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Participation of Ethylene in Common Purslane Response to Dicamba[1,2]

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ABSTRACT

The responses of common purslane (*Portulaca oleracea* L.) plants to 2-methoxy-3,6-dichlorobenzoic acid (dicamba) were found to be similar in many respects to ethylene fumigation effects. Dicamba and ethylene increased the permeability of cell membranes in purslane tissues. An increased efflux of electrolytes was observed in the bending region of the stems of dicamba-treated plants. Epinastic leaves after dicamba (10 micrograms) and ethylene (microliter per liter) treatments showed an increased efflux of rubidium. The permeability effects were observable within 1 day after dicamba or ethylene application. Protein metabolism in purslane leaves was not influenced by dicamba until 2 days after treatment, as indicated by reduced nitrate reductase activity. Inhibition of phenylalanine-U-^{14}C incorporation into protein was observed 3 days after treatment. Ethylene reduced both phenylalanine-U-^{14}C incorporation into protein and nitrate reductase actvity within 1 day. Dicamba caused a rapid increase in ethylene production in purslane plants to levels many times greater than those observed in untreated plants. It was concluded that the dicamba-enhanced production of ethylene is responsible for many of the observed effects of the herbicide.

Dicamba[4] is a systemic herbicide which has been used effectively to control many broad-leaved weeds resistant to other herbicides. Common purslane, a troublesome weed which is extremely drought-resistant, is not easily controlled by 2,4-D or 2,4,5-T but is sensitive to dicamba (23, 39). Very little is known about the mode of action of dicamba, but the responses of treated plants suggest that it is auxin-like. Stem bending was reported for dicamba-treated soybeans (43) and Canada thistle (11). Secondary responses included killing of the terminal bud and subsequent lateral branching in soybeans (43) and flax (33). Stem swelling was produced by dicamba in bean plants (38).

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[4] Abbreviations: dicamba: 2-methoxy-3,6-dichlorobenzoic acid; 2,4-D: 2,4-dichlorophenoxyacetic acid; 2,4,5-T: 2,4,5-trichlorophenoxyacetic acid; picloram: 4-amino-3,5,6-trichloropicolinic acid.

The literature suggests that many herbicides act upon protein synthesis (7, 27, 31) and cell membrane permeability (6, 26). Dicamba was reported to inhibit leucine incorporation into protein in soybean hypocotyl sections and to inhibit induction of α-amylase in barley aleurone layers (31). Magalhaes and Ashton (26) found a decrease in the permeability of cell membranes of dicamba-treated nutsedge leaves. There are also a number of reports that some systemic herbicides, apparently acting as synthetic auxins, stimulate ethylene production in treated plants and that their physiological effects can be attributed in part to the effects of this natural hormone (1–3, 16, 20, 28, 32). In this report the action of dicamba is examined by comparing the effects of the herbicide and ethylene on protein synthesis and cell membrane permeability.

MATERIALS AND METHODS

Common purslane (*Portulaca oleracea* L.) seeds were sown in sand in 10-cm plastic pots placed in trays filled with Hoagland's nutrient solution (19) for subirrigation. The plants were grown in a growth room under a 14-hr light (1100 ft-c) and 10-hr dark period at a continuous temperature of 25 ± 1 C and 60% relative humidity. At 2 weeks of age the plants were thinned to three plants per pot.

Dicamba and Ethylene Treatments. At 4 weeks of age the purslane plants were treated with various levels of dicamba dissolved in water. Foliar application was performed by placing 20-μl droplets of a desired concentration on each of five fully developed leaves of each plant. Root application was accomplished by watering each pot with 30 ml of dicamba solution.

Ethylene studies were carried out with 4-week-old potted purslane plants placed in 10-liter desiccators. Saturated KOH solution was included in each desiccator to serve as a CO_2 trap. The desiccator outlets were sealed with rubber vaccine caps through which ethylene was injected with a gas-tight syringe to obtain the desired concentration of the gas.

Efflux of Electrolytes from Stem Segments. The foliage of 4-week-old purslane plants was treated with 0.05, 0.5, or 5 μg of dicamba per plant. After 6 hr 1-cm sections were excised from the basal part of the stems. Ten replications with three segments per sample were used for each treatment. The samples were weighed and placed in 25 ml of deionized water at 25 ± 2 C for 24 hr. The efflux of electrolytes from the stem sections was measured according to the method of Wilner (44). After the incubation period the specific conductance of the medium was recorded using a Serfass conductivity bridge, model RCM15. The segments were than boiled at 15 p.s.i. for 30 min, and the specific conductance was measured again. The efflux of

electrolytes was calculated as a percentage of the total electrolyte content released by boiling.

