J Plant Growth Regul (1983) 2:31–45

Journal of
Plant
Growth
Regulation

Effect of Chlorsulfuron on Ethylene Evolution from Sunflower Seedlings*

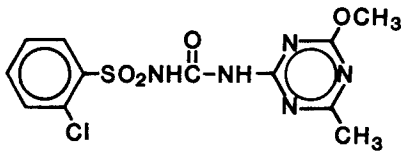
Jeffrey C. Suttle

United States Department of Agriculture, Agricultural Research Service, Metabolism and Radiation Research Laboratory, State University Station, Fargo, North Dakota 58105 USA

Received October 25, 1982; accepted March 21, 1983

Abstract. The effect of the herbicide chlorsulfuron (2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide) on ethylene production in light-grown sunflower (*Helianthus annuus* L.) seedlings was examined. Application of chlorsulfuron to the apex stimulated ethylene production in all tissues examined: cotyledons, hypocotyls, and roots. The greatest stimulation occurred in the upper portion of the hypocotyl adjacent to, and including, the cotyledonary node. Ethylene evolution from hypocotyls excised from treated seedlings was stimulated over control levels 1 day after herbicide application and reached a maximum (approx. 75 x control or 17 nl/g f wt/h) 2 to 3 days after treatment. Labeling and inhibitor studies indicated that the ethylene produced was derived primarily from methionine. Chlorsulfuron treatment stimulated the rate of accumulation of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), as well as the ability of the tissue to convert exogenous ACC to ethylene. Chlorsulfuron had little effect on ethylene production when administered to the hypocotyls *in vitro*. Removal of the cotyledons from treated seedlings reduced the rate of ethylene evolution from the hypocotyls. These results suggest that stimulation of ethylene production in sunflower hypocotyls by chlorsulfuron is not a wound response but rather is dependent on factors derived from the cotyledons.

* Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.



Chlorsulfuron
(DPX-4189)

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

The effects of herbicides and other xenobiotics on basic aspects of plant metabolism (photosynthesis, respiration, membrane function) are well documented (for review see Ashton and Crafts 1981). Much less is known about the interaction of herbicides and other plant processes, such as phytohormone content and activity. A more detailed knowledge of this latter interaction is important in order to develop a more thorough understanding concerning the phytotoxic actions of herbicides and pesticides in general.

A series of investigations have been initiated in this laboratory that are aimed at identifying interactions between herbicides and endogenous phytohormones. Once identified, these interactions may be of importance for at least two reasons: (1) the identification of novel mechanisms of action of herbicides; and (2) the identification of herbicides that could be used to modify phytohormone status in a nonlethal manner (i.e. growth modification).

Chlorsulfuron (2 - chloro - *N* - [(4 - methoxy - 6 - methyl - 1,3,5 - triazin - 2 - yl)aminocarbonyl]benzenesulfonamide) (Fig. 1) is an experimental postemergence herbicide currently being evaluated for broad-leaf weed control in small grains. Chlorsulfuron is active in minute quantities (recommended rates of approximately 10–40 g/hectare).

In laboratory studies, growth inhibition was observed 2 h after chlorsulfuron treatment, and this inhibition was attributed to a cessation of cell division (Ray 1982). Other basic cellular processes were found to be initially unaffected (Ray 1982). The potency of chlorsulfuron suggests that it alters plant growth in a novel fashion. Alterations of endogenous phytohormone levels may constitute a possible physiological target for chlorsulfuron action.

Because epinasty and leaf abscission often occur in plants treated with chlorsulfuron, the effect of chlorsulfuron on ethylene production was examined. Chlorsulfuron was shown to induce elevated rates of ethylene production and alterations in enzyme activity in soybeans (Suttle and Schreiner 1982). Other broadleaf crops exhibited similar physiological alterations with sunflower seedlings exhibiting the most dramatic response. As sunflowers are often grown adjacent to or in rotation with small grains, chlorsulfuron injury to this crop represents a real concern.

This report describes the effect of chlorsulfuron on ethylene evolution from sunflower seedlings and attempts to localize the biochemical mechanism by which chlorsulfuron exerts its effect on ethylene evolution. A preliminary report concerning a portion of this work has appeared previously (Suttle 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 $\mu Em^{-2}sec^{-1}$). 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 m*M* TRIS, pH 8.0) containing varying amounts of herbicide to the apex of each seedling. In all experiments involving hypocotyl segments, a 1.5-cm hypocotyl segment including the cotyledonary node was excised from the treated plants. Except where otherwise noted, excised hypocotyl segments were allowed to stand for 4 h prior to the determination of the rate of ethylene evolution.

Chemicals

Chlorsulfuron was obtained from E.I. duPont deNemours and Company. Chloramphenicol, aminooxyacetic acid, α-aminoisobutyric acid, 6-benzylaminopurine, indole-3-acetic acid, and MES (2[N-morpholino]ethanesulfonic acid) were purchased from Sigma Chemical Company. 1-Aminocyclopropane-1-carboxylic acid was purchased from Calbiochem Company. Cobalt chloride was purchased from Mallinckrodt Company. TRIS (tris-[hydroxymethyl]-aminomethane) was purchased from Swartz Mann Biochemical Company. L-[^{14}C-U]-Methionine was purchased from Amersham. Aminoethoxyvinylglycine was a gift of Dr. R. W. Bagley of HLR Sciences, Inc.

