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An Evaluation of the Role of Ethylene in Herbicidal Injury Induced by Picloram or Clopyralid in Rapeseed and Sunflower Plants[1]

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J. CHRISTOPHER HALL*[2], PAWAN K. BASSI, MARY S. SPENCER, AND WILLIAM H. VANDEN BORN
Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

ABSTRACT

The role of ethylene in herbicidal injury induced by 4-amino-3,5,6-trichloropicolinic acid (picloram) or 3,6-dichloropicolinic acid (clopyralid) was investigated in sunflower (*Helianthus annuus* L.) and rapeseed (*Brassica napus* L. cv Altex). Picloram induces herbicide injury in both species, whereas clopyralid induces injury only in sunflower. Picloram applied to the third leaf of a rapeseed plant increased ethylene evolution several-fold. Clopyralid had no effect on ethylene production in rapeseed. In sunflower, both picloram and clopyralid elevated ethylene production. Ethylene biosynthesis induced by the herbicide treatment was not restricted to treated areas. When clopyralid was applied only to the lower stem and cotyledons of sunflower, the herbicide treatment resulted in an increase in the rate of ethylene production from the true leaves. Increased ethylene production preceded or coincided with the onset of morphological responses induced by a herbicide application to a susceptible species. The contrast in ethylene production by these two plant species cannot be accounted for by differences in absorption and translocation of clopyralid and picloram.

Treatment with aminoethoxyvinylglycine (AVG) before picloram or clopyralid application prevented an increase in ethylene production. Pretreatment with AVG also delayed the development of morphological changes induced by picloram or clopyralid. It appears that enhanced ethylene biosynthesis after application of picloram or clopyralid to the susceptible plant species was a factor involved in resulting morphological changes.

Herbicides have been reported to stimulate ethylene production in a number of plant species (1, 3, 11, 13, 14, 17, 18, 20, 21). In particular, the auxin-type herbicides such as 2,4-D, 2,4,5-T, picloram, 2,5-dichlorophenoxyacetic acid, and dicamba promote ethylene biosynthesis (5, 8, 15–19). A controversy exists among researchers as to whether there is an association between plant sensitivity to auxin-type herbicides and the increase in ethylene production brought about by this herbicide treatment (16). The separation of some of the effects of auxinic herbicides from those of ethylene has been shown (1, 4, 5, 16). There is some evidence to indicate that the auxinic herbicide-induced responses that precede death, such as leaf and stem epinasty and leaf abscission, may be accounted for by ethylene (5, 16). Auxinic herbicides have been shown to promote ethylene biosynthesis more in susceptible than in resistant species (16, 18). Furthermore, analogs of auxinic herbicides that are inactive as synthetic auxins are also inactive as ethylene biosynthesis promoters. For example, phenoxyacetic acid, several chloro-substituted phenoxyacetic acids, and 3,4,5-trichlorophenoxyisobutyric acid, are all known analogs of auxinic herbicides but have little auxin activity.

Most studies describing the biosynthesis of ethylene induced by auxin-type herbicides have been conducted with closed systems. Although simple in terms of gas sampling, the closed system is subject to many sources of error. Specifically, the concentrations of CO_2, O_2, and ethylene will constantly change as a result of the plant's metabolism. Variations in ambient concentrations of these gases have been shown to have an effect on the rate of ethylene production by the plant. Moreover, because in such experiments the tissue is sealed in a chamber for several hours before ethylene determinations can be made, it is often difficult to distinguish whether changes in the rate of ethylene production precede or follow the response of the plant. Thus, a continuous flow system is highly desirable for measurement of the rate of ethylene production from plant tissue (6).

Clopyralid and picloram, members of the pyridine class of herbicides, have auxin-type activity, including the induction of severe epinasty, hypertrophy, fasciation of crown and leaf petioles, and the induction of premature abscission of leaves. Clopyralid is registered for use in Europe and Canada to selectively control weeds belonging to the Compositae and Polygonaceae families, while members of the Cruciferae family, particularly rapeseed, are quite resistant. Picloram has a wider spectrum of broadleaved weed control than clopyralid and will damage rapeseed crops.

The purpose of this research was to determine whether ethylene is involved in the herbicidal activity of picloram and clopyralid. Sunflower and rapeseed plants were chosen as representative plant species for this investigation because of the susceptibility differences of these plants to the herbicides. A continuous flow system was used to evaluate the possible role of ethylene in the herbicidal activity of picloram and clopyralid in sunflower and rapeseed.

MATERIALS AND METHODS

Seeds of sunflower (*Helianthus annuus* L. cv Mammoth Grey Stripe) were obtained from Robertson Seed Inc., Edmonton, Alberta and rapeseed (*Brassica napus* L. cv Altex), from the University of Alberta research farm. Seeds were planted in individual pots containing sand, soil, and peat moss (1:1:1). After 10 d, plants were thinned to three plants per pot. Plants were grown in a growth cabinet maintained at 20/16 ± 1°C day/night with a

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16-h photoperiod and RH of 80%. The intensity of light (400–725 nm) was constant at 450 $\mu E \cdot m^{-2} \cdot s^{-1}$.

