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EFFECTS OF SENESCENCE AND GIBBERELLIC ACID TREATMENT ON STEROL LEVELS IN DETACHED LEAVES OF DANDELION (*TARAXACUM OFFICINALE*)

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Key Word Index—*Taraxacum officinale*; Compositae; dandelion; leaf senescence; gibberellic acid; sterols; sitosterol; cycloartenol.

Abstract—Excised leaf laminae of dandelion incubated in water in the dark for 5 days lost 60% of their chlorophyll but showed large increases in levels of sitosterol (140%) and its ester (119%) and glycoside (298%). Free and esterified 4,4-dimethyl sterols were also higher by approximately 350 and 200%, respectively. Treatment with 3×10^{-4} M GA_3 halved the pigment loss and largely abolished increases in esters and glycosides but had little influence on free sterols.

INTRODUCTION

Changes in the nature and properties of membranes, particularly those of chloroplasts [1] and endoplasmic reticulum [2], are currently considered to be important early events in plant cell senescence. Recently, membranes of senescing cells were reported [3–5] to exhibit large increases in their sterol:phospholipid ratio and this was postulated [3] as being due mainly to an increase in sterol levels.

Senescence in many excised plant tissues can be delayed by treatment with growth regulators such as gibberellic acid (GA_3) [6]. The biochemical basis of this hormonal effect is not clear but it is not thought to be mediated via gene expression [7]. Gibberellins, however, are known to influence the fluidity and permeability of both synthetic [8] and plant cell membranes [9, 10] as well as sterol metabolism in plant tissues [11, 12]. Such observations suggest that the senescence-delaying properties of GA_3 might be due to, or associated with, stabilization of sterol levels in cell membranes. This paper describes preliminary investigations to explore this possibility.

RESULTS AND DISCUSSION

A study of the effect of GA_3 on senescence of excised *T. officinale* leaf tissue revealed a halving of the 60% loss in total chlorophyll which occurred in control tissue after 5 days of incubation.

Esterified, free and glycosylated 4-demethyl sterols were present in leaf tissue (in that order of abundance), with sitosterol being the most abundant compound in each category. Cholesterol could not be detected, nor could acylated glycoside derivatives. Contrary to expectations, free and (particularly) esterified 4,4-dimethyl sterols (cycloartenol and 24-methylenecycloartanol) were also prominent.

Free sitosterol (but not stigmasterol or campesterol) and free 4,4-dimethyl sterols increased significantly (approximately 140 and 300%, respectively) after the 5-day incubation, but similar changes occurred in GA_3-treated tissue (Table 1). Other workers (e.g. [3, 5]) have also reported increases in free sterols during senescence, but the lack of effect of GA_3 on levels of these compounds raises doubts about the causality between free sterol changes and senescence. Similar doubts have recently been expressed elsewhere [13].

Sitosterol ester and glycoside and esters of cycloartenol and 24-methylenecycloartanol also increased during the 5-day incubation (by 119, 298, 195 and 171%, respectively), but these increases were markedly reduced by GA_3 to 31, 63, 24 and 12% of respective day 0 control (Table 1). Our data therefore raise the possibility of a general stimulation of sterol biosynthesis during senescence. In addition, the ability of GA_3 to abolish largely increases in esters and glycosides suggests that the formation of sterol derivatives may be more closely correlated with senescence than accumulation of free sterols. Sterol esters have also been shown to increase in ageing intact leaves of soybean [14]. An explanation of this apparent hormonal inhibition of sterol derivative synthesis is hindered by the paucity of information on the functions of such compounds in plants [15]. Storage and transport hypotheses have attracted little experimental support [16, 17]. Interestingly, these forms apparently do not possess the membrane-stabilizing properties of free sterols [18] and could possibly contribute directly to membrane destabilization [19].

The correlations observed here between sterol derivatives and leaf senescence add a new dimension to studies in this area, but further work is still required. In particular, it remains to be ascertained whether similar changes in sterols occur in naturally-senescing attached leaves and, if so, whether such changes constitute a primary event in senescence or are merely a consequence of other events.

EXPERIMENTAL

Plant material. Dandelion plants (*Taraxacum officinale* Weber) were collected on the University of Exeter campus. Whole plants of approximately the same size and leaf form were dug up and

Table 1. Effect of senescence and GA_3 on sitosterol and 4,4-dimethyl sterols of excised dandelion leaf laminae

Sterol	Derivative	Concentration (μg/g dry wt)		
		Fresh tissue	5 days old (control)	5 days old (GA_3)
Sitosterol	Free	1083	2608	2323
	Ester	4484	9815	5884
	Glycoside	265	1054	432
Cycloartenol	Free	554	2549	2599
	Ester	4750	13991	5886
24-Methylenecycloartanol	Free	500	2260	2150
	Ester	2528	6851	2843

Leaf tissue was analysed fresh or after incubation for 5 days in the dark at 20° in Petri dishes containing 3.5 ml of distilled water (control) or 3×10^{-4} M GA_3.

potted into John Innes No. 2 compost. Pots were normally kept in an open, north-facing cold frame but were transferred to an unheated glasshouse during the winter months.

Harvesting and treatment. As far as possible, only fully-expanded mature leaves of approximately the same size were harvested for experiments. To accelerate senescence and facilitate short-term incubation in non-sterile solutions, scored leaf discs were not removed but whole leaf laminae, minus midrib and petiole, were used. Approximately 10 g of tissue was placed, abaxial side down, on a single layer of Whatman No. 4 filter paper moistened with 3.5 ml of either 3×10^{-4} M GA_3 or distilled water (pH 6.0) in a 150 mm diameter Petri dish. Dishes were incubated at 20° in the dark for 5 days, after which leaf tissue was lyophilized, weighed and analysed for sterols. Separate samples were similarly treated but analysed for chlorophylls by the method of Arnon [20] as an index of senescence. Sterol and chlorophyll analyses were also conducted on material lyophilized immediately after harvest.

Extraction, separation and quantitation of sterols. Methods for extraction of 4-demethyl and 4,4-dimethyl sterols and derivatives, separation by CC and TLC and quantitation by GLC were as described previously [21]. All experiments were duplicated and data are means of the two samples.

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