Rubidium Efflux from Excised Leaves. The permeability of leaves after dicamba and ethylene treatment was examined by incubating excised leaves with rubidium and then following its efflux. The leaves of 4-week-old purslane plants were treated with 10 μg of dicamba per plant or fumigated with 1 μl/liter ethylene in desiccators. After 24 hr the leaves were excised, avoiding the ones to which dicamba was directly applied. Duplicate 5-g samples were immersed for 2 hr in 50 ml of a 10 mM RbCl solution. Following the incubation period the leaves were washed three times with 50 ml of demineralized water and then immersed in another 50-ml portion of this deionized medium. At various time intervals samples were removed, blotted, and dried at 70 C overnight. Subsequently, the rubidium content of the leaves in the samples was determined by atomic absorption spectrophotometry (29).

Nitrate Reductase Activity. Triplicate 4-g leaf samples were excised at various times after dicamba (10 μg per plant) or ethylene (1 μl/liter) treatment and nitrate reductase activity was assayed according to the procedure of Hageman and Flescher (15). Protein was determined by the biuret method (24).

Incorporation of L-Phenylalanine-U-^{14}C. Following the application of dicamba or cycloheximide or fumigation with ethylene, triplicate 1-g samples of leaves were excised at various times. The samples were placed in 0.5 ml of the incubation mixture containing 10 mM potassium phosphate buffer, pH 6; 1% sucrose; 10 μg of streptomycin; and 0.05 μc of L-phenylalanine-U-^{14}C (specific radioactivity 224 mc/mmole). The samples were incubated at 25 ± 2 C under 500 ft-c for 2 hr, then washed three times with 50 ml of 25 μM phenylalanine, and ground in a prechilled mortar with a minimal amount of 10 mM $HgCl_2$. The homogenized material was made up to 9 ml with 10 mM $HgCl_2$, and 1-ml aliquots were dried on planchets and counted in a Nuclear-Chicago gas flow counter. To determine phenylalanine incorporation, protein was precipitated from an 8-ml aliquot by the addition of 2 ml of 50% trichloroacetic acid, extracted three times with 5 ml of 1 N NaOH at 100 C for 15 min, and precipitated again with 50% trichloroacetic acid to obtain a final concentration of 10% trichloroacetic acid. Further purification of the protein was done according to the method of Peterson and Greenberg (34). The purified protein was dried on planchets, weighed, and counted as before.

Ethylene Evolution Measurements. Purslane or bean (*Phaseolus vulgaris* L. cv. Red Kidney) plants in pots were enclosed in 10-liter desiccators provided with CO_2 traps and sealed with vaccine caps. Also, 5 g of detached purslane leaves were placed in 50-ml Erlenmeyer flasks, and 5 ml of water or dicamba solution were added. The flasks were sealed with vaccine rubber caps, and samples of air were periodically withdrawn for ethylene determination (25) from both flasks and desiccators. After every sampling the flasks were opened, flushed with air, and resealed. Evolution of ethylene by the vaccine rubber caps (21) was not detectable. The gas samples were injected into a HI-FI Aerograph model 600D gas chromatograph equipped with a hydrogen flame detector and stainless steel column (1.8 m × 3.5 mm) packed with aluminum oxide, 60/80 mesh, activated overnight at 110 C. The chromatography was run at ambient oven temperature. Nitrogen was used as the carrier gas with a flow rate of 25 ml/min. Hydrogen flow rate was also 25 ml/min, and that of air was 250 ml/min. Under these conditions ethylene had a retention time of 4 min. The amount of ethylene was estimated from a chart integrator.

RESULTS

Morphological Response of Common Purslane Plants to Dicamba and Ethylene. The symptoms of dicamba injury invariably started with the treated leaf exhibiting epinasty. The lowest amount of dicamba producing this effect was 0.1 μg per plant. The sequence of epinasty proceeded from the youngest leaves to the older ones. Parallel to the epinastic response was the disappearance of leaf nyctinasty. Another rapid effect of dicamba in young upright plants was the bending of the stems in the region of the first node, from which the plants recovered when the amount of dicamba was lower than 1 μg per plant. Both the epinasty of the treated leaf and bending of the stem occurred within 4 hr after the application of 10 μg dicamba to 4-week-old plants. The actual time of change in stem position was about 30 min.

After a week the main apex produced a cluster of callus intumescences. Apical dominance was lost, and there was a proliferation of young branches from the lateral buds in the leaf axils which normally remained dormant. The new leaves were small, narrow, and twisted. The stems were abnormally swollen in their upper parts. The plants treated with dicamba defoliated quickly. At the rate of 5 and 10 μg of dicamba per plant the defoliation was complete and the plants died within 1 to 2 weeks.

Since many of these dicamba effects were similar to typical ethylene responses, the effect of ethylene on purslane was also examined. Common purslane was sensitive to ethylene in the range of 0.5 to 100 μl/liter C_2H_4. Within 4 to 6 hr after application of 20 to 100 μl/liter C_2H_4 epinasty of all the leaves occurred and abscission began. Defoliation was complