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THE EFFECT OF LONG- AND SHORT-DAY PHOTOPERIODS ON THE STEROL LEVELS IN THE LEAVES OF *SOLANUM ANDIGENA*

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Abstract—The changes which occur in the levels of free sterols, their esters and glycosides in the leaves of a wild species of potato, *Solanum andigena*, when the plants are transferred from long-day to short-day conditions has been studied. This transfer causes an initial sharp decrease in the level of β-sitosterol and cycloartenol and a rise in the level of cholesterol; however, after 2–3 weeks the levels of these sterols return to those of the long-day leaves.

INTRODUCTION

Solanum andigena is a wild species of potato in which the tuberization response is strongly photoperiodic; plants grown under long-day (LD) conditions will not tuberize whereas those grown under short-day (SD) conditions will. For full induction of tuberization, twenty-five SD cycles are required. The locus of the photoperiodic reaction is the leaves, where it is presumed a tuberization stimulus is produced. This is then translocated to the site of tuberization and initiates the formation of tubers. The nature of this mobile tuberization stimulus or hormone is unknown.

The induction of flowering in many plants is also controlled by day length. It has been shown that floral induction in *Xanthium pennsylvanicum*,[1] *Pharbitis nil*[1,2] and *Lolium temulentum*[3] is suppressed by certain inhibitors of steroid biosynthesis. This has been interpreted as suggesting that the flowering hormone is a steroid or a polyisoprenoid compound. We have found that inhibitors of steroid biosynthesis also inhibit the development of tubers by *S. andigena* grown under SD conditions.[4] The implication of steroids in the tuberization process has led us to investigate the effect of LD and SD photoperiods on the levels of sterols in the leaves of *S. andigena*.

RESULTS AND DISCUSSION

Two groups of cuttings were taken from *Solanum andigena* plants, which had been grown under LD conditions. Both groups were grown for 4 weeks under LD conditions; the cuttings rooted within 2 weeks. One group was then transferred to SD conditions and allowed to grow for a further 6 weeks; the other group remained under LD conditions for this period.

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Small tubers were present after 3 weeks of SD conditions. The free sterol, sterol ester and sterol glycoside content of the leaves of these two groups of plants was determined every week commencing at the third week after planting.

The most abundant of the 4-demethyl sterols present in the species were β-sitosterol, stigmasterol and cholesterol, β-sitosterol constituting about 80 per cent of the total. Campesterol and brassicasterol were also present but at very low levels. The major 4,4′-dimethyl

TABLE 1. FREE STEROL CONTENT OF LEAVES OF *Solanum andigena* GROWN UNDER SHORT- (SD) AND LONG- (LD) DAY PHOTOPERIODS

	Free sterols (μg/g dry weight)											
	4-Demethyl sterols						4,4′-Dimethyl sterols					
Weeks after planting	β-Sitosterol		Stigmasterol		Cholesterol		Cycloartenol		24-Methylene cycloartanol		β-Amyrin*	
	LD	SD†	LD	SD†	LD	SD†	LD	SD†	LD	SD†	LD	SD†
3	239·3	239·3	43·9	43·9	22·5	22·5	21·7	21·7	11·6	11·6	3·3	3·3
4	256·9	256·9	47·3	47·3	26·5	26·5	29·3	29·3	12·0	12·0	3·4	3·4
5	323·3	218·9	47·3	41·4	32·7	29·8	33·8	19·1	16·9	8·5	5·7	2·4
6	354·2	252·0	39·8	37·8	31·4	38·4	28·1	8·9	12·6	5·2	7·3	2·3
7	345·1	261·4	45·3	41·9	34·3	49·5	24·0	33·0	9·2	14·5	3·9	5·2
8	357·0	249·4	52·8	37·9	38·9	34·5	26·9	25·6	10·4	7·6	2·2	3·3
9	—	361·7	—	61·5	—	26·5	—	28·4	—	11·2	—	9·7
10	323·0	316·1	40·2	47·0	31·8	21·2	15·3	19·2	10·3	10·6	10·5	4·4

* β-Amyrin is a pentacyclic triterpene present in the 4,4′-dimethyl sterol fraction.
† SD photoperiods commenced 4 weeks after planting.