Ethylene Analysis

Samples of the headspace of sealed flasks were removed using a 1-ml plastic syringe. Ethylene analysis was performed on an activated alumina column as previously described (Kende and Hanson 1976). The identity of ethylene was confirmed by absence of the peak when the tissues were incubated in the presence of $Hg(ClO_4)_2$.

Ethylene Production from Various Tissues

Seedlings were treated with buffer alone or buffer containing 10 μg chlorsulfuron. Two days after treatment, the plants were dissected into roots, cotyledons, and upper, middle, and lower hypocotyls. The excised tissues were weighed and placed into flasks. The flasks were sealed with serum stoppers and were incubated in the dark at 25°C. After 4 h (the minimal time required to detect ethylene evolution from control tissues), a gas sample was removed

with a syringe and the ethylene content was determined by gas chromatography (GC). Ethylene production was calculated on a gram fresh weight basis.

Dose-Response Experiments

Seedlings were treated with varying amounts of chorsulfuron in 50 μl of buffer, and hypocotyl segments were excised 2 days after treatment. Groups of five hypocotyl segments were then placed in 25-ml flasks containing a piece of wetted filter paper (#1). The flasks were sealed and incubated in the dark at 25°C. After 4 h the ethylene content of the headspace was analyzed by GC.

Time Course Experiments and the Extraction and Determination of 1-aminocyclopropane-1-carboxylic acid

Seedlings were treated with buffer alone or buffer containing 10 μg chlorsulfuron. Each day post-treatment, hypocotyl segments were excised and placed in 25-ml flasks, and the flasks were then sealed. The flasks were incubated in the dark at 25°C. After 4 h the ethylene content of the headspace was determined by GC. The sections were then removed from the flasks and were homogenized in ice-cold 80% EtOH with a glass tissue grinder. The extracts were clarified by centrifugation (10,000 g/15 min). Following decantation the pellet was re-extracted with additional ice-cold 80% EtOH. Following centrifugation (as above) the combined supernatants were dried under a stream of nitrogen (35°C). The dried extracts were eluted with distilled water and were assayed for 1-aminocyclopropane-1-carboxylic acid (ACC) as described by Lizada and Yang (1979). The assay was corrected for efficiency and inhibitory material through the use of an internal standard. While not routinely employed, TLC of the extract followed by ACC assay demonstrated that the zone yielding the bulk of the ethylene liberated by the assay co-chromatographed with authentic ACC.

In Vitro *Treatment*

Hypocotyl segments were isolated from untreated 6-day-old seedlings and were allowed to stand in the dark (25°C) for 4 h. At this time groups of five hypocotyls were placed in 50-ml flasks that contained 5 ml of treatment solution. Chemicals to be tested for their ability to induce ethylene evolution were dissolved in 1×10^{-2} M potassium phosphate buffer (pH 5.4) containing 3×10^{-3} M calcium chloride and 50 μg/ml chloramphenicol. The flasks were sealed and incubated in the dark (25°C) for 24 h. At this time a gas sample was removed for ethylene analysis.

Effect of Selected Organ Removal

The following tissues were removed from 6-day-old seedlings: none, roots, or cotyledons. These plants were transferred to a hydroponic culture system and

were grown in aerated nutrient solution. One half of these seedlings were treated with 1 μg chlorsulfuron in buffer. Two days after treatment, hypocotyl segments were excised. Groups of five segments were placed in 25-ml flasks that were then sealed. The flasks were incubated in the dark (25°C) for 4 h. A gas sample was then removed for ethylene analysis.

Effect of Inhibitors

Seedlings were treated with 10 μg chlorsulfuron. Hypocotyl segments were excised from the seedlings 1 and 2 days after herbicide treatment. The following inhibitors were dissolved in 10 m*M* TRIS (pH 8.0) containing 50 μg/ml chloramphenicol:aminooxyacetic acid (0.1 m*M*), aminoethoxyvinylglycine (0.1 m*M*), cobalt chloride (1.0 m*M*), and α-aminoisobutyric acid (10.0 m*M*). Immediately following excision, the hypocotyl segments were floated on these inhibitor solutions, in 50-ml flasks for 5 h. Following this incubation, the flasks were sealed and incubated for 4 h in the dark (25°C). Ethylene evolution was determined by GC.

Specific Radioactivities of Ethylene and ACC

0.11 μCi of L-[U-^{14}C]-Methionine (282 mCi/mmole) in water was applied twice daily to the apex of 5-day-old sunflower seedlings (for a total of five applications/seedling). When the seedlings were 6 days old, 10 μg chlorsulfuron was applied to each apex as before. Two days after herbicide treatment, hypocotyl segments were excised and were washed with distilled water for 15 min. Groups of five hypocotyl segments were placed in flasks containing a CO_2 trap (filter paper wetted with saturated $Ba(OH)_2$ in 1 *N*(NaOH), and the flasks were sealed. The sealed flasks were incubated in the dark (25°C) for 4.5 h. At this time an aliquot of the headspace was withdrawn and the specific radioactivity of ethylene was determined as described previously (Suttle and Kende 1980). The hypocotyl segments were extracted in ice-cold 80% EtOH, the extract centrifuged (10,000 g/15 min), and the supernatant was evaporated under a stream of nitrogen (35°C). The ethanol solution material was eluted with distilled water, and ethylene was liberated from carbon atoms 2 + 3 of ACC as described above. The specific radioactivity of the ACC-derived ethylene was determined as described above.

