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C-GLYCOSYLFLAVONE ACCUMULATION IN SANDOZ 6706-TREATED BARLEY SEEDLINGS IS A PHOTOCONTROLLED RESPONSE

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Key Word Index—*Hordeum vulgare*; Gramineae; *Phaseolus aureus*; Leguminosae; pyridazinone herbicides; blue light response; plastids.

Abstract—Sandoz 6706 pretreatment of white light grown barley seedlings causes a 60% increase in saponarin (6-*C*-glucosyl-7-*O*-glucosylapigenin) but a 300% increase in lutonarin (3′-hydroxysaponarin). Norflurazon has little effect on saponarin levels but is almost as effective as Sandoz 6706 in enhancing lutonarin net synthesis. Barley roots contain saponarin and lutonarin only after herbicide treatment. Mung bean seedlings respond to Sandoz 6706 by accumulating higher levels of rutin and delphinidin 3-glucoside. The results are discussed in relation to the site of action of the herbicides, the High Energy photoresponse, and control of flavonoid 3′-hydroxylation.

INTRODUCTION

The pyridazinone herbicides Sandoz (San) 6706 and Norflurazon are potent inhibitors of chloroplast development. They block carotenoid cyclization [1] allowing chlorophylls to be photooxidized and plastid ribosomes degraded in the light [2]. In a previous study of these compounds we reported that blue light was involved in herbicide-induced increases in phenylalanine ammonia-lyase (PAL) in barley and mung bean seedlings [3]. Reported effects of pyridazinone herbicides on polyphenolics are limited to observations that anthocyanin levels are increased in wheat seedlings treated with San 6706 [4].

RESULTS

The general effects of these herbicides on growth and chlorophyll content in barley and mung bean seedlings have been described in the previous paper [3]. The major flavonoids of light grown barley shoots are saponarin and lutonarin; 7-*O*-glucosides of the 6-*C*-glucosides of apigenin and luteolin. Traces of lutonarin 3′-methyl ether are also present, but as this minor constituent is separated from lutonarin only with great difficulty [4], and is likely formed from lutonarin, by a late 3′-methoxylation reaction [6], lutonarin and its 3′-methyl ether were determined as a mixture and designated as lutonarin in the present study.

The effects of San 6706 or Norflurazon on the accumulation of flavonoids in white light grown barley shoots are shown in Fig. 1. Both herbicides markedly increase the level of lutonarin while San 6706 is much more effective in increasing saponarin.

Examination of two dimensional paper chromatograms from San 6706-treated plants disclosed small amounts of flavonoids not detected in controls. Based on chromatographic behaviour and color reactions, these new compounds are thought to be the corresponding aglycones, isovitexin and isoorientin.

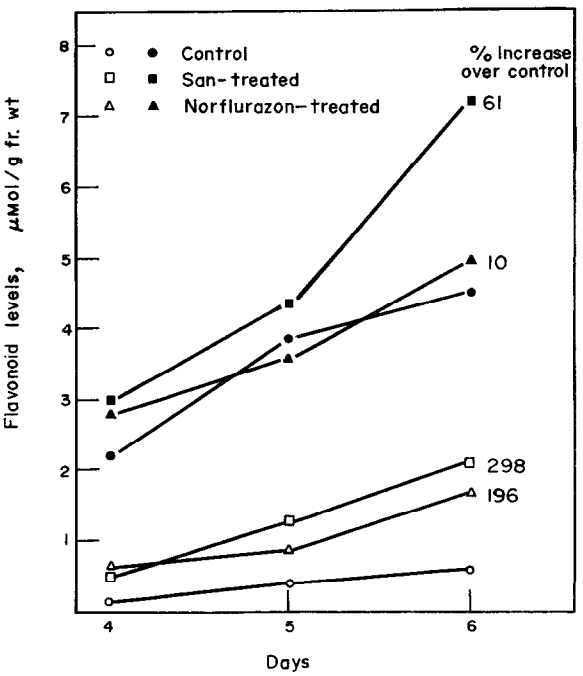

Fig. 1. Seeds were germinated for 48 hr on filter paper with H_2O or aq. solns of San 6706 (100 μM) or Norflurazon (200 μM, see ref. [3]), transplanted to vermiculite, and maintained under continuous cool white fluorescent light until harvest. Closed symbols = saponarin, open symbols = lutonarin.

San 6706 has no effect on PAL levels [3] or on flavonoids (Table 1) in dark-grown barley shoots. Under continuous red light this herbicide promotes only

* Part 8 in the series 'Phenolic Biosynthesis in Barley Seedlings'. For Part 7 see ref. [3].

Table 1 Effects of San 6706 on flavonoid levels in barley seedlings

Light treatment	Saponarin (μmol/g fr. wt)	Lutonarin (μmol/g fr. wt)
WHOLE SHOOTS		
Dark—Control	0.26	none†
San 6706	0.25	none
Red—Control	1.97	0.10
San 6706	2.54	0.11
Blue—Control	3.12	0.25
San 6706	6.50	0.89
White—Control	4.45	0.52
San 6706	7.17	2.07
ROOTS		
White—Control	none	none
San 6706	0.04	0.01

Seedlings were germinated for 48 hr on wet filter paper with or without prior treatment with 100 μM San 6706, transplanted to vermiculite, and grown under continuous photoconditions as indicated for 5 (dark) or 6 (light treated) days.

† Sensitivity of detection is *ca* 10 nmol (ref. [16]).

saponarin; blue light (and presumably the blue component of white light) is required for increased lutonarin.

C-glycosylflavones were not detected in the roots of etiolated barley [5] or in roots of control plants grown in continuous white light (Table 1). However, San 6706 treated plants grown in white light accumulate small amounts of saponarin and lutonarin in roots buried under several centimeters of vermiculite (Table 1).