The absorption and translocation patterns of [2,6-^{14}C]clopyralid (2,6-^{14}C; 429 MBq·mmol^{-1}, 11.6 mCi·mmol^{-1}), and [2,6-^{14}C]picloram (2,6-^{14}C; 370 MBq·mmol^{-1}, 10.0 mCi·mmol^{-1}) were investigated in both plant species. Herbicides were dissolved in ethanol:water (1:9 v/v) containing polyoxyethylene sorbitan monolaurate (Tween 20) 0.5% (v/v) to obtain a concentration with approximate radioactivity of 2.0×10^5 dpm (3333 Bq)/10 μl, which is equivalent to 777 and 901 μM of [^{14}C]clopyralid and [^{14}C]picloram, respectively. The quantity of radiolabeled herbicide in the 10 μl of solution applied to the plants was 77.7×10^{-4} or 90.1×10^{-4} μmol of [^{14}C]clopyralid and [^{14}C]picloram, respectively. Cotyledons were removed from sunflower and rapeseed plants prior to treatment. A micropipette (Wiretrol, Drummond Scientific Co., Broomall, PA) was used to apply a total of 10 μl of herbicide solution as 8 to 10 drops across the midsection (perpendicular to the midvein) of a leaf. Herbicide treatments were applied to the third leaf of rapeseed plants at the five-leaf stage, and to one leaf of the second leaf pair of sunflower plants at the three-leaf stage. Plants were harvested 24 h after treatment and dissected into the treated leaf, tissue above the treated leaf, and tissue below the treated leaf. The amount of herbicide present on the treated leaf surface was quantified by means of a leaf rinse technique (9). The technique was performed by holding the treated leaf in a plastic funnel and directing 10 ml of an ethanol:water (1:9 v/v) wash solution over the treated area of the leaf. A 5-ml aliquot was taken from the rinse solution and added to a 22-ml scintillation vial containing 10 ml of scintillation liquid (Aquasol-2; New England Nuclear). Radioactivity was quantified by standard liquid scintillation spectrometry (model Tri-Carb 460 CD; Packard Instr. Co. Inc., Downers Grove, IL). The plant tissue was dried for 48 h at 50°C and combusted in a biological sample oxidizer (model OX300; R. J. Harvey Instr. Corp., Hillsdale, NJ) and the $^{14}CO_2$ trapped in the scintillation fluid (Carbon 14 Cocktail—CO_2 trapping; R. J. Harvey Instr. Corp) and quantified by liquid scintillation spectrometry.

In all experiments in which ethylene production was measured, commerical formulations (Dow Chemical Co.) of picloram (Tordon 22K, containing 4-amino-3,5,6-trichloropicolinic acid as the K^+ salt, 240 g ae[3]/L and clopyralid (Lontrel 360, containing 3,6-dichloropicolinic acid, 360 g ae/L) were used. Preliminary experiments on the selectivity of picloram and clopyralid in sunflower and rapeseed were done in a growth chamber. The herbicides were applied with a motorized laboratory sprayer at a rate of 5 kg/ha for clopyralid and 100 g/ha for picloram. Plant response was monitored for 18 d for herbicide symptoms. In all other experiments, herbicides were applied as 10 μl drops with an automatic pipet, to the third leaf of rapeseed or to one leaf of the second leaf pair of sunflower. Concentrations of the herbicide solutions used were 0.52, 2.6, 13.0, or 25.0 mM, and 0.41, 2.1, 10.4, or 19.9 mM for clopyralid and picloram, respectively. These concentrations are equivalent to 0.1, 0.5, 2.5, or 4.8 g ae/L for both herbicides. Depending on the experiment, the quantity of herbicide applied to a plant was either 0.052, 0.26, 1.3 μmol in 100 μl or 5.0 μmol in 200 μl of solution for clopyralid; and either 0.04, 0.21, 1.04 μmol in 100 μl or 3.98 μmol in 200 μl of solution for picloram. These quantities are equivalent to 10, 50, 250 μg in 100 μl or 960 μg in 200 μl of solution for both herbicides. In some experiments a pretreatment of a 31- or 125-μM solution of AVG (Fluka Chemicals) was applied with an atomizer by spraying the plants until runoff. Tween 20 (0.05% v/v) was added to the AVG solution applied to rapeseed, in order to achieve adequate wetting of the waxy leaves.

For each experiment on the measurement of rate of ethylene production, one plant in the four-leaf stage was selected for similar morphological characteristics. Cotyledons were removed and the plant was sealed around the stem in a glass cuvette previously described (6). The system was allowed to equilibrate for several hours in a stream of air to avoid problems arising from ethylene production resulting from mechanical stimulation during insertion of the plant into the cuvette. To remove hydrocarbon contaminants, air containing 500 μl/L of CO_2 (Matheson Company Inc.) was passed through a stainless steel tube (2.54 cm diameter) packed with platinized asbestos maintained at 700°C (10). The purified cool air was passed through the cuvette at a flow rate of 200 ml/min. The total volume of the system was 3.8 L, requiring approximately 20 min for equilibration and complete gas turnover. Temperature of the cuvette was maintained at 27 ± 1°C by water from a constant temperature bath, circulating within a jacket enclosing the cuvette. Light (400–725 nm) intensity was constant at 125 $\mu E \cdot m^{-2} \cdot s^{-1}$, provided by six 150-w incandescent bulbs. The light was passed through a Plexiglas filter containing 12 mm $CuSO_4$:$5H_2O$ (2% w/v) solution with a cutoff point at 730 nm.