Effect of Exogenous 1-aminocyclopropane-1-carboxylic acid

Seedlings were treated with 10 μg chlorsulfuron or buffer alone. Day 0 refers to the day of herbicide treatment. Each morning, groups of hypocotyl segments were excised from control and treated seedlings. Groups of five segments were transferred to 25-ml flasks containing 3.5 ml of buffer solution ± 0.1 m*M* ACC. The buffer solution was 10 m*M* MES (pH 6.0) containing 50 μg/ml chloramphenicol. The flasks were sealed and transferred to a dark incubator (25°C). After 4 h a gas sample was withdrawn and the ethylene content assayed by

GC. The ACC response was calculated by taking the difference between segments floated on buffer alone and buffer containing ACC.

Statistical Procedures

In all cases, groups of five hypocotyl segments were treated as a replication. Each treatment within an experiment was run with three to five (usually five) replications. With one exception (see below), each experiment was repeated from one to three times, all yielding comparable data. The data from a typical experiment are presented. The experiment comparing the specific radioactivities of ethylene and ACC was performed only once with three replications.

Results

Under the conditions employed in this study, hypocotyl growth (elongation and dry weight increase) had essentially ceased by 6 days post-sowing (i.e. the day of herbicide treatment). Thus the effects of chlorsulfuron described in this report were not compounded by alterations of hypocotyl growth.

Treatment of intact sunflower seedlings with chlorsulfuron (10 μg/seedling) resulted in an increased rate of ethylene evolution from all tissues examined (Fig. 2). Maximum rates of ethylene evolution (per gram fresh weight) from treated tissues were as follows (roots: 9.63 nl/4 h; cotyledons: 29.90; upper hypocotyl: 162.43; mid-hypocotyl: 132.94; and lower hypocotyl: 76.97). Because the largest increase in the rate of ethylene evolution was found to have occurred in the upper portion of the hypocotyl, this portion of the seedling was used in all further studies. Unless otherwise noted, intact seedlings were treated with the herbicide and at various times thereafter the upper portion of the hypocotyl was excised and used for the subsequent determinations.

Application of 100 ng or more of chlorsulfuron resulted in observable growth inhibition and a marked stimulation of ethylene evolution 2 days post-treatment (Fig. 3). The rate of ethylene evolution increased in a linear fashion as the dose of chlorsulfuron was increased logarithmically. There was no evidence of saturation or inhibition of the response at the highest dose employed in this study (10 μg/seedling). The emission of other hydrocarbons (notably ethane) was also examined in chlorsulfuron-treated tissues. Untreated tissues evolved a small but detectable amount of ethane, but chlorsulfuron treatment (10 μg/seedling) had no effect on the rate of evolution (data not shown).

The increase in ethylene evolution following chlorsulfuron treatment was evident within 1 day following herbicide treatment (Fig. 4). The rate of ethylene evolution in herbicide-treated tissue reached a maximum 2 days post-treatment and declined thereafter. Seven days post-treatment ethylene evolution from untreated hypocotyl segments exceeded that from treated tissue.

Treatment of excised sunflower hypocotyl segments with 100 μ*M* chlorsulfuron *in vitro* (Table 1) had only a marginal effect on ethylene evolution (1.6 x control). The observed ability of the phytohormones indole-3-acetic acid (IAA) and 6-benzyladenine (BA) to elicit a substantial increase in ethylene evolution

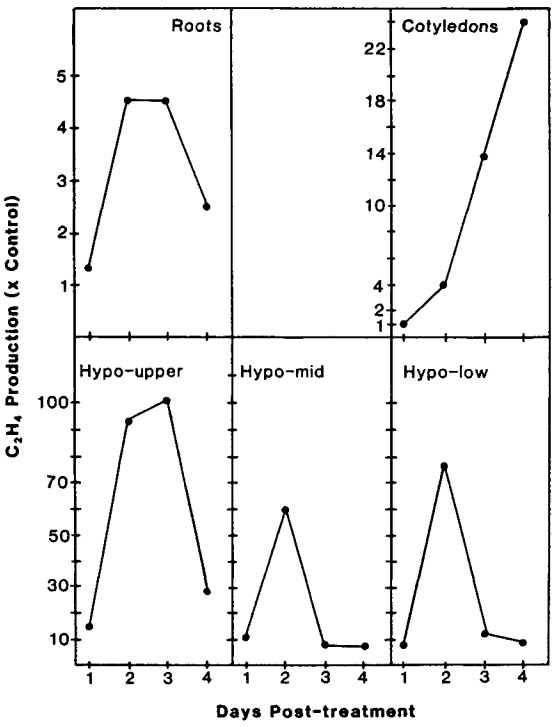


Fig. 2. Effect of chlorsulfuron treatment (10 μg/seedling) on the rate of ethylene production from various sunflower seedling tissues excised 2 days post-treatment. Hypo = hypocotyl segment. Ethylene production rates were calculated on a per gram fresh weight basis and are expressed as the ratio of ethylene production in treated tissues divided by the production rate in control tissues (values >1 denote stimulation by chlorsulfuron).

(33 x and 4 x control, respectively) indicated that these tissues were fully competent to produce ethylene. These results suggested that the ability of chlorsulfuron to increase the rate of ethylene evolution from hypocotyl segments was not the result of a direct action of the herbicide on the tissue (i.e. contact activity) but rather was mediated by a factor or factors derived from other portions of the seedling.