PAL levels in mung bean seedlings decrease after 100 μM San 6706 treatment [3] yet there is an increased level of rutin and delphinidin-3-glucoside (Table 2).

DISCUSSION

Studies of the mode of action of pyridazinone herbicides have pointed to specific blocks in carotenoid cyclization within plastids [1, 2, 4]. Since these effects of San 6706, at least on plastid development, can be overcome by concomitant applications of phytol or certain unsaturated fatty acid esters [7], many metabolic aspects of plastids are apparently unaltered by such treatments. Here, we have shown that herbicide-treated barley shoots show striking increases in flavonoids, especially lutonarin which raises the question of plastid involvement in these processes.

Table 2. Effects of San 6706 on flavonoid levels in mung bean seedlings grown under continuous white light

Treatment	Rutin (μmol/g fr. wt)	Delphinidin 3-glucoside (μmol/100 mg dry wt)
Control	1.34	0.27
San 6706	2.23	0.67

Seeds were germinated in the light for 48 hr on wet filter paper with or without prior treatment with 100 μM San 6706, transplanted to vermiculite, and grown under continuous cool white fluorescent light for an additional 72 hr. Rutin was determined in primary leaves, delphinidin 3-glucoside in hypocotyls.

Phenolases from plastid thylakoids hydroxylate *p*-coumaric acid [8] and various flavonoids [9], and PAL has been reported in thylakoids [10]. Although plastids in light grown San 6706 treated plants lack grana, they develop thylakoids which become more disorganized as white light intensity is increased [2, Blume unpublished], and their membrane lipid composition is changed [7]. This suggests that San 6706 treatment may change the enzyme composition of thylakoids and divert plastid metabolism to increased polyphenolic production. The accumulation of small amounts of aglycones of saponarin and lutonarin in herbicide treated plants may indicate reduced activity of UDP-glucose: *C*-glucosylflavone 7-*O*-glucosyltransferase [11].

Norflurazon is thought to be the active product to which San 6706 is degraded within the plant [12], and the two herbicides are essentially equal in inducing albino plants [12] or in increasing PAL in barley shoots [3]. However, San 6706 is considerably more effective than Norflurazon in increasing saponarin accumulation in barley shoots (Fig. 1). Also, 100 μM San 6706 treatments decrease PAL levels in barley roots [3] but only after such treatment do we find any *C*-glycosylflavones in roots (Table 1). Finally, 100 μM San 6706 decreases PAL levels in mung bean leaves [3] but promotes rutin in the same organs (Table 2). It is apparent that these herbicides alter polyphenolic accumulation at several points and in a complex fashion.

San 6706 treated barley shoots respond to red light by increasing saponarin levels, yet blue (or white) light is required for increased lutonarin (Table 1). We suggest that the absence of blue light absorbing carotenoids in herbicide treated plants allows more complete expression of the blue light High Energy photoresponse effective in increasing 3′-hydroxylated flavonoids in several grasses including sorghum [13], corn [14] and barley [15].

Action spectra are considerably different for increased accumulation of saponarin and lutonarin in barley shoots [16]. While saponarin levels seem to be explained largely by a low energy red–far-red photoreversible phytochrome response, lutonarin is more complex and it has been proposed [16] that photoreceptors involved in the 3′-hydroxylation of lutonarin are associated with plastids. This view of plastid involvement in phenolic biosynthesis has been subsequently reinforced by our work on photocontrol of PAL [17] and saponarin [18] in etioplasts and chloroplasts isolated from barley shoots.

There are several problems in a simple scheme where San 6706 blocks carotenoid formation, allows maximal expression of blue light within plastids, and diverts primary phenolic metabolism into polyphenols. For example, San 6706 effects on PAL are inversely correlated with flavonoid accumulation in barley roots (Table 1, and ref. [3]) and these tissues are not themselves exposed to light. Also, mung bean leaf PAL is decreased by San 6706 at doses which markedly increase rutin (Table 2, and ref. [3]). Furthermore, while plastid phenolase may be involved in the 3′-hydroxylation of flavonoids [6, 9] we have been unable to detect phenolase activity in plastids isolated from barley shoots (Muhitch and McClure, unpublished). Finally, although plastids isolated from either dark or light grown barley shoots contain appreciable levels of saponarin, and this accumulation is strikingly enhanced by a red–far-red revers-

ible photoresponse, the plastids contain no detectable lutonarin [18]. Mung bean plastids contain no traces of flavonoids although whole leaf extracts are rich in rutin [19]. If critical stages of flavonoid biosynthesis involve plastids [20, 21], there are no clear patterns for the (transitory?) accumulation of flavonoids within plastids isolated from a wide range of plants [19]. Investigations are currently underway to determine the effects of San 6706 on several enzymes of flavonoid biosynthesis, both in whole shoots and in isolated plastids, in an attempt to resolve some of these apparent inconsistencies.

EXPERIMENTAL

Plant material, herbicides and light treatments were as in ref. [3].

Flavonoid determination. Methanolic homogenates of barley shoots were separated by 2-D PC and flavonoids quantitated by techniques detailed in ref. [5]. Saponarin was determined by *A* at 333 nm (ε 1.75 × 10^4), lutonarin (including traces of its 3′-methyl ether) at 349 nm (ε 1.88 × 10^4). Similar techniques were applied to determine rutin at 358 nm (ε 1.76 × 10^4) in mung bean primary leaves. Delphinidin 3-glucoside was extracted from freeze dried mung bean hypocotyls into 1% aq. HCl and measured at 540 nm (ε 2.5 × 10^4). All data points are means of at least three separate determinations.

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