Air leaving the cuvette was bubbled through a 15-ml ice-cooled, saturation solution of KOH contained in a gas washing bottle, to remove CO_2 and water vapor from the air. To collect the ethylene, air was then passed through a trap containing silica gel (0.5 g, 60–80 mesh) kept at −86°C in a dry ice-acetone bath (10). Ethylene concentrations were determined on a gas chromatograph (Hewlett Packard 5830A) equipped with a Porapak Q column (80–100 mesh) and a flame ionization detector. The helium carrier gas flow rate was 60 ml/min. The gas chromatograph oven temperature was maintained at a constant 60°C. Concentrations of CO_2 were determined on a gas chromatograph (Hewlett Packard 5880) equipped with the same column, and a thermal conductivity detector. Helium carrier gas flow was 60 ml/min, while the gas chromatograph oven temperature was maintained at a constant 50°C.

A study of changes in morphology and leaf axil angle after treatment with AVG, herbicide, or AVG plus herbicide, was conducted with sunflower plants. The plants were transferred to a growth chamber maintained at 27 ± 1°C and RH of 90%, 24 h prior to treatment. The light (400–725 nm) intensity was constant at 250 $\mu E \cdot m^{-2} \cdot s^{-1}$. The details of chemical treatments are described under "Results". Morphological changes were assessed by observation at 0, 4, 8, 12, and 24 h after treatment.

Each experiment involving the measurement of ethylene production was repeated at least three times. Data from a typical experiment are presented for each treatment.

RESULTS

In a preliminary experiment, rapeseed plants were treated with a clopyralid dose of 5 kg/ha, delivered by a laboratory sprayer. This dose of clopyralid is about 16 times the recommended field rate for weed control in rapeseed. The plants did not show any herbicide symptoms up to at least 18 d following treatment. However, a picloram dose as low as 100 g/ha induced leaf curling and epinasty 3 d after treatment. Sunflower plants were found to be susceptible to both clopyralid and picloram. In this species, both herbicides had approximately the same phytotoxic activity on a molar basis.

It may be argued that the lack of response of rapeseed plants to clopyralid could be a result of little herbicide uptake and/or translocation. An experiment was done to assess whether there were differences in absorption and translocation patterns of clopyralid and picloram in the two plant species. The data (Table I) indicate that after foliar application of radiolabeled clopyralid or picloram, there was substantial absorption and translocation

[3] Abbreviations: ae, active ingredient; AVG, amino-ethoxyvinylglycine.

Table I. *Distribution of ^{14}C as a Percentage of Total Radioactivity Recovered 24 h after Application*

The third leaf of rapeseed plants at the five-leaf stage and one leaf of the second leaf pair of sunflower plants in the three-leaf stage were treated with herbicide in 10 μl of solution.

Species	Treatment	Amount Applied	Radioactivity[a] Recovered			
			Leaf wash	Treated leaf	Above treated leaf	Below treated leaf
		nmol	%			
Sunflower	Clopyralid	7.77	1.3 ± 0.5	53.3 ± 9.9	45.1 ± 10.0	0.3 ± 0.2
Sunflower	Picloram	9.01	5.7 ± 3.0	40.6 ± 3.8	52.7 ± 5.0	1.0 ± 0.3
Rapeseed	Clopyralid	7.77	1.4 ± 0.6	55.4 ± 2.9	40.6 ± 2.5	2.6 ± 0.3
Rapeseed	Picloram	9.01	14.5 ± 3.3	58.8 ± 3.0	16.0 ± 2.5	10.6 ± 2.1

[a] Means ± SE based on five replicates.