The source or sources of these "seedling factors" was determined by examining the effect of selective organ removal on the ability of chlorsulfuron to stimulate ethylene evolution. With no organ removed, chlorsulfuron treatment induced a 17.7-fold increase in the rate of ethylene evolution (Table 2). Removal of the roots had no effect on the basal rate of ethylene evolution but did reduce the chlorsulfuron effect by 43%. The most dramatic effect occurred when the cotyledons were excised. Basal ethylene evolution was reduced by 26%, and the chlorsulfuron effect was reduced by 89%. The 2-fold stimulation of ethylene production induced by chlorsulfuron in seedlings without cotyledons is nearly equal to the 1.6-fold stimulation of ethylene evolution observed when excised hypocotyls were treated with chlorsulfuron *in vitro* (Table 1).

In order to confirm the biochemical site or sites at which chlorsulfuron acts to stimulate ethylene evolution, it was necessary to confirm the role of the methionine pathway of ethylene biosynthesis in treated tissues. Initially, the effects of several well-known inhibitors of the methionine pathway were examined in chlorsulfuron-treated tissues. Inhibitors of ACC synthase (aminooxy-

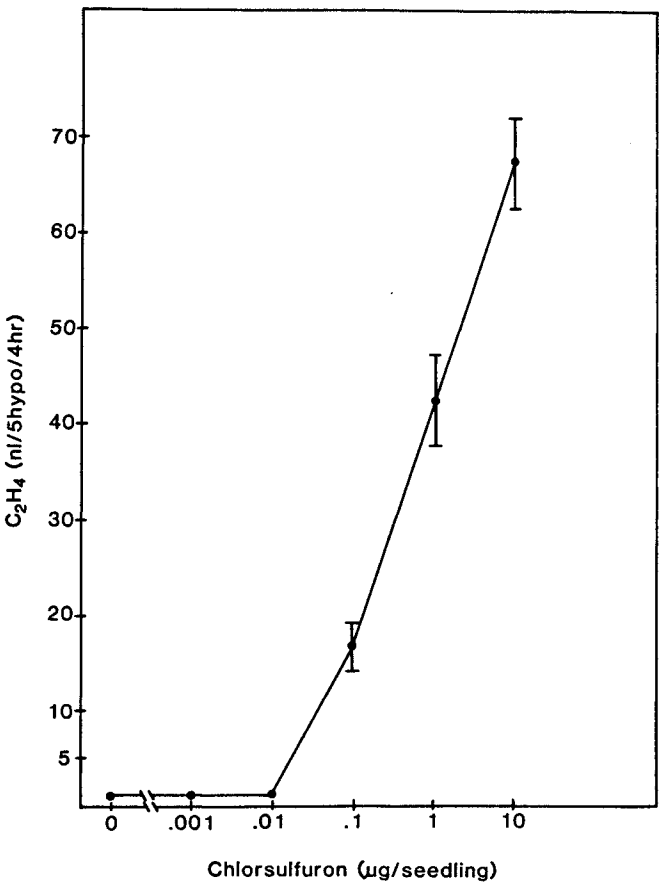


Fig. 3. Effect of various doses of chlorsulfuron on the rate of ethylene evolution from hypocotyl segments isolated 2 days post-treatment. Bars indicate standard error. Hypo = 1.5 cm hypocotyl segment.

acetic acid [AOA], aminoethoxyvinylglycine[AVG]) were effective 1 day post-treatment (Fig. 5). However, both of these compounds were without effect 2 days post-treatment. In contrast, inhibitors of the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Co^{++} and α-aminoisobutyric acid [αAIBA]) were effective on either day, although the degree of inhibition was reduced 2 days post-treatment.

As a second test of the role of methionine in treated tissues, the specific radioactivities of ethylene and carbon atoms 2 + 3 of ACC were determined following application of L-[U-^{14}C]-methionine to herbicide-treated seedlings. Two days post-treatment, radioactivity was found in both ethylene and ACC (Table 3). Moreover, comparisons of the specific radioactivities of both ACC and ethylene revealed no significant differences. Thus it was concluded that the methionine pathway is operative in chlorsulfuron-treated tissues. The large reduction in the specific radioactivities of ACC and ethylene relative to the administered methionine indicated a substantial reduction by endogenous pools.

Having established methionine as the major source of ethylene in chlorsulfuron treated hypocotyls, we next attempted to determine at what points along this pathway chlorsulfuron treatment stimulates. The effect of chlorsulfuron