TABLE 2. STEROL ESTER CONTENT OF LEAVES OF *Solanum andigena* GROWN UNDER SHORT- (SD) AND LONG- (LD) DAY PHOTOPERIODS

	Sterols occurring in esterified form (μg/g dry weight)											
	4-Demethyl sterols						4,4′-Dimethyl sterols					
Weeks after planting	β-Sitosterol		Stigmasterol		Cholesterol		Cycloartenol		24-Methylene cycloartanol		β-Amyrin*	
	LD	SD†	LD	SD†	LD	SD†	LD	SD†	LD	SD†	LD	SD†
3	24·9	24·9	trace	trace	16·0	16·0	28·6	28·6	6·4	6·4	2·6	2·6
4	55·2	55·2	4·1	4·1	18·0	18·0	41·7	41·7	7·9	7·9	2·3	2·3
5	72·2	17·0	5·4	0·9	16·9	12·8	55·2	3·7	11·3	trace	3·4	trace
6	80·2	21·8	trace	trace	15·2	10·7	63·9	trace	12·6	trace	3·3	trace
7	68·6	29·2	8·9	2·0	14·4	22·9	61·9	12·7	11·0	3·7	5·6	1·5
8	81·8	41·2	trace	trace	27·4	25·6	51·8	20·0	9·0	5·5	2·6	trace
9	—	53·2	—	3·5	—	16·6	—	49·6	—	11·2	—	2·4
10	95·3	40·6	6·0	2·2	43·0	8·5	63·0	38·5	21·0	7·6	8·6	2·7

* β-Amyrin is a pentacyclic triterpene present in the 4,4′-dimethyl sterol fraction.
† SD photoperiods commenced 4 weeks after planting.

sterols were cycloartenol and 24-methylenecycloartanol. The pentacyclic triterpene β-amyrin was also present. The level of 4α-methyl sterols was very low.

The content of the main sterols, present in free form, as esters and as glycosides in the leaves of plants grown under LD or SD conditions, expressed as μg/g dry weight of tissue, is shown in Tables 1, 2 and 3. The level of the free 4-demethyl sterols in both LD and SD

TABLE 3. STEROL GLYCOSIDE CONTENT OF LEAVES OF *Solanum andigena* GROWN UNDER SHORT- (SD) AND LONG- (LD) DAY PHOTOPERIODS

Weeks after planting	Sterols occurring as glycosides (μg/g dry weight)					
	β-Sitosterol		Stigmasterol		Cholesterol	
	LD	SD*	LD	SD*	LD	SD*
3	8·9	8·9	trace	trace	2·1	2·1
4	13·4	13·4	1·2	1·2	8·9	8·9
5	72·2	23·3	7·7	2·5	7·2	5·5
6	22·8	18·2	2·1	1·9	3·5	3·4
7	151·8	26·4	7·1	2·6	3·3	5·7
8	43·5	—	3·1	—	4·5	3·9
9	—	18·8	—	1·8	—	2·3
10	12·6	—	3·7	—	trace	—

* SD photoperiods commenced 4 weeks after planting.

leaves is very much higher than that of their esters or glycosides. However, the level of free cycloartenol in both LD and SD leaves is considerably lower than that of esterified cycloartenol, whilst the levels of free and esterified 24-methylenecycloartanol and β-amyrin are of the same order. The content of 4,4′-dimethyl sterol glycosides was too low to be measured accurately in the quantities of LD and SD leaves assayed.

The most marked changes in sterol level relative to photoperiod are seen with β-sitosterol, cholesterol and cycloartenol (Fig. 1). In LD leaves the level of β-sitosterol and cycloartenol, in both free and esterified form, rises from the third to the sixth week of the experimental period, remains steady and then begins to fall slightly. However, when the plants are transferred to SD conditions after 4 weeks of growth, the level of each of these sterols falls sharply for 2 weeks, then recovers and climbs back to a level comparable with that of the LD leaves. The same picture is also seen, but to a lesser extent, with stigmasterol, 24-methylenecycloartanol and β-amyrin. In the case of cholesterol the effect of SD conditions is quite different. In LD leaves the level of free and esterified cholesterol rises steadily throughout the experimental period. However when the plants are transferred to SD conditions their levels rise, reach a maximum and then fall away; the maximum level of free cholesterol occurs after 3 weeks of SD conditions whilst that of esterified cholesterol occurs 1 week later.