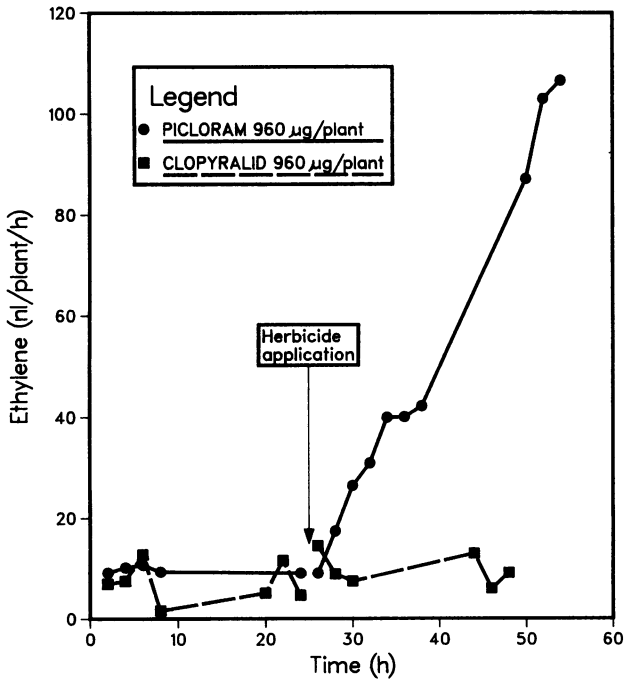

FIG. 1. Effect of picloram (●) or clopyralid treatment (■) on ethylene production by rapeseed plants. The *x* axis indicates the time lapse from insertion of the plant into the cuvette. Each plant (four-leaf stage) was allowed to equilibrate for several hours in the cuvette before measurements of ethylene production were initiated. After 24 h, each plant received 960 μg of picloram or clopyralid, applied in 200 μl (20 10-μl drops) to the third leaf.

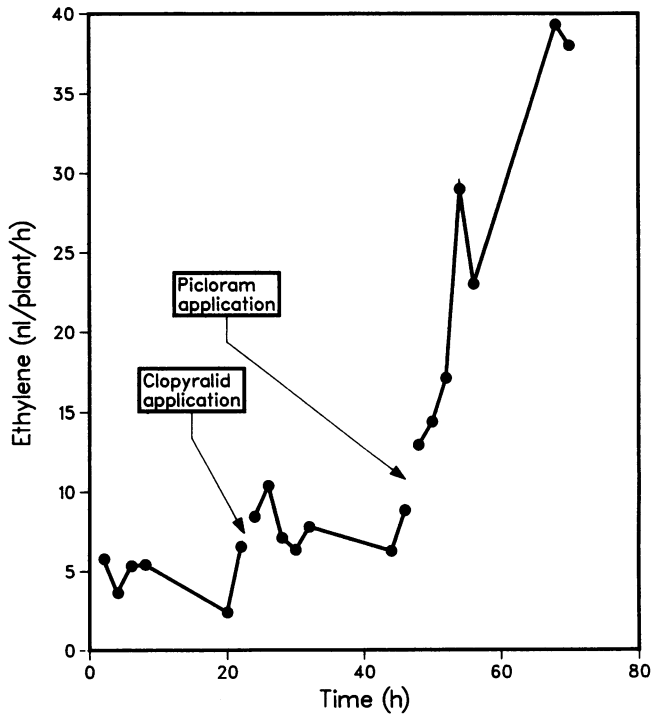

FIG. 2. Effect of a sequential treatment of clopyralid followed by picloram on ethylene production by a rapeseed plant. Conditions and *x* axis as in Figure 1.

of ^{14}C in both sunflower and rapeseed plants.

Inasmuch as auxin-type herbicides are known to increase ethylene production in plants, and in view of the fact that some of the symptoms induced by the two herbicides in susceptible species are typical of those induced by ethylene, it was of interest to determine the changes in rates of ethylene production following application of herbicide treatments to both plant species. When picloram was applied to the third leaf of a rapeseed plant there was a lag period of approximately 5 h before ethylene levels rose significantly above values obtained prior to the herbicide treatment (Fig. 1). No morphological injury symptoms were evident at this time. The same dose of clopyralid applied to another rapeseed plant did not elevate ethylene concentrations above basal values (Fig. 1).

In another experiment, a rapeseed plant received a 960-μg/plant dose of clopyralid followed 23.5 h later by a similar dose of picloram (Fig. 2). Both treatments were applied to the third leaf. Clopyralid treatment had little effect on ethylene production. However, 1.5 h after application of picloram, the ethylene production began to increase. This increased ethylene production occurred before any morphological changes became apparent. The rate of ethylene production was 4 to 5 times greater than the basal level, 24 h after picloram treatment. The apparent increase in the rate of ethylene production before picloram treatment (Fig. 2) represents fluctuations in basal rates of ethylene production (for comparison see Fig. 1).

Having determined that ethylene production increases in response to the herbicide to which the plant is susceptible, an experiment was done to assess whether this increase in ethylene production could be prevented with AVG, a known inhibitor of ethylene biosynthesis (22). The treatment of leaves and cotyledons of rapeseed with AVG prevented the generation of ethylene above basal levels by the plant after receiving a picloram dose of 250 μg (Fig. 3). In contrast, a plant that did not receive the AVG pretreatent generated approximately six times more ethylene 20 h after the picloram treatment (Fig. 3).

In sunflower, a species susceptible to both herbicides, ethylene production increased in response to both chemicals. The data are presented only for clopyralid. Clopyralid (10 μg) increased ethylene production in sunflower several-fold (Fig. 4). When

