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Effect of Chlorsulfuron on Phenylpropanoid Metabolism in Sunflower Seedlings*

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Abstract. The effect of the herbicide chlorsulfuron on phenylpropanoid titer and metabolism and the role of endogenous ethylene in this response was examined in light-grown sunflower (*Helianthus annuus* L.) seedlings. Application of chlorsulfuron to the apex resulted in large increases in both total phenolic and hydroxycinnamic acid content in hypocotyls isolated from the treated seedlings. Both of these parameters were increased within 24 h of herbicide treatment, and both reached a maximum level 3–4 days post-treatment. An increase in ethylene evolution was found to proceed in parallel with the alterations of phenolic content. The extractable activities of phenylalanine ammonia lyase, *trans*-cinnamic-4-hydroxylase, and soluble peroxidase were increased by chlorsulfuron treatment. Chlorsulfuron had little effect on total phenolic content when administered directly to isolated hypocotyl segments. Exogenous ethylene had no effect on the endogenous titer of phenolic compounds. Root-fed cobalt chloride (5×10^{-4} M) inhibited chlorsulfuron-induced ethylene production by 92% and also inhibited the accumulation of phenolic materials by 56%. Exogenous ethylene was unable to reverse the inhibition caused by cobalt chloride. It was concluded that chlorsulfuron-induced increases in phenolic compounds were not mediated solely by endogenous ethylene.

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The herbicide chlorsulfuron (Fig. 1) has recently been introduced as a herbicide for use in small grains (Levitt et al. 1981). Chlorsulfuron possesses a type of chemistry not previously exploited for herbicidal activity. It is extremely active against most dicots and certain monocots as well (Levitt et al. 1981). Chlorsulfuron is unique in that application rates for weed control range from 10–40 g (ai) per hectare. In laboratory situations, chlorsulfuron produces marked biological effects when applied at sub-microgram doses (Suttle 1982). Research has shown that chlorsulfuron treatment results in a promotion of anthocyanin accumulation in soybean seedlings (Suttle and Schreiner 1982). Like many other secondary plant products, anthocyanins are synthesized from simple C_6-C_3 precursors ultimately derived from phenylalanine via phenylalanine ammonia lyase activity (Camm and Towers 1973).

Many compounds derived from this phenylpropanoid skeleton are phenolic in nature and have been implicated in the internal regulation of plant growth and development (Kefeli and Kadyrov 1971). In addition, many of these phenylpropanoid derivatives are both medically and commercially useful (Towers and Wat 1979). Because of these considerations, we decided to investigate the effect of chlorsulfuron on phenlypropanoid metabolism in greater detail.

This report describes the effect of chlorsulfuron on phenylpropanoid metabolism and titer in light-grown sunflower seedlings. Also, because elevated rates of ethylene evolution have been shown to result from chlorsulfuron treatment (Suttle 1982), the role of endogenously produced ethylene in chlorsulfuron-mediated increases in phenolic metabolism will be assessed. A preliminary report describing aspects of this research has appeared previously (Suttle et al. 1982).

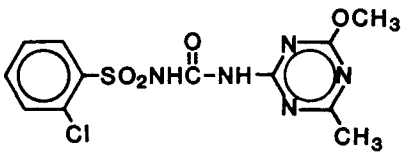
Materials and Methods

Plant Material

Seeds of sunflower (*Helianthus annuus* L.) were sown in plastic pots (10 cm diameter) containing vermiculite. Plants were grown in a growth chamber under a 14-h photoperiod (light intensity at plant height, 500 $\mu Em^{-2}sec^{-1}$, PAR). Growth-chamber day temperature was 25°C and night temperature was 21°C with a relative humidity of 50%. Plants were treated when they were 6 days old. The treatments consisted of applying a 50-µl droplet of a solution (10 mM TRIS, pH 8.0) containing varying amounts of herbicide to the apex of each intact seedling. In all experiments involving hypocotyl segments, a 1.5-cm hypocotyl segment including the cotyledonary node was subsequently excised from the treated plants.

