2 uptake (10) is related to photocontrolled development. Four successive dark periods cause a 4-fold increase in the labeling of chlorogenic acid, and a 10-fold increase in the labeling of isochlorogenic acid from $C^{14}O_2$ in *Xanthium* leaves in succeeding (fifth) dark period. A single long-dark period caused an approximate 30 % increase in the uptake of activity from labeled CO_2 into both caffeoylquinic acid isomers. The actual and relative concentrations (mg/g dry wt) of the caffeoylquinic acids in *Xanthium* leaves do not change however, after 1 or several long-dark periods. This photoperiodically induced increase in the specific activity of the caffeoylquinic acids could be caused by higher specific activity precursors from the organic acid pool, or may be a reflection of a faster rate of metabolic turnover. The first alternative seems the more likely because no indication of a change in their turnover rates is seen in radiochemical investigations involving phenylalanine-C^{14}.

The less photoperiodically sensitive Mexican form of *Xanthium pensylvanicum* contained the major flavonoid glycosides and cinnamic acid esters of the normal form at approximately half the concentration on a dry weight basis. Radiochemical investigations, however, showed a similar pattern of incorporation of label into these derivatives in both forms. The major difference between these 2 plants as seen in this work, is the Mexican form's marked increase in incorporation of C^{14} labeled CO_2 and phenylalanine into the methylated flavonol aglycones unknown 1 and unknown 2 (table III). Spectroscopic analysis of these aglycones extracted from leaves showed that on a dry weight basis the Mexican form had approximately 3 times the concentration of unknown 1, and one-third the concentration of quercetin-3-methyl ether of the normal form. The difference between these 2 morphologically similar forms of *Xanthium* in their endogenous levels of these phenolic compounds may be made more interesting when the detailed structure of the unknown flavonoid aglycones is determined, and when there is a greater understanding of the metabolic function of the chlorogenic acids (19).

Summary

Investigations were made of the influence of 26 days of photoperiodic treatment on the endogenous levels of phenolic phenylpropane derivatives in the whole of the aerial parts of *Xanthium pensylvanicum* plants; and, of the influence of a single dark period on the biosynthesis of these derivatives in a single leaf, as judged by their relative incorporations of C^{14} from CO_2, phenylalanine and tyrosine.

Chlorogenic acids make up 93 % of the soluble phenylpropane derivatives and 0.3 % dry weight of

Xanthium shoots (1–1.5 % dry wt of young leaves). After the 26-day photoperiodic treatments a change of approximately 40 % in the concentration of these esters, was seen between long-day and short-day treatments. Total phenol levels in the shoots changed from 36 to 21 mg equivalents of ferulic acid per g dry weight under the same conditions. Both these light-induced changes appear to be a consequence of the length of exposure of the plants to high light energies.

Light breaks given during a long-dark period had only a small effect on the endogenous levels of the major phenylpropane derivatives. It seems unlikely that morphogenic responses elicited by low energy light systems in *Xanthium* could be mediated by compounds of this type. There was no evidence of a differential biosynthesis of mono-and ortho-dihydroxy substituted phenylpropane derivatives under the photoperiodic treatments given.

A 4-fold increase in the labeling of phenylpropane derivatives from $C^{14}O_2$ in *Xanthium* leaves occurs during a long-dark period when plants have previously been given 4 successive inducive cycles. This enhanced uptake appears largely in the chlorogenic acids, apparently via higher specific activity precursors from the organic acid pool.

Acknowledgments

The author wishes to thank Drs. R. M. Horowitz and A. H. Williams for their generous gifts of quercetin-3-methyl ether and 3-*o*-*p*-coumaroylquinic acid, respectively. Controlled climate facilities were kindly provided by Dr. K. J. Mitchell of Plant Physiology Division. The help of Drs. R. G. Thomas and M. Zucker in the preparation of this manuscript is also gratefully acknowledged. This work is part of that carried out for a Ph.D. under the tenure of an ICI (N.Z.) Research Scholarship.