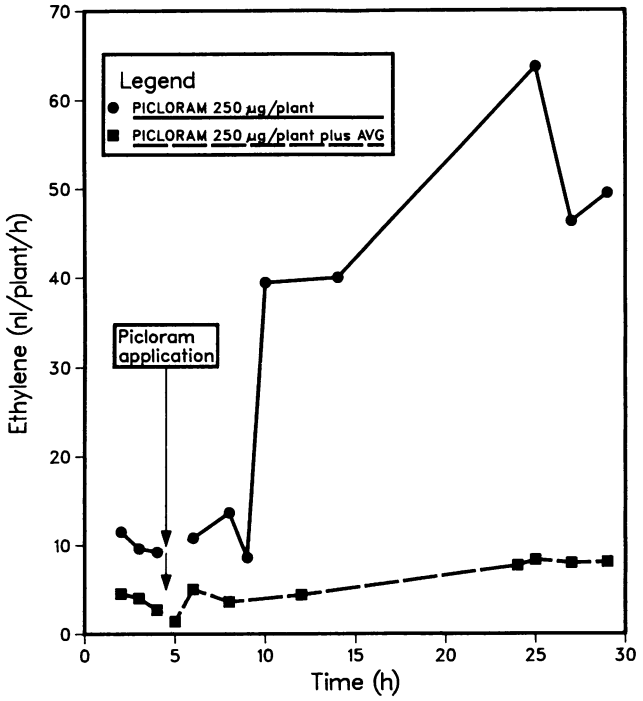

FIG. 3. Effects of picloram (●) or a pretreatment with AVG followed by picloram (■) on ethylene production by rapeseed plants. AVG (125 µM) solution was sprayed on the entire plant 48 and 24 h prior to insertion into the cuvette. Herbicide dose was delivered in 100 µl of solution (10 10-µl drops).

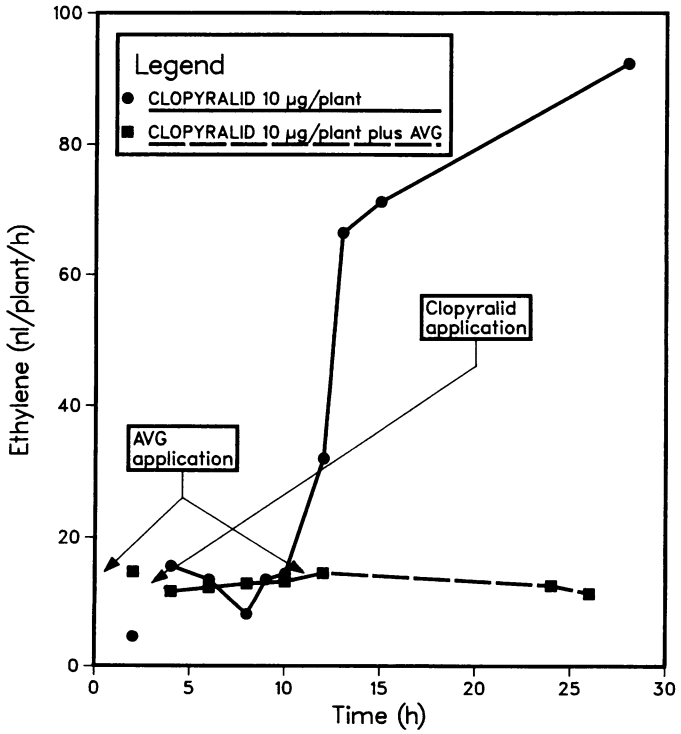

FIG. 4. Effect of clopyralid treatment on ethylene production by sunflower plants. One leaf of the second leaf pair received the herbicide dose delivered in 100 µl of solution. AVG (31 µM) was applied after insertion of the plant into the cuvette.

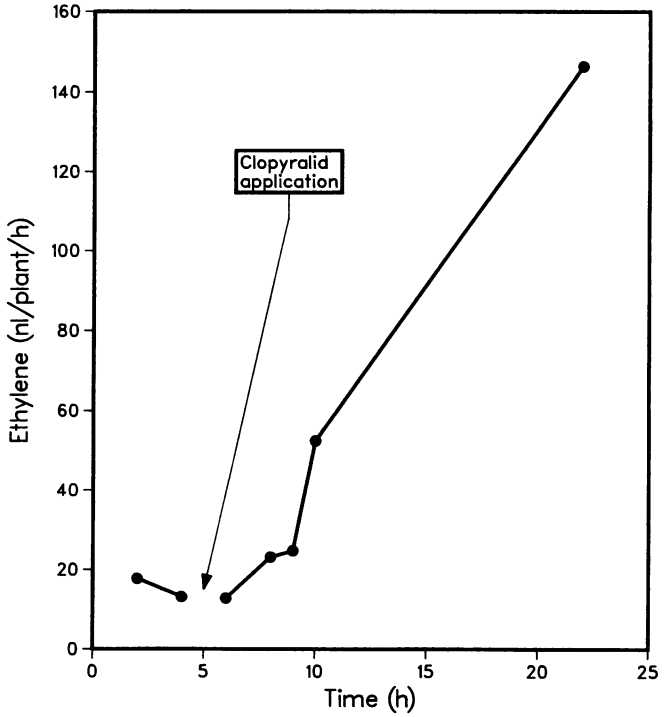

FIG. 5. Effect of clopyralid on ethylene production when applied to sunflower cotyledons. Cotyledons were isolated outside the sealed cuvette. A herbicide dose of 50 µg was delivered in 100 µl of solution.

AVG (31 µM) was applied before treatment with clopyralid, AVG prevented generation of ethylene above levels recorded before the herbicide treatment was applied (Fig. 4).