Chemicals

Chlorsulfuron was obtained as a 75% (w/w) wettable powder from E.I. DuPont DeNemours and Company and was purified by successive recrystalizations from an acetone solution with water and hexane. All solvents used in this study were reagent-grade and were distilled in glass. TRIS (tris-[hydroxymethyl]-



Chlorsulfuron

Fig. 1. Structure of chlorsulfuron: 2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide.

aminomethane) was purchased from Schwarz Mann Biochemical Company. Cobalt chloride and magnesium chloride were purchased from Mallinckrodt Company. Sodium borate was purchased from Matheson, Coleman, and Bell Company. S-adenosyl-L-[methyl-^{14}C]methionine, *trans*-[3-^{14}C]cinnamic acid, and L-[U^{14}C]phenylalanine were purchased from Amersham. Sinapic acid (3-5-dimethoxy-4-hydroxycinnamic acid) was purchased from Aldrich Chemical Company, Inc. All other chemicals were purchased from Sigma Chemical Company.

Phenolic Determinations and Ethylene Analysis

Total phenolic materials were determined in ethanolic extracts with the Folin-Ciocalteu phenol reagent (Folin and Ciocalteu 1927) using *p*-coumaric acid as a standard. The ethylene content of the headspace was determined in 1 ml gas samples by Gas chromatography (Kende and Hanson 1976).

Dose-response Experiments

Seedlings were treated with varying amounts of chlorsulfuron in buffer. Four days after treatment, hypocotyl segments were isolated and were homogenized in 80% ethanol with a glass tissue grinder (Kontes). The homogenates were clarified by centrifugation (10,000 × *g*). The resulting pellets were re-extracted with ethanol and centrifuged as above, and 0.1 ml aliquots of the combined supernatants were assayed for total phenolic content.

Time-course Experiment

Seedlings were treated with buffer alone or buffer containing 10 μg chlorsulfuron, and 1.5-cm hypocotyl segments were isolated from these seedlings. The excised segments were allowed to stand (dark, 25°C) for 4 h to allow for the dissipation of wound ethylene. The segments were then transferred to 25-ml flasks, which were sealed and placed in a dark incubator (25°C) for 4 h. A gas sample was then removed for ethylene analysis. The segments were then removed from the flasks, homogenized in 80% ethanol and centrifuged as before, and the phenolic content was determined.

Quantitation of Hydroxycinnamic Acids

Seedlings were treated with buffer alone or buffer containing 10 μg chlorsulfuron. The procedures for the extraction, hydrolysis, and isolation of phenolic acids were essentially those of Krygier et al. (1982). At various times after treatment, 50 hypocotyl segments were excised and homogenized in methanol:acetone:water (7:7:6) with a Brinkman polytron homogenizer. The homogenates were filtered and were partially evaporated under reduced pressure at 35°C. The remaining liquid was adjusted to 2 N using NaOH. This solution was capped and flushed with nitrogen and allowed to stand (dark, 21°C) for 4 h to facilitate hydrolysis of any phenolic esters present. The hydrolysates were then adjusted to pH 2.0 with concentrated HCl. These acidified solutions were partitioned once against hexane and the hexane phase discarded. The aqueous phase was then partitioned three times against diethylether:ethylacetate (1:1). The combined organic phases were evaporated under a stream of nitrogen (40°C). The dried extracts were redissolved in methanol and aliquots were fractionated by HPLC. Fractionation was accomplished on C_{18} reverse-phase columns using the following solvent program run at 2.5 ml/min: starting conditions 15% acetonitrile in water for 3 min, then a 15-min linear gradient up to 20% acetonitrile in water, then hold. All solvents contained 1% (v/v) glacial acetic acid. Typical elution times for standards were: caffeic acid—5.50 minutes; *p*-coumaric acid—9.80 minutes; ferulic acid—11.58 minutes; and sinapic acid—11.58 min. Fractions were collected at the appropriate times and were evaporated under nitrogen (40°C). They were dissolved in methanol and assayed against the appropriate standards using the Folin-Ciocalteu reagent.