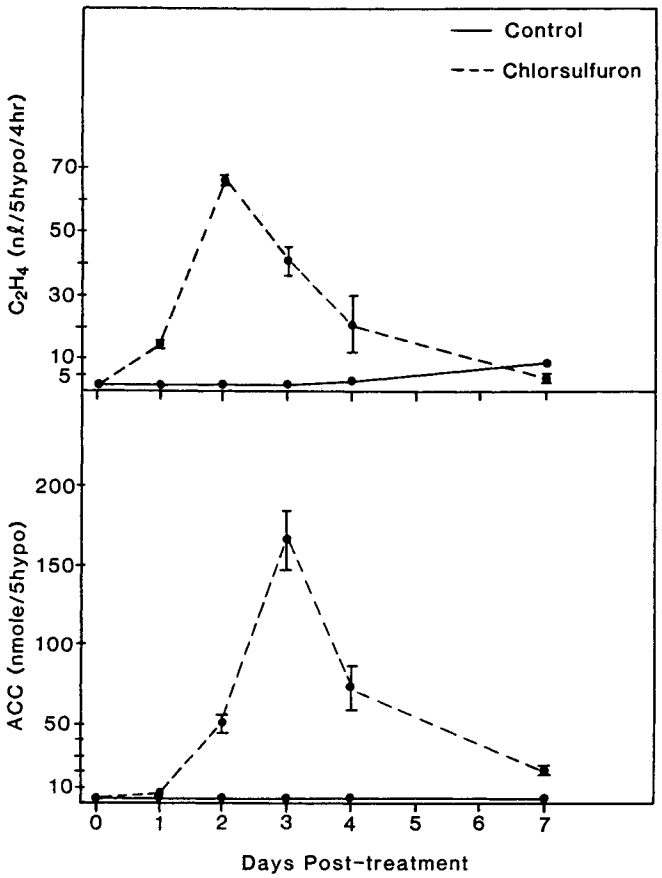


Fig. 4. Time course of chlorsulfuron effects on the rate of ethylene evolution (upper) and endogenous ACC content (lower) from hypocotyl segments isolated at various times following herbicide treatment (10 μg/seedling). Bars indicate standard error.

Table 1. Effect of chlorsulfuron, indole-3-acetic acid (IAA), and 6-benzyladenine (BA) on ethylene production from sunflower hypocotyl segments treated *in vitro*.

Treatment	Concentration (m*M*)	Ethylene production (nl/5 hypo/24 h, ± SE)
None	—	3.41 ± 0.45
Chlorsulfuron	0.1	5.44 ± 0.78
BA	0.1	15.13 ± 1.82
IAA	0.1	115.11 ± 11.36

Hypocotyl segments (1.5 cm) were isolated from 6-day-old sunflower seedlings and were floated on solutions of 10 m*M* potassium phosphate (pH 5.4) containing 3 m*M* $CaCl_2$ and 50 μg/ml chloramphenicol.

treatment on the endogenous levels of ACC was determined in hypocotyls isolated from treated seedlings. In control hypocotyls both the rate of ethylene evolution and the endogenous levels of ACC were low and relatively constant (Fig. 4). One day post-treatment there was a substantial increase in the rate of ethylene evolution. Although not evident from the figure, chlorsulfuron treat-

Table 2. Effects of selected organ removal on chlorsulfuron-induced ethylene production from sunflower hypocotyl segments.

Organ removed	Ethylene production (nl/5 hypo/4 h)		Stimulation (× control)
	Control	Chlorsulfuron	
None	0.97 ± 0.00	17.20 ± 3.46	17.7
Roots	1.04 ± 0.04	10.47 ± 0.85	10.1
Cotyledons	0.72 ± 0.07	1.43 ± 0.47	2.0

Organ removal was performed on 6-day-old sunflower seedlings prior to chlorsulfuron treatment (1 μg/seedling). Ethylene production was determined 2 days following herbicide treatment.

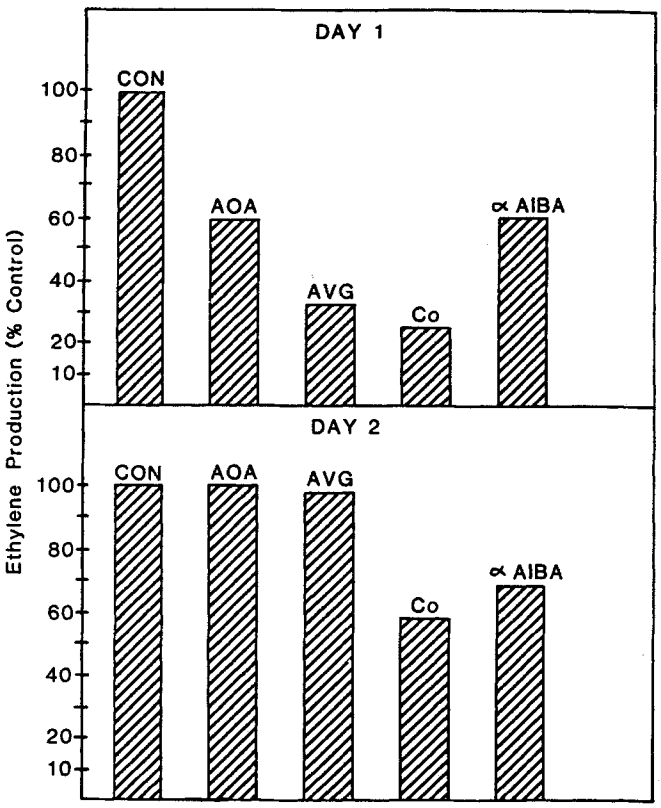


Fig. 5. Effects of various inhibitors on chlorsulfuron-induced ethylene evolution from hypocotyl segments isolated 1 and 2 days post-treatment (10 μg/seedling). Con: no treatment; AOA: 0.1 m*M* aminooxyacetic acid; AVG: 0.1 m*M* aminoethoxyvinylglycine; Co: 1.0 m*M* $CoCl_2$; αAIBA: 10.0 m*M* α-aminoisobutyric acid.

ment also induced a 12-fold increase in endogenous ACC levels within 1 day of treatment (0.13 nmole in control hypocotyls vs. 1.61 nmole in chlorsulfuron-treated segments). Both the rate of the ethylene evolution and the content of ACC continued to rise 2 days post-treatment. At this point the rate of ethylene evolution began to decline. However, the maximum titer of ACC was reached 3 days post-treatment. Thereafter it declined as well.

Table 3. Specific radioactivities of ethylene and carbon atoms 2 + 3 of ACC isolated from chlorsulfuron-treated sunflower hypocotyl segments following administration of L-[U-^{14}C]-Methionine.

Compound	Specific radioactivity (pCi/nmole ± SE)
Ethylene	9.11 ± 0.94
ACC	11.82 ± 2.67

0.53 μCi of L-[U-^{14}C]-Methionine (282 mCi/mmole) were administered to sunflower seedlings over a period of 3 days prior to harvesting. Chlorsulfuron (10 μg/seedling) was applied to the seedlings 2 days prior to harvesting.

The effect of chlorsulfuron treatment on the ability of the tissue to convert ACC to ethylene was examined by providing a relatively high concentration of exogenous ACC (Fig. 6). The ability of control tissues to convert ACC to ethylene was virtually constant over the duration of the experiment (the slight reduction observed on day 3 occurred consistently). Chlorsulfuron treatment led to an enhanced ability to convert ACC to ethylene 1 day post-treatment (160% of control). Two days post-treatment exogenous ACC had only a marginal effect on the rate of ethylene evolution from treated tissues, and by day 3 it had no effect.

Discussion

The results presented in Figs. 2–4 clearly demonstrate that treatment of sunflower seedlings with chlorsulfuron results in a marked elevation of ethylene evolution that is sustained for at least 4 days post-treatment. Under the conditions employed in this study, elevations of ethylene evolution were detected only in those seedlings that exhibited an inhibition of apical growth. The ability of the well-known inhibitors of the methionine pathway of ethylene biosynthesis to reduce the rate of ethylene evolution in treated seedlings (Fig. 5) coupled with the similarity between the specific radioactivities of ethylene and ACC (Table 3) support the hypothesis that methionine is the major, if not sole, precursor of ethylene in these tissues.

The observed loss of efficacy of both AOA and AVG 2 days post-treatment (Fig. 5) could be interpreted as indicating an alternate pathway of ethylene biosynthesis. This conclusion is discounted by the results of the labeling study (Table 3) that was conducted 2 days post-treatment. Since both of these inhibitors block the formation of ACC (i.e. ACC synthase activity) an alternate explanation for the observed loss of efficacy is that the endogenous pool(s) of ACC is (are) sufficient to sustain ethylene evolution over the measurement period (4 h). The very high levels of endogenous ACC found in these tissues 2 days post-treatment (Fig. 4) is consistent with this explanation. Assuming a constant rate of ethylene evolution, there is sufficient endogenous ACC to sustain the observed rate of production for at least 68 h. A similar loss of efficacy of AVG has been noted previously during the transition of ripening tomato tissues from the green to pink stages (Baker et al. 1978). Subsequent

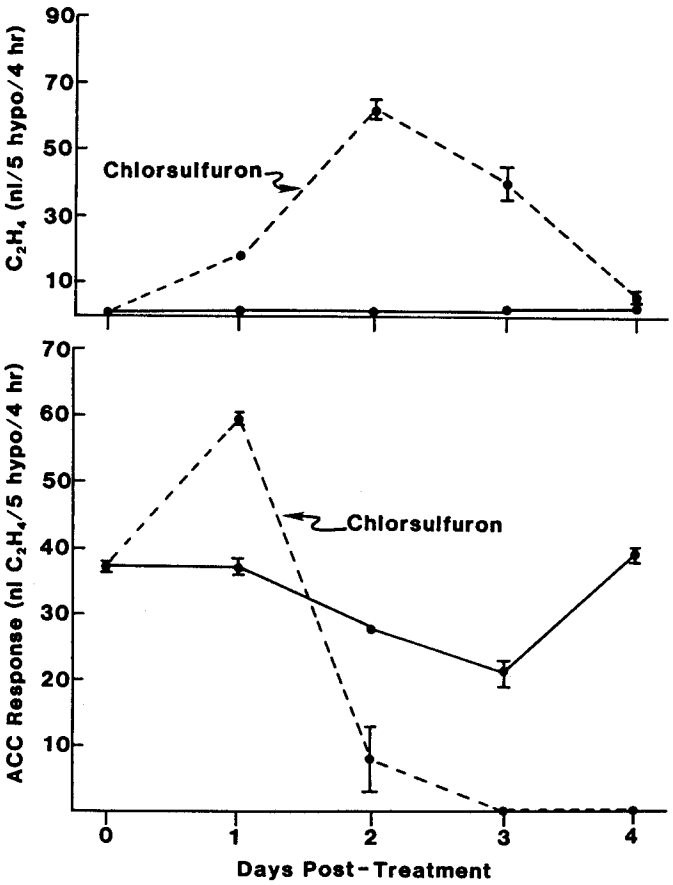


Fig. 6. Effect of chlorsulfuron treatment (10 μg/seedling) on the rate of ethylene evolution (upper) and on the ability of the tissues to utilize exogenous ACC (0.1 m*M*). See text for details. Bars indicate standard error. Hypo = 1.5 cm hypocotyl segment. Controls = solid lines.

work demonstrated that this loss of efficacy was correlated with elevated levels of endogenous ACC in the pink tissues (Boller et al. 1979).