It might be argued that an increase in ethylene production is a result of localized tissue wounding caused by application of the herbicide solution to a plant. Therefore, an experiment was designed to minimize any ethylene production as a result of tissue wounding. A 50-µg dose of clopyralid was applied to the stem and cotyledons of a sunflower plant (Fig. 5). The treated zone of the plant was situated below the cuvette, which enclosed the true leaves of the plant. Five h after treatment with clopyralid, ethylene concentration in the cuvette began to rise and was still increasing after 24 h.

As is evident by the data presented in Figures 1 to 5, there was considerable variation in the absolute rates of ethylene production between different plant species and within a species, in spite of selection for uniformity of plants in terms of age and size. Such variations in basal rates of ethylene production became apparent when measurements are made using a continuous flow system (7). (In closed systems, where ethylene can be quantified only by sealing the plant for several hours in a chamber before the samples are withdrawn, the net measurement of ethylene accumulation tends to obscure the variability in basal rates of ethylene produced.) Even with this variation, the times at which changes in ethylene production occurred in response to a treatment were consistent for all replications of an experiment.

For detailed experiments on the changes in morphology induced by various treatments, sunflower was chosen as the representative plant because of its susceptibility to both herbicides. Leaves of sunflower plants became epinastic 4 h after a 50-µg clopyralid dose was applied to the cotyledons (Fig. 6). The symptoms were virtually identical to the ones observed when plants were fumigated with ethylene (C. Hall, P. K. Bassi, M. S. Spencer, W. H. Vanden Born, unpublished data). Symptoms progressed rapidly and stem curvature was apparent 8 h after clopyralid treatment. Treatment of plants with AVG, before the

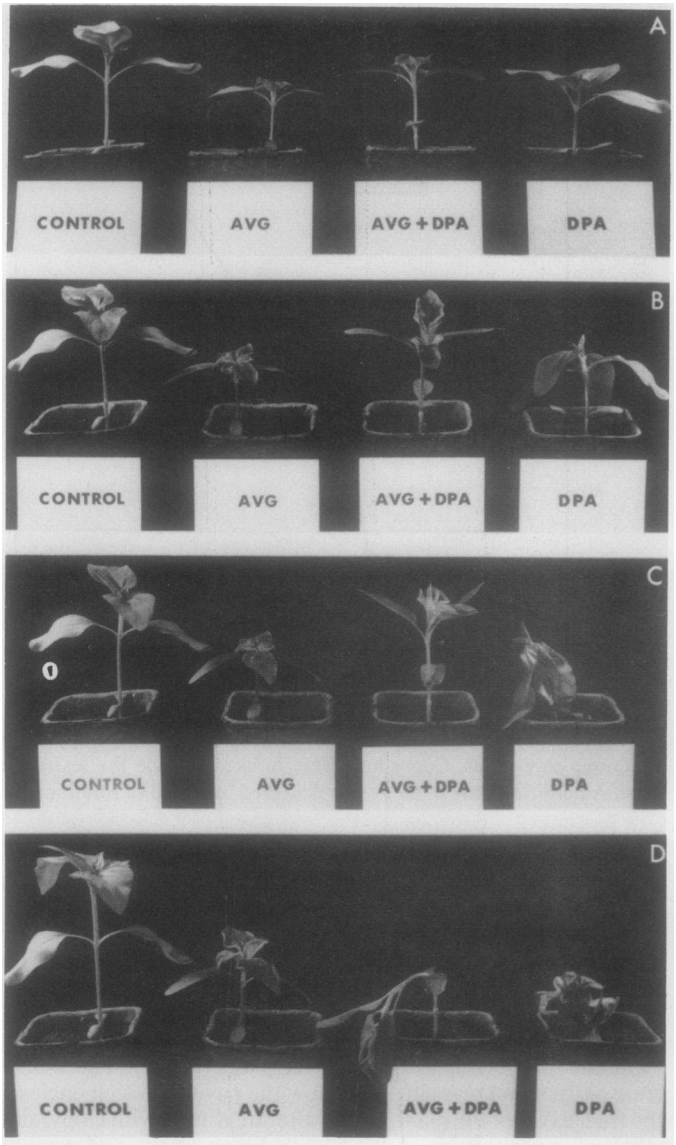

FIG. 6. Morphological changes in sunflower treated with AVG, clopyralid (3,6-dichloropicolinic acid [DPA]), or AVG + clopyralid (AVG + DPA). Pictures represent symptoms 0, 4, 12, and 24 h after clopyralid was applied (A, B, C, D). AVG (125 μM) was applied to leaves until runoff, 24 and 12 h before and 0, 4, 12, and 24 h after cotyledons were treated with clopyralid (50 μg/100 μl, 10 10-μl drops).

application of herbicide, delayed the development of symptoms. The plant treated with AVG plus clopyralid displayed hyponasty of cotyledons and leaves, as well as leaf rolling, 8 h after treatment, although some hyponasty was evident after 4 h. Stem bending did not occur until approximately 16 h after the application of the AVG plus clopyralid treatment. Application of AVG alone did not appear to affect the morphological status of a sunflower plant, although, 24 h after treatment with AVG, the leaves emerging from the apex were somewhat chlorotic with some rolling evident at leaf margins. Symptoms were followed for 5 d after herbicide treatment; there was little change in morphological characteristics of the plant after 24 h. The morphology of sunflower plants treated with clopyralid was affected regardless of whether an AVG pretreatment was applied. Leaves of plants treated with AVG plus clopyralid remained hyponastic, while crown tissue showed little hypertrophy. Leaves of clopyralid-treated plants were epinastic, while crown tissue showed marked hypertrophy.