Enzyme Preparation

Seedlings were treated with buffer alone or buffer plus herbicide (10 μg/seedling). Hypocotyl segments (50) were then isolated at various times after treatment. The segments were weighed and were then homogenized in 3 vol of 0.1 M potassium phosphate buffer (pH 7.5) containing 1×10^{-2} M mercaptoethanol, Polyclar AT (0.1 vol), and sand (0.1 vol). The brine was strained through four layers of cheesecloth and was centrifuged at $1,500 \times g$ for 10 min. The homogenate was centrifuged at $10,000 \times g$ for 20 min, and the supernatant was then centrifuged at $100,000 \times g$ for 60 min. Both the pellet and supernatant from this last centrifugation were retained. The pellet was used without delay for the determination of *trans*-cinnamic acid-4-hydroxylase activity (see below). The supernatant was brought to 30% of saturation by the addition of ammonium sulfate. The resulting precipitate was removed by centrifugation at $10,000 \times g$ for 10 min. The supernatant was then brought to 60% of saturation with additional ammonium sulfate and recentrifuged as above. This pellet was dissolved in a minimum amount of glass-distilled water and was divided into three portions. Each portion was dialyzed for 18 h (4°C) against the following solutions, depending on the subsequent enzyme assay: phenylalanine ammonia lyase: 5×10^{-2}M sodium borate (pH 8.8) containing 1×10^{-2}M mercaptoethanol; 0-methyltransferase: 1×10^{-1}M potassium phosphate (pH 7.5) also containing 1×10^{-2}M mercaptoethanol; peroxidase: 1×10^{-1}M potassium

phosphate (pH 6.5). Following dialysis, the solutions were brought to volume and were used directly as the source of enzymes.

Preliminary experiments demonstrated proportionality between the amount of product formed vs. either the amount of enzyme added or incubation time for phenylalanine ammonia lyase, *trans*-cinnamic-4-hydroxylase, and soluble peroxidase. As has been found by others (Lamoureux and Rusness 1980 and references cited therein), the activity of caffeic acid-0-methyltransferase did not exhibit proportionality. For this reason, comparisons were made only between enzyme aliquots representing equivalent amounts of tissue. A note of caution accompanies the data as they are presented in the *Results* section.

Enzyme Assays

Phenylalanine ammonia lyase: the reaction mixture contained 1 ml of enzyme preparation, 5×10^{-6} mole L-phenylalanine, 5×10^{-5} mole sodium borate buffer (pH 8.8), and 0.02 μCi L-[U-^{14}C]-L-phenylalanine (521 mCi/mmole) in a total volume of 2 ml. After incubation for 4 h at 30°C, the reaction was terminated by the addition of 0.5 ml 1N HCl followed by 4 ml of diethylether. Aliquots of the acidic-ether phase were dried in scintillation vials, and the radioactivity therein was determined by scintillation spectroscopy. Product identity (*trans*-cinnamic acid) was confirmed by TLC. In addition, a similar assay employing the spectrophotometric determination of *trans*-cinnamic acid confirmed the results.

Cinnamic-4-hydroxylase: the reaction mixture contained 0.5 ml of microsomal enzyme preparation, 3×10^{-5} mole potassium phosphate buffer (pH 7.5), 6.7×10^{-2} mole NADPH, 1.4×10^{-7} mole *t*-cinnamic acid, and 0.5 μCi *t*-[3-^{14}C]cinnamic acid (55 mCi/mmole) in a total volume of 1 ml. The mixture was incubated for 30 min at 25°C and was then lyophilyzed. The residue was dissolved in absolute methanol and was fractionated by TLC. The zone corresponding to authentic *p*-coumaric acid was scraped from the plate and was quantitated by scintillation counting.

Caffeic acid-0-methyltransferase: the reaction mixture contained 1 ml of enzyme preparation, 5×10^{-5} mole potassium phosphate buffer (pH 7.5), 1×10^{-7} mole caffeic acid, 1×10^{-8} mole magnesium chloride, 5×10^{-8} mole S-adenosyl-L-methionine, and 0.09 μCi S-adenosyl-L-[methyl-^{14}C]methionine (58 mCi/mmole) in a total volume of 2 ml. After incubation for 4 h at 30°C, the reaction was terminated by the addition of 0.5 ml of 1N HCl followed by 4 ml of diethylether. The acid-ether phase was fractionated by TLC, and the zone co-chromatographing with authentic ferulic acid was scraped from the plate. The radioactivity associated with ferulic acid was quantitated by scintillation spectroscopy as before.

Peroxidase: the reaction mixture contained 0.05 ml of enzyme preparation, 3×10^{-4} mole potassium phosphate (pH 6.2), 1 μmole 0-dianisidine, and 2.6 μmole H_2O_2 in a total volume of 3 ml. The reaction was followed spectrophotometrically (A_{460}) for 1–2 min. The results of all enzyme assays were expressed as unit product formed per unit time per 50 hypocotyls. There was no qualitative difference found when the enzyme activities were expressed on a

per gram fresh weight or per mg protein basis. However, because neither of these latter two values remained constant over the experimental period (4 days) it was decided to express the data on a per unit organ basis.

Effect of Cobalt Chloride

Five-day-old sunflower seedlings were transferred to a hydroponic system containing a standard nutrient solution plus or minus 5×10^{-4}M cobalt chloride. Preliminary experiments demonstrated that concentrations of cobalt chloride less than 5×10^{-4}M had only a marginal effect on the rate of ethylene evolution from treated seedlings. When they were 6 days old, the seedlings were treated with buffer alone or buffer containing chlorsulfuron (10 μg/seedling). After allowing the treatment solution to dry, certain groups of seedlings were enclosed in a Plexiglass chamber, and sufficient ethylene was added to bring the internal chamber concentration to 25 ppm (v/v). The chamber was vented every 12 h, then closed, and additional ethylene was added to return it to the initial conditions. Over the 36 h of the experiment, the ethylene concentration of the chamber was monitored and was found to vary between 22 and 30 ppm (v/v). After 38 h, hypocotyl segments were excised and were further manipulated as described above in the section concerning the time-course experiments.

Statistical Procedures

In most cases (see below) groups of five hypocotyl segments were treated as a replication. Each treatment within an experiment was run with three to five replications. In addition, the experiments were repeated from one to three times; all yielded comparable data. The quantitation of individual hydroxycinnamic acid derivatives was run twice with groups of 50 hypocotyl segments. The experiments concerning enzyme activities were run three times with groups of 50 hypocotyl segments. In all cases, the data from a typical experiment are presented.

Results

Application of 0.1 μg or more chlorsulfuron to the apex of sunflower seedlings resulted in an inhibition of apical growth as well as a large increase in the phenolic content of hypocotyl segments isolated from the treated seedlings (Fig. 2). The maximum stimulation of phenolic accumulation occurred at chlorsulfuron doses of between 1 and 10 μg per seedling. Because of solubility limitations, the effects of doses greater than 10 μg were not evaluated. The observed accumulation of phenolic material following administration of 10 μg chlorsulfuron reached a value in excess of 3 mg *p*-coumaric acid eq./5 hypocotyl segments (approximately 1 g fresh weight). This represented a stimulation of over 10 × control values. As there was no evidence of inhibition of the response at a dose of 10 μg per seedling, this dose was used in the subsequent