Literature Cited

1. Bogorad, L. 1958. The biogenesis of flavonoids. Ann. Rev. Plant Physiol. 9: 417–48.
2. Dawson, R. F. and E. Wada. 1957. Flavonoids and depsides of the green tobacco leaf. I. Rutin and chlorogenic acid. Tobacco Sci. 1: 47–50.
3. Furuya, M. and R. G. Thomas. 1964. Flavonoid complexes in *Pisum sativum*. II. Effects of red and far-red light on biosynthesis of kaempferol complexes and on growth of etiolated plumules. Plant Physiol. 39: 634–42.
4. Gage, T. B. and S. H. Wender. 1950. Quantitative determination of certain flavonol-3-glycosides by paper partition chromatography. Anal. Chem. 22: 708–11.
5. Garay, A. and F. Sagi. 1962. The importance of phenols in the adaptive formation and action of auxin oxidase. Acta Botanica 8: 51–57.
6. Hare, R. C. 1964. Indoleacetic acid oxidase. Botan. Rev. 30: 127–65.
7. Jurd, L. 1962. Spectral properties of flavonoid compounds. In: The Chemistry of Flavonoid Compounds. T. A. Geissmann, ed. Pergamon Press. p 107–55.
8. Klein, W. H., R. B. Withrow, V. Elstad, and L. Price. 1957. Photocontrol of growth and pigment synthesis in the bean seedling as related to irradiance and wavelength. Am. J. Botany 44: 15–19.
9. Konishi, M. and A. W. Galston. 1964. Light-induced changes in phenolic inhibitors of indoleacetic acid oxidase in cotyledons of *Pharbitis nil*. Phytochemistry 3: 559–68.
10. Langston, R. and A. C. Leopold. 1954. Dark fixation of CO_2 as a factor in photoperiodism. Plant Physiol. 29: 436–39.
11. Levy, C. C. and M. Zucker. 1960. Cinnamyl and *p*-coumaryl esters as intermediates in the biosynthesis of chlorogenic acid. J. Biol. Chem. 235: 2418–25.
12. Neyland, M., Y. L. Ng, and K. V. Thimann. 1963. Formation of anthocyanins in leaves of *Kalanchoe blossfeldiana*; a photoperiod response. Plant Physiol. 38: 447–51.
13. Politis, J. 1948. Recherches cytologiques sur le mode de formation de l'acide chlorogenique. Rev. Cytol. Biol. Vegetables 10: 229–31.
14. Prokoshev, S. M. and V. Z. Baranova. 1959. Chlorogenic acid in plants. Biokhimiya Pladov. i. ovaschei. 5. Moscow, Akad. Nauk SSSR. 204.
15. Reid, W. W. 1959. Incorporation of carbon^{14} into tobacco leaves with particular reference to polyphenols. I. Tobacco Sci. 3: 109–12.
16. Scarpati, M. L. 1963. Acidi caffeil-chinici del caffe e del mate. Ann. Chim. Rome 53: 1315–28.
17. Siegelman, H. W. and S. B. Hendricks. 1958. Photocontrol of alcohol, aldehyde, and anthocyanin production in apple skin. Plant Physiol. 33: 409–13.
18. Smith I. 1960. In: Chromatographic and Electrophoretic Techniques. I. Smith, ed. Heinemann.
19. Sondheimer, E. 1962. The chlorogenic acids and related compounds. In: Plant Phenolics and Their Industrial Significance. Proc. Symp. Plant Phenolics Group N. America 15–37.
20. Sondheimer, E. and D. H. Griffin. 1960. Activation and inhibition of indoleacetic acid oxidase activity from pea. Science 31: 672.
21. Swain, T. and W. E. Hillis. 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agr. 10: 63–68.
22. Taylor, A. O. 1964. Ph.D. Thesis, Victoria University of Wellington, New Zealand.
23. Wong, E. and A. O. Taylor. 1962. The chromatography of flavonoid aglycones in the solvent system benzene-acetic acid-water. J. Chromatog. 9: 449–54.
24. Zucker, M., C. Nitsch, and J. P. Nitsch. 1964. The induction of flowering in *Nicotiana*. II. Photoperiodic alteration of the chlorogenic acid concentration. Am. J. Botany. In press.