DISCUSSION

Data presented in Table I show that the difference in rate of ethylene production in response to either herbicide treatment cannot be attributed to differences in absorption and translocation in rapeseed plants. Furthermore, we have data that indicate that differences in the rate of ethylene production probably cannot be accounted for by differences in the extent of metabolism of the two herbicides in rapeseed plants. After 24 h, the percentage of total applied herbicide remaining in the unmetabolized form was 72.7 ± 1.9 and 62.4 ± 1.8 for picloram and clopyralid, respectively. These results, along with the fact that the two herbicides have similar chemical structures, suggest that these compounds interact differently with some target sites within the cell. We attempted to inhibit the generation of ethylene by rapeseed plants in response to picloram treatments by pretreating the plants with large doses of clopyralid. No clear indication of competitive inhibition of the herbicidal action of picloram was evident.

It could be argued that the commercial formulations used in the ethylene experiments were designed to optimize selectivity differences within rapeseed plants while the formulation used in the absorption and translocation studies was designed to favor penetration. Therefore, it was important to determine if rapeseed plants would respond similarly, in terms of the amount of ethylene generated, when the commercial formulations were compared with the formulation used in the absorption and translocation experiments. Consequently, in a control experiment, a dose of 300 μg/plant of clopyralid or picloram, spiked with the corresponding radiolabeled herbicide, was dissolved in a solution containing 10% ethanol plus 0.5% Tween 20 and applied to rapeseed plants. Ethylene levels rose at least 22-fold 24 h after picloram application, whereas there was no increase in ethylene evolution above basal levels in rapeseed plants treated with clopyralid. The percentage of the total recovered radioactivity that translocated acropetally out of the treated leaf 24 h after application of [^{14}C]clopyralid and [^{14}C]picloram was 32.9 ± 3.1 and 19.7 ± 4.3, respectively. These findings indicate that the selectivity differences within rapeseed plants, to the commercially formulated mixtures containing clopyralid and picloram, were not a result of differences in the amount of absorption and subsequent translocation of the two herbicides.

This is the first paper in which herbicide-induced ethylene production has been shown to occur, using intact plants in a continuous flow system. This approach eliminates the potential pitfalls of previously published reports of experiments, using closed systems, where it is often very difficult to interpret the data because of other interfering factors such as changes in the gaseous environment around the plant. Moreover, closed systems are not suitable for following the time course of changes in rate of ethylene production. This makes it difficult to distinguish whether changes in rate of ethylene production occur prior to or following the development of symptoms in response to herbicide treatment. The data presented in this paper indicate that the application of picloram and clopyralid to susceptible species induces the increased biosynthesis of ethylene. This increase in ethylene production preceded or coincided with the onset of morpholocigal changes induced by application of a herbicide to the susceptible species. However, when clopyralid treatments are applied to a resistant species, rapeseed, ethylene production does not increase above basal levels.

Wounding has been shown to increase ethylene production (2). Visible wounding of the treated leaf was apparent only in some cases when a 960-μg dose of clopyralid per plant was applied to sunflower. This local wounding, when observed, oc-

curred several hours after the increase in rate of ethylene production started. Moreover, increased ethylene production was observed with the herbicide treatment regardless of whether wounding was visible or not. In order to further reduce the effects of possible wounding on ethylene production, sunflower cotyledons were treated with clopyralid and isolated below the sealed cuvette. (Hallmen [12] has shown that [^{14}C]picloram, applied to the cotyledons of sunflower, will move acropetally 24 h after treatment.) The treatment of isolated cotyledons with clopyralid led to an increase in ethylene production from the portion of the plant enclosed in the cuvette approximately 4 h after treatment. Thus, ethylene production cannot be attributed to localized wounding of the treated tissue.

Abeles (1) found that 2,4-D stimulated ethylene production in corn and soybean. Ethylene had an inhibitory effect on growth of the two plant species. However, CO_2, a competitive inhibitor of ethylene biosynthesis, could not be demonstrated to reverse the supposed ethylene effect. Several other researchers indicated that the manifestation of auxinic-type herbicide action is independent from, or in addition to, the action of these compounds in inducing ethylene (4, 5, 16). Our results indicate that picloram- and clopyralid-induced ethylene production could well be responsible for some of the morphological symptoms displayed by the plants.