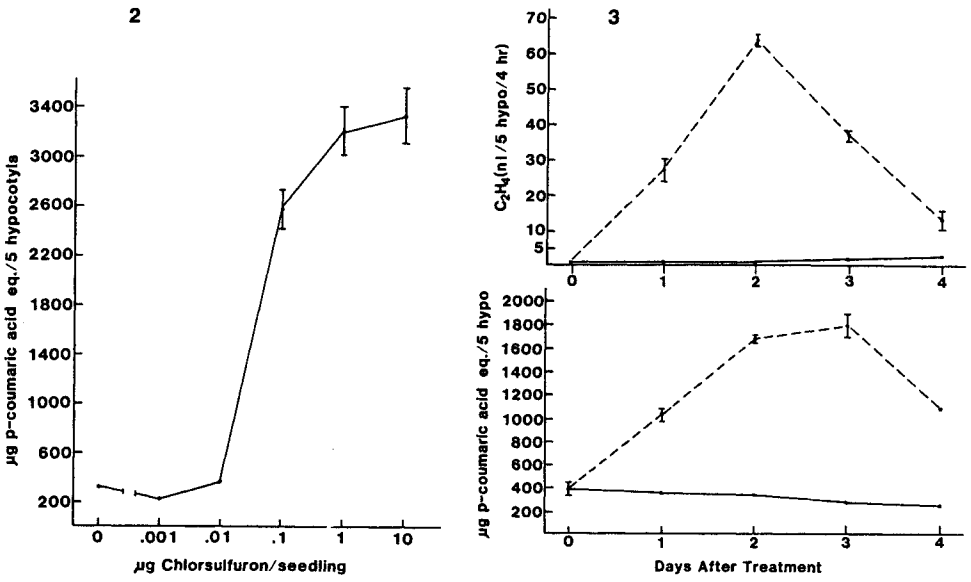


Fig. 2 and 3. Effect of various doses of chlorsulfuron on the endogenous content of phenolic material isolated from sunflower hypocotyls 4 days post-treatment. Bars indicate S.E. **Fig. 3.** Time-course of chlorsulfuron effects on the rate of endogenous ethylene evolution (upper) and phenolic content (lower) from hypocotyl segments isolated at various times following treatment (10 µg/seedling). Solid lines = control seedlings; dashed lines = chlorsulfuron-treated seedlings; bars indicate S.E.

studies. In general, it was found that increases in phenolic content were observed only in those seedlings whose growth had been inhibited by chlorsulfuron treatment.

The increase in phenolic content following chlorsulfuron treatment was readily observable 24 h posttreatment (Fig. 3). The levels of total phenolics in treated seedlings continued to rise 2 days after treatment and reached a maximum level 3 days following treatment. A conspicuous decline in phenolic content was found to have occurred between 3 and 4 days after treatment. Because intact seedlings were used in these studies, the observed decline could be explained by at least two hypotheses that are not mutually exclusive: (1) the phenolic material was metabolized to other products (i.e. lignin), or (2) a portion of the phenolic material was translocated out of the hypocotyl segments to other portions of the seedling.

Because our interests primarily concerned the effects on phenylpropanoid metabolism, we next examined the effects of chlorsulfuron treatment on the levels of several hydroxycinnamic acid derivatives. Hydroxycinnamate derivatives were readily detected in hypocotyl segments isolated from untreated seedlings (Table 1). Caffeic acid was the predominant species found in control tissues. Following chlorsulfuron treatment, large increases in the titer of all hydroxycinnamate derivatives examined were found. As with the amount of total phenolics, maximum levels of hydroxycinnamates were found 3 days after treatment. Similar to untreated tissue, caffeic acid represented the predominant

Table 1. Effect of chlorsulfuron treatment on the endogenous levels of hydroxycinnamic acids in light-grown sunflower seedlings.

Group	Days Post-treatment	Hydroxycinnamic acid (μg/50 hypocotyl)		
		p-Coumaric	Caffeic	Ferulic + Sinapic[a]
		Experiment 1		
Control	1	40	160	29
	3	28	166	32
Chlorsulfuron	1	124	832	102
	3	1052	3880	430
		Experiment 2		
Control	2	46	283	34
	4	34	77	20
Chlorsulfuron	2	840	3672	226
	4	919	3840	254

[a] Under the HPLC conditions employed, these two acids were unresolved and were therefore assayed together.

species in treated seedlings. In spite of its predominance, the largest increase over control values 3 days post-treatment was found with *p*-coumaric acid (37 × control). Because alkaline hydrolysis was employed in our isolation procedure, the degree to which these acids are esterified *in situ* is not known.