Two sites of regulation in the pathway of ethylene biosynthesis are currently recognized: (1) the conversion of S-adenosylmethionine (SAM) to ACC; and (2) the conversion of ACC to ethylene (see Yang 1980 for review). One day post-treatment, there was a 19-fold increase in the rate of ethylene evolution coupled with a 12-fold increase in ACC content (Fig. 4). Also the ability of the tissue to convert ACC to ethylene was enhanced 1.6 times (Fig. 6). Endogenous ACC reached a maximum level of over 160 nmoles/5 hypo (5 hypo = 1 g fresh weight). This peak in ACC content was found to occur at the same time that the tissue lost its responsiveness to exogenous ACC (Figs. 4 and 6). These results suggest that 3 days post-treatment the system converting ACC to ethylene is saturated. Seven days post-treatment the ACC levels in treated tissues are still 35 × that found in the controls, yet the rate of ethylene evolution in treated tissue has declined below control values. These results suggest that the system converting ACC to ethylene is more susceptible to herbicide damage than is ACC synthase. Indeed, many studies have shown that this final step is

very sensitive to a variety of stresses: thermal (Yu et al. 1980), chemical, and osmotic (Abelbaum et al. 1981). The ability to simultaneously stimulate these two steps in the pathway of ethylene biosynthesis has also been shown to occur when mannitol is administered to citrus leaf tissues (Riov and Yang 1982).

The mechanism(s) by which chlorsulfuron exerts its stimulatory effects on ethylene evolution is not clear at present. As reviewed by Abeles (1973), two classes of chemicals have been shown to stimulate ethylene evolution: (1) substances with hormonal activity (i.e. IAA, 2,4-D); and (2) chemicals that elicit a stress or wound reaction (i.e. paraquat). It would be tempting to include chlorsulfuron in either or both of these classes. However, that inclusion seems premature in light of several observations made in this study. First is the observed ineffectiveness of chlorsulfuron to induce ethylene evolution when administered to excised plant tissues (Table 1). This is in marked contrast to the stimulatory effects of IAA and BA observed in the same experiment. Second, when tested in a variety of bioassay systems that respond to chemicals with auxin- or cyto-kinin-like activity, chlorsulfuron failed to induce any detectable hormonal response (data not presented). Thus, it is unlikely that chlorsulfuron possesses any hormone-like activity.

That many herbicides induce a chemical stress or wounding response in plants is clear. Equally well documented is the fact that elevated ethylene evolution is a characteristic of the herbicide-stress syndrome in plants (Yang and Pratt 1978, Morgan 1976). However, chlorsulfuron behaves quite differently from those herbicides that elicit a stress reaction. First, observable tissue damage (necrosis) is slow to develop in chlorsulfuron-treated tissues. This is in marked contrast to the rapid action of contact herbicides such as paraquat. Since the wounding phenomenon and the resultant stress reaction is apparently a cellular event (Elstner and Konze 1976), the failure of chlorsulfuron to induce ethylene evolution in excised tissues is also not consistent with it being a chemical stress agent. Well-known inducers of the wound response in plants (Cd^{++}, Cu^{++}, SO_3^{2-}) have been shown to elicit the typical symptoms (i.e. elevated ethylene evolution) when administered to excised tissues (Fuhrer 1982, Yu and Yang 1980, Bressan et al. 1979). Ethane emission, considered to result from lipid peroxidation, has proved to be a far-superior assay for both mechanical (Elstner and Konze 1976) and chemical wounding (Bressan et al. 1979). The absence of any effect of chlorsulfuron treatment on the rate of ethane emission is also not consistent with its inclusion as a chemical stress agent.

The results presented in Table 2 suggest that a factor or factors originating in the cotyledons is necessary for the observed effect of chlorsulfuron to stimulate ethylene evolution from the hypocotyls. The interpretation of excision experiments can be problematical owing to the multiple interactions that occur between the various plant organs. In young seedlings, the cotyledons are a source of both nutritional and hormonal substances. The ability of excised hypocotyls to respond to IAA and BA (Table 1) demonstrates that, at least in the short run, the hypocotyls have sufficient substrate and energy for elevated ethylene production. Furthermore, comparative experiments in this laboratory have shown that cotyledon excision has a much more dramatic effect on chlor-

sulfuron-induced ethylene evolution as compared to 2,4-D-induced ethylene evolution (data not presented). Cotyledons have been shown to be required for the development and expression of a number of seedling responses. Cotyledon excision in sunflower seedlings has been shown to reduce phototropic curvature (Bruinsma et al. 1975) and adventitious rooting (Fabijan et al. 1981). In the latter case, application of an aqueous extract of cotyledons has been shown to partially restore the response (Fabijan et al. 1981). The nature of the cotyledon factor(s) implicated in this investigation is currently being explored. Presently we speculate that it is (or they are) hormonal in nature.