This conclusion is supported by our work with AVG. Yu and Yang (22) suggested that AVG inhibits ethylene biosynthesis by inhibiting the conversion of methionine to 1-aminocyclopropane-1-carboxylic acid. AVG has been used with nonauxinic-type herbicides to determine whether herbicide symptoms can be attributed to increased ethylene production brought about by the herbicide treatment (20). In our experiments, treatment of plants with AVG prevented the generation of ethylene induced by massive doses of picloram or clopyralid applied to rapeseed and sunflower plants. AVG delayed the development of symptoms after herbicide treatment. When the symptoms did develop, they were quite distinct from those induced by the herbicide alone. AVG acted to delay stem curvature and prevented epinasty. Hypertrophy was less severe when AVG was applied as a pretreatment before sunflower was treated with clopyralid. However, leaves were hyponastic 4 h after herbicide treatment and remained in this position for at least 5 more days. Conversely, when only the herbicide was applied, epinasty and stem curvature were apparent 4 h after treatment. Furthermore, the morphological manifestations of epinasty and stem curvature, brought about by herbicide treatment, appeared soon after, or simultaneously with, the rise of ethylene levels above basal levels in plants sealed in the cuvette. Morphological responses to ethylene fumigation or clopyralid treatment were similar. These results, taken together, suggest that enhanced ethylene biosynthesis, in response to herbicide application, is a factor involved in the resulting morphological changes.

LITERATURE CITED

1. ABELES FB 1968 Herbicide-induced ethylene production: role of the gas in sublethal doses of 2,4-D. Weed Sci 16: 498–500
2. ABELES FB 1973 Ethylene in Plant Biology. Academic Press, New York
3. ABU-IRMAILEH BE, LS JORDAN, J KUMAMOTO 1979 Enhancement of CO_2 and ethylene production and cellulase activity by glyphosate in *Phaseolus vulgaris*. Weed Sci 27: 103–106
4. APELBAUM A, SP BURG 1972 Effect of ethylene on cell division and deoxyribonucleic acid synthesis in *Pisum sativum*. Plant Physiol 50: 117–124
5. ASHTON FM, AS CRAFTS 1981Mode of Action of Herbicides. John Wiley and Sons, New York
6. BASSI PK, MS SPENCER 1979 A cuvette designed for measurement of ethylene production and carbon dioxide exchange by intact shoots under controlled environment conditions. Plant Physiol 64: 488–490
7. BASSI PK, MS SPENCER 1982 Effect of carbon dioxide and light on ethylene production in intact sunflower plants. Plant Physiol 69: 1222–1225
8. BAUR JR, PW MORGAN 1969 Effect of picloram and ethylene on leaf movement in huisache and mesquite seedings. Plant Physiol 44: 831–838
9. DEVINE MD, HD BESTMAN, C HALL, WH VANDEN BORN 1984 Leaf wash techniques for estimation of foliar absorption of herbicides. Weed Sci 32: 418–425
10. EASTWELL KC, PK BASSI, MS SPENCER 1978 Comparison and evaluation of methods for the removal of ethylene and other hydrocarbons from air for biological studies. Plant Physiol 62: 723–726
11. HAGEMAN LH, R BEHRENS 1984 Chlorsulfuron induction of leaf abscission in velvetleaf (*Abutilon theophrasti*). Weed Sci 32: 132–137
12. HALLMEN U 1974 Translocation and complex formation of picloram and 2,4-D in rape and sunflower. Physiol Plant 32: 78–83
13. HOLM RE, FB ABELES 1968 The role of ethylene in 2,4-D-induced growth inhibition. Planta 78: 293–304
14. LEE TT, T DUMAS 1983 Effect of glyphosate on ethylene production in tobacco callus. Plant Physiol 72: 855–857
15. MAXIE EC, JC CRANE 1967 2,4,5-Trichlorophenoxyacetic acid: effect on ethylene production by fruits and leaves of fig tree. Science 155: 1548–1550
16. MORGAN PW 1976 Effects of ethylene physiology. *In* LJ Audus, ed. Herbicides: Physiology, Biochemistry, and Ecology, Ed 1, Vol 1. Academic Press, New York, pp 256–280
17. MORGAN PW, JR BAUR 1970 Involvement of ethylene in picloram-induced leaf movement response. Plant Physiol 46: 655–659
18. MORGAN PW, WC HALL 1962 Effect of 2,4-dichlorophenoxyacetic acid on the production of ethylene by cotton plants and grain sorghum. Physiol Plant 15: 420–427
19. MORGAN PW, RE MEYER, MG MERKLE 1969 Chemical stimulation of ethylene evolution and bud growth. Weed Sci 17: 353–355
20. PARADISE I, E EBERT, EF ELSTNER 1981 Metolachlor (2-chloro-N-[2-ethyl-6-methylphenyl]-N-[2-methoxy-1-methylethyl] acetamide) and the metolachlor safener CGA 43089 [alpha-(cyano-methoximino) benzacetonitrile] in sorghum seedlings: correlations between morpholigical effects and ethylene formation. Pestic Biochem Physiol 15: 209–212
21. STACEWICZ-SAPUNCAHIS M, HV MARSH, J VENGRIS, PH JENNINGS, T ROBINSON 1973 Participation of ethylene in common purslane response to dicamba. Plant Physiol 52: 466–471
22. YU YB, SF YANG 1979 Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. Plant Physiol 64: 1074–1077