Having determined the effect of chlorsulfuron treatment on the content of several hydroxycinnamic acids, we next turned our attention to the effects of chlorsulfuron treatments on the extractable activities of four enzymes thought to be involved in hydroxycinnamate synthesis and metabolism. The extractable activity of phenylalanine ammonia lyase (PAL) was found to increase 24 h after treatment (Fig. 4). In agreement with the time-course studies on both phenolic and hydroxycinnamate content (Fig. 3, Table 1), PAL activity reached a maximum (6 × control) 3 days after treatment. The extractable activity of a particulate, NADPH-requiring, *trans*-cinnamic-4-hydroxylase system was also stimulated 24 h post-treatment, and this activity, while declining slightly, remained above control levels throughout the experiment. Soluble peroxidase activity was greatly stimulated 1 and 2 days post-treatment and declined to near-control levels after this period. Of the enzymes assayed, the 0-methyltransferase catalyzing the methylation of caffeic acid to ferulic acid was the only enzyme system not appreciably affected by chlorsulfuron treatment. The failure to observe an increase in 0-methyltransferase activity following chlorsulfuron treatment may not represent the actual situation within the seedling. Studies on the *in vitro* activity of other 0-methyltransferases isolated from higher plants have demonstrated that the enzyme is inhibited by S-adenosyl-L-homocysteine, a product of its activity (Lamoureux and Rusness 1980). Thus, the results of the *in vitro* assays of this enzyme must be interpreted with caution.

The observed increase in phenolic content previously observed 24 h post-treatment (Fig. 3) suggested that treatment of excised hypocotyl segments with chlorsulfuron might allow us to better characterize the biochemical mechanism

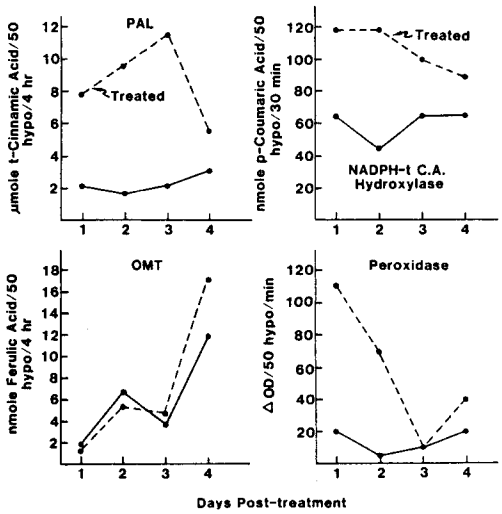


Fig. 4. Time-course of chlorsulfuron effects on the extractable activities of: phenylalanine ammonia lyase (upper left); *t*-cinnamic-4-hydroxylase (upper right); o-methyltransferase (lower left) and soluble peroxidase (lower right). Seedlings were treated with buffer ± 10 µg chlorsulfuron on day 0. Solid lines = control seedlings; dashed lines = chlorsulfuron-treated seedlings.

by which this compound exerts its stimulatory effect on phenylpropanoid metabolism. However, it was found that treatment of excised hypocotyl segments with chlorsulfuron at 1 or 100 µM had no effect on phenolic titer (data not presented). The presence of 100 µM L-phenylalanine had no effect on phenolic levels in the presence or absence of chlorsulfuron. This observation indicated that substrate availability was not limiting in the *in vitro* system. The presence, absence, or intensity of light during the 24-h *in vitro* incubation had no effect on the ability of chlorsulfuron to elicit phenolic accumulation (data not shown).

Seedlings treated with chlorsulfuron have previously been shown to exhibit large and sustained increases in the rate of ethylene evolution (Suttle and Schreiner 1982, Suttle 1982). In fact, an increase in the rate of ethylene evolution was found to parallel the increase in phenolic content following chlorsulfuron treatment (Fig. 3). Exogenous applications of ethylene have often been shown to stimulate PAL activity and result in elevated phenolic levels (Hyodo et al. 1978, also see Abeles 1973 for review). Because of these considerations, we attempted to determine the role of endogenously produced ethylene in chlorsulfuron-mediated increases in phenolic levels.

The rationale for these experiments was twofold: (1) if ethylene does mediate the response to chlorsulfuron, then treatment of seedlings with exogenous ethylene in the absence of chlorsulfuron should elicit a similar response, and (2) inhibition of endogenous ethylene synthesis in chlorsulfuron-treated seedlings should greatly reduce or eliminate the response. The inhibition of endogenous ethylene production was accomplished by the use of root-applied cobalt chloride (5×10^{-4}M).

Exposure of sunflower seedlings to exogenous ethylene (approximately 25 ppm) for 38 h had no effect on the endogenous content of phenolic compounds (Table 2). Administration of 5×10^{-4}M cobalt chloride to the root system of seedlings treated with chlorsulfuron inhibited the endogenous rate of ethylene evolution by 92% and reduced the phenolic content by 56%. However, exog-

Table 2. Effect of ethylene, chlorsulfuron, chlorsulfuron-cobalt chloride, and chlorsulfuron-cobalt chloride-ethylene on endogenous ethylene evolution and phenolic content in sunflower hypocotyl segments.

Treatment	Ethylene production (nL/5 hypo/4 hr)	Phenolic content (μg *p*-coumaric acid eq./5 hypo)
None	1.36 ± 0.10[a]	113 ± 7[a]
Ethylene	7.46 ± 0.78	88 ± 4
Chlorsulfuron	84.75 ± 8.85	643 ± 24
Chlorsulfuron/Co^{++}	6.78 ± 1.36	282 ± 26
Chlorsulfuron/Co^{++}/Ethylene	17.40 ± 0.99	273 ± 16

[a] Average ± S.E.M.

enous ethylene was not able to restore the phenolic content in cobalt chloride treated seedlings back to levels found in seedlings treated only with chlorsulfuron. These results suggested that the reduction in phenolic levels by cobalt chloride was not due to its inhibition of ethylene product but rather to some other effect, perhaps heavy metal toxicity. While high, this concentration of root-applied cobalt chloride was the lowest dose found to be effective in inhibiting ethylene evolution from sunflower seedlings. The elevated rates of ethylene evolution observed in hypocotyls isolated from seedlings exposed to 25 ppm ethylene as compared to their respective controls could have been due to the slow outward diffusion of the applied ethylene.

Discussion

The results presented in this study demonstrate that treatment of sunflower seedlings with chlorsulfuron results in a marked increase in phenolic materials present in the hypocotyl. While these investigations concerned only the hypocotyl of the seedling, it is possible that chlorsulfuron treatment also affected the content of phenolic materials in other portions of the seedling. Although the effect of chlorsulfuron treatment on the content of individual hydroxycinnamic acids was substantial, it was not as great as its effect on total phenolics (Fig. 2 vs. Table 1). The nature of the Folin-positive, non-hydroxycinnamic acid materials that accumulated following chlorsulfuron treatment is not known.

Analysis of the extractable activities of several enzymes thought to be involved in phenylpropanoid metabolism demonstrated good correlations between increases in their respective activities and the overall accumulation of phenylpropanoids (Fig. 4, Table 1). While the qualitative patterns of increased enzyme activity vs. hydroxycinnamic acids accumulated agree reasonably well with each other, the absolute values for the enzymatic activities are low relative to the amount of phenolic materials that accumulated. One possible explanation for this discrepancy is that much enzymatic activity was irrevocably lost during workup. Phenolic materials and their oxidation products are well-known nonspecific enzyme inhibitors, and it is quite reasonable to assume that given the large amounts of phenolic materials present in the initial homogenates, considerable interference and/or inactivation occurred during enzyme isola-

tion. Supporting this contention, initial experiments demonstrated that enzymatic activities were below detection in crude homogenates of these seedlings and could only be measured following protein precipitation and overnight dialysis (data not shown).

A temporal correlation between the accumulation of phenolic material and increases in PAL activity has often been demonstrated (for review see Camm and Towers 1973). This correlation has supported two hypotheses: (1) the measured increase in phenolic material is due to *de novo* synthesis, and (2) PAL may be considered to be the rate-limiting enzyme in phenylpropanoid metabolism. The temporal correlation between PAL activity and hydroxycinnamic acid levels found in this study suggests that the dramatic accumulation of phenolics can be ascribed to increased synthesis. Other studies in this laboratory (data not presented) have shown that application of α-aminooxy-β-phenylproprionic acid, a competitive inhibitor of PAL (Amrhein et al. 1976), substantially reduces the total amounts of phenolic materials found in chlorsulfuron-treated seedlings. This observation also supports the hypothesis of increased synthesis.

It can be argued that the observed ability of chlorsulfuron to stimulate phenolic levels is merely a reflection of its herbicidal properties (i.e. phytotoxicity). In fact, mechanical or chemical wounding of many plant tissues has been shown to stimulate PAL activity and elevate phenolic levels (Rhodes and Wooltorton 1978). Since the wounding phenomenon and the resulting stress reduction are apparently cellular events (Elstner and Konze 1976), the inability of chlorsulfuron to stimulate phenolic accumulation when administered to excised hypocotyl segments is not consistent with it being a chemical stress agent. Other investigations have failed to demonstrate any positive correlation between phytotoxicity and elevated PAL levels (Jangaard 1974, Hoagland and Duke 1981).

The inability of chlorsulfuron to elicit an appreciable stimulation of phenolic titer when administered *in vitro* suggests that the compound does not directly affect phenolic metabolism. The possibility exists that a factor or factors produced elsewhere in the seedling in response to chlorsulfuron treatment migrates or is translocated to the hypocotyl and subsequently induces the stimulation. The ineffectiveness of L-phenylalanine to alter the lack of *in vitro* response either in the presence or absence of chlorsulfuron suggests that it is not this factor.

Ethylene, whether endogenously produced or administered, has often been found to increase PAL activity and lead to elevated phenolic levels (Rhodes and Wooltorton 1971, 1973, Abeles 1973). The temporal correlation between increased ethylene evolution and elevated phenolic levels (Fig. 3) is suggestive of a causal relationship. However, exogenous ethylene had no effect on the level of phenolic material found in the hypocotyl segments (Table 2). The inability of exogenous ethylene to reverse the effect of cobalt chloride suggests that this ion exerts physiological effects in these seedlings that are unrelated to ethylene evolution. Taken on the whole, these results do not support the contention that endogenous ethylene is the sole mediator of chlorsulfuron-induced elevations in phenolic content. Similar situations where increased ethylene evolution was found to proceed in parallel with increases in phenolic

content but was not the causative agent have been found with elicitor-induced increases in phytoalexin accumulation in soybean cotyledons (Paradies et al. 1980) and with chlorsulfuron-mediated increases in anthocyanin accumulation in soybean hypocotyls (Suttle and Schreiner 1982).

The results presented in this paper could be of both practical and academic importance. Phenolic compounds have been assigned an important role in the regulation of plant growth and development. This assignment has been based primarily on the biological effects produced when phenolic compounds are exogenously supplied. However, considerations arising from both the relatively high dosages supplied, coupled with the problems of intracellular compartmentation, have precluded universal acceptance of a regulating role for endogenous phenolics. The ability to manipulate endogenous levels by chemical treatment might allow for types of experimentation that would be more conclusive. Secondly selected types of phenolic materials have been implicated in many aspects of plant and environment interactions (Harborne 1982). These include resistance to fungal invasion, pollination ecology, feeding preferences of insects and herbivores, and other ecological interactions. The potential for altering the endogenous spectrum and/or titer of phenolic materials present as natural plant constituents could be an exciting avenue for future research in plant bioregulation.

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