While the effect of chlorsulfuron on ethylene evolution from treated seedlings is well documented in this study, the role of ethylene in chlorsulfuron-mediated injury is not known. Sunflower seedlings treated with chlorsulfuron exhibit the following visible symptoms: inhibition of apical growth, apical chlorosis, vein discoloration and hypocotyl browning, progressive necrosis, and ultimately death. Like chlorsulfuron, ethylene treatment has been shown to inhibit cell division in higher plants (Apelbaum and Burg 1972, Rost and Sammut 1982). Furthermore, analyses of both chlorsulfuron- and ethylene-treated tissues have shown that, although there is a marked reduction in the number of mitotic figures, the frequency distribution of the mitotic stages remains unchanged (Ray 1982, Apelbaum and Burg 1972). Continuous exposure of sunflower seedlings to 10 ppm ethylene resulted in the inhibition of apical growth as well as apical chlorosis (data not shown). In addition we have found that elevations of ethylene evolution can be detected within 9 h of chlorsulfuron treatment (not presented). It is therefore possible that endogenous ethylene mediates, or in some manner participates in, at least these two aspects of chlorsulfuron action. Further studies are needed to resolve this question.

Although physiologically potent, ethylene, even at high concentrations, is not in itself lethal (Abeles 1973). Normal growth typically resumes following removal of the gas. Thus, it appears unlikely that the ultimate cause of seedling death following chlorsulfuron treatment is solely the result of excessive ethylene production. Nevertheless, the large and sustained elevation of ethylene evolution that follows chlorsulfuron treatment undoubtedly affects many sensitive physiological processes. These alterations in combination with other metabolic perturbations that follow chlorsulfuron treatment certainly contribute to the seedling's demise.

Acknowledgments. The following individuals are gratefully acknowledged for their help: Mrs. Jeri Schmidt for typing this manuscript, Mr. Tom Hlavaty for preparing and photographing the figures, and A.K. Mattoo and my associates in the plant science section of the MRRL for their thoughtful suggestions.

References

Abeles FB (1973) *Ethylene in Plant Biology*. Academic Press, New York

Apelbaum A, Burg SP (1972) Effect of ethylene on cell division and deoxyribonucleic acid synthesis in *Pisum sativum*. Plant Physiol 50:117–124

Apelbaum A, Burgoon AC, Anderson JD, Solomos T, Lieberman M (1981) Some characteristics of the system converting 1-aminocyclopropane-1-carboxylic acid to ethylene. Plant Physiol 67:80–84

Ashton FM, Crafts AS (1981) *Mode of Action of Herbicides*. John Wiley & Sons, Inc, New York
Baker JE, Lieberman M, Anderson JD (1978) Inhibition of ethylene production in fruit slices by rhizobitoxine analog and free radical scavengers. Plant Physiol 61:886–888
Boller T, Herner RC, Kende H (1979) Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. Planta 145:293–303
Bressan RA, LeCureux L, Wilson LG, Filner P (1979) Emission of ethylene and ethane by leaf tissue exposed to injurious concentrations of sulfur dioxide or bisulfite ion. Plant Physiol 63:924–930
Bruinsma J, Karssen CM, Benschop M, VanDort JB (1975) Hormonal regulation of phototropism in the light-grown sunflower seedling, *Helianthus annus* L.: Immobility of endogenous indole-acetic acid and inhibition of hypocotyl growth by illuminated cotyledons. J Exp Bot 26:411–418
Elstner EF, Konze JR (1976) Effect of point freezing on ethylene and ethane production by sugar beet leaf discs. Nature 263:351–352
Fabijan D, Yeung E, Mukherjee I, Reid DM (1981) Adventitious rooting in hypocotyls of sunflower (*Helianthus annuus*) seedlings. I. Correlative influences and developmental sequence. Physiol Plant 53:578–588
Fuhrer J (1982) Ethylene biosynthesis and cadmium toxicity in leaf tissue of beans (*Phaseolus vulgaris* L.). Plant Physiol 70:162–167
Kende H, Hanson AD (1976) Relationship between ethylene evolution and senescence in morning glory flower tissue. Plant Physiol 57:523–527
Lizada MCC, Yang SF (1979) A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100:140–145
Morgan PW (1976) Effects on ethylene physiology. In: Audus LJ (ed) Herbicides: Physiology, biochemistry, ecology. Academic Press, New York, pp 255–280
Ray TB (1982) The mode of action of chlorsulfuron: A new herbicide for cereals. Pest Biochem Physiol 17:10–17
Rost ThL, Sammut M (1982) Regulation of cell division in pea root tips after wounding: A possible role for ethylene. Protoplasma 111:1–9
Riov J, Yang SF (1982) Stimulation of ethylene production in citrus leaf discs by mannitol. Plant Physiol 70:142–146
Suttle JC (1982) Effect of chlorsulfuron on ethylene production. Plant Physiol 69:S–18
Suttle JC, Kende H (1980) Methionine metabolism and ethylene biosynthesis in senescing petals of *Tradescantia*. Phytochem 19:1075–1079
Suttle JC, Schreiner DR (1982) Effects of DPX-4189 (2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide) on anthocyanin synthesis, phenylalanine ammonia lyase activity and ethylene production in soybean hypocotyls. Can J Bot 60:741–745
Yang SF (1980) Regulation of ethylene biosynthesis. HortiSci 15:238–243
Yang SF, Pratt HK (1978) The physiology of ethylene in wounded plant tissues. In: Kahl G (ed) Biochemistry of wounded plant tissues. deGruyter, Berlin, pp 595–622
Yu Y, Yang SF (1980) Biosynthesis of wound ethylene. Plant Physiol 66:281–285
Yu Y, Adams DO, Yang SF (1980) Inhibition of ethylene production by 2,4-dinitrophenol and high temperature. Plant Physiol 66:286–290