Thus SD conditions affect the level of these sterols in different ways; β-sitosterol and cycloartenol, in particular, show a marked decrease in level whilst that of cholesterol increases. However, these upward or downward trends begin to be reversed by the second or third week of the SD conditions and the sterols approach the LD levels again. It is significant that twenty-five short-day cycles are required for full induction of tuberization in *S. andigena*, for it is within this period that the changes in sterol levels occur. It is tempting to suggest that these changes are concerned with the initiation of tuberization, perhaps with the formation

of a hormone. Cholesterol is known to be the precursor of steroid hormones in animals[5] and has recently been shown to be metabolized in plant tissues to progesterone[6] and ecdysterone,[7] both of which are animal hormones. It could equally well be the precursor of the androgens and oestrogens (e.g. 5α-androstane-3β,16α,17α-triol,[8] oestrone[9,10] and oestriol[11]) which have been found in plant tissues. If cholesterol were the precursor of a hormone concerned in tuberization whose production is initiated by a change to SD conditions, one could

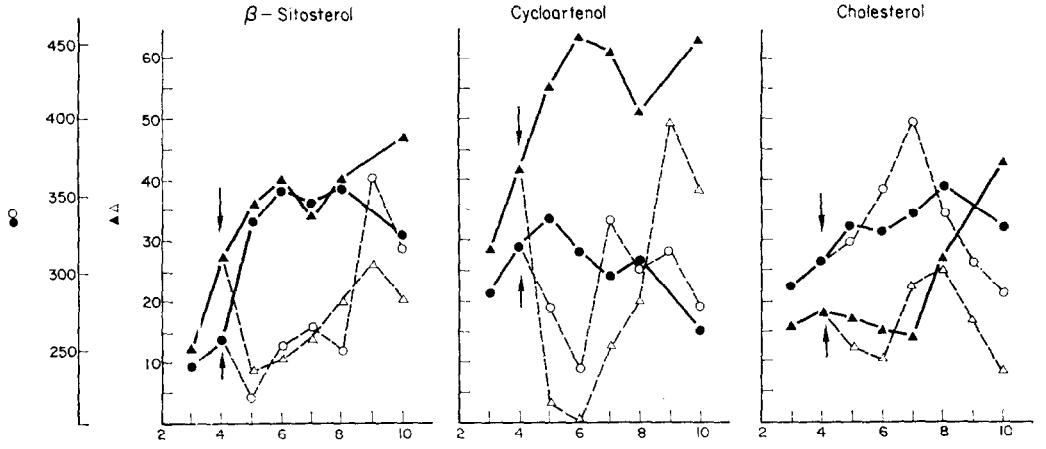

FIG. 1. CHANGES IN THE LEVELS OF FREE AND ESTERIFIED β-SITOSTEROL, CYCLOARTENOL AND CHOLESTEROL IN THE LEAVES OF *Solanum andigena* PLANTS UNDER LD AND SD PHOTOPERIODS.

x axes—weeks after planting
y axes—μg/g dry weight of leaf
●——●—free sterol under LD conditions
o------o—free sterol under SD conditions
▲——▲—esterified sterol under LD conditions
△------△—esterified sterol under SD conditions
↓—start of SD conditions.
↑

envisage an initial rapid increase in cholesterol synthesis followed by a decrease as induction of tuberization nears completion. The changes in the level of cholesterol during the initiation of tuberization by SD conditions may also be due to its utilization for steroid alkaloid synthesis. Cholesterol is known to be converted into steroid alkaloids in plants;[12,13] moreover SD conditions stimulate solanine synthesis in the tubers.[14] The transient increase in cholesterol

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synthesis could occur at the expense of synthesis of the main leaf sterol β-sitosterol and would explain its decreased synthesis and that of its likely precursor, cycloartenol.[15–18]

EXPERIMENTAL

Plant Material and Growth Conditions

Plants of *Solanum andigena* were grown, from cuttings taken from tubers, under LD conditions (16 hr light:8 hr dark) in a greenhouse. Fifty cuttings were taken from these plants and planted in John Innes No. 1 potting compost. They were grown for 4 weeks under LD conditions and then divided into two groups of twenty-five plants. One group was grown for a further 6 weeks under LD conditions whilst the other was grown for the same period under SD conditions (8 hr light:16 hr dark). Leaves were harvested from each group of plants every 7 days from the fourteenth day of planting. At each harvest a selected number of plants were used; leaflets were taken from each of the compound leaves in an attempt to get as representative a sample of leaf tissue as possible. These plants were not used again in subsequent harvests. The leaf tissue (15 g wet weight at each harvest) was divided into two portions of 5 g and 10 g wet weight. The former was dried for 24 hr at 80° and then at 110° to constant weight. The latter was washed with deionized water and stored at −20° until analysed.

The cuttings were taken on 13 November 1968 and grown in a greenhouse until 22 January 1969. In order to produce LD conditions during this period of the year it was necessary to supplement daylight with artificial light. This was accomplished with a combination of 700-W mercury vapour lamps and 100-W tungsten lamps suspended 24 in. above the plants.

Lipid Extraction

Each 10 g batch of leaf tissue was homogenized in an MSE Atomix Blender with boiling ethanol and filtered through a sintered funnel. The residue was re-extracted once with boiling ethanol and twice with ethyl acetate. The extracts were combined and taken to dryness under reduced pressure in a rotary evaporator. The residue was redissolved in a 2:1 (v/v) mixture of petroleum ether (b.p. 40–60°) and ethanol and thoroughly shaken with water in a separatory funnel. The petroleum ether phase, containing the sterols and sterol esters, was collected and the aqueous phase, containing the sterol glycosides, re-extracted with petroleum ether. The two petroleum ether extracts were bulked; further petroleum ether extracts of the aqueous phase did not contain any sterol or sterol ester. The petroleum ether extract was dried, taken to dryness and subjected to column chromatography. The aqueous phase was concentrated to 100 ml prior to hydrolysis of the sterol glycosides.

Column Chromatography

The lipid from the petroleum ether extract was chromatographed on a 10 g column of neutral, Brockmann Grade 3 alumina (Woelm) using 100-ml vols. of 1% E/P, 6% E/P and 20% E/P for development (E/P = ether in petrol ether, b.p. 40–60°). The 1% E/P fraction contained the sterol esters; the 6% E/P and 20% E/P fractions contained the 4,4′-dimethyl sterols and the 4-demethyl sterols respectively.

Hydrolysis of the Sterol Esters and Sterol Glycosides

The sterol ester fraction was refluxed for 90 min in 6% (w/v) KOH in 90% (v/v) aqueous ethanol and the unsaponifiable material containing the sterols extracted with diethyl ether. The sterol glycoside fraction was refluxed for 3 hr in 2 N HCl. The mixture was then cooled, adjusted to pH 7·5 and extracted with ether.

Thin-layer Chromatography

The sterols from the 6% E/P and 20% E/P fractions, and from hydrolysis of the esters and glycosides were purified by chromatography on thin layers (0·25 mm) of silica gel G impregnated with Rhodamine 6G using $CHCl_3$ for development.[19] Samples of lanosterol (R_f 0·48) and stigmasterol (R_f 0·36) were run on each plate as markers of the 4,4′-dimethyl- and 4-demethyl sterol zones respectively. The zones were scraped off the plates and the sterols eluted with ether.

Gas–Liquid Chromatography

The 4,4′-dimethyl- and 4-demethyl sterols were analysed and quantitatively determined by gas–liquid chromatography using a Packard Gas Chromatograph Series 7500 fitted with a glass column (6 ft × 2 mm i.d.) packed with 3% OV-1 supported on 80–100 mesh silane-treated Gas-Chrom Z. The chromatograms were

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developed at 230° with an argon flow rate of 40 ml/min. A known aliquot (1–2 μg) of each sterol fraction was chromatographed and the quantity of each sterol present determined by comparing its peak area with a standard curve relating peak area to weight (μg) of that sterol. The standard curves were prepared using authentic sterols and were linear over the range 0·1–1·2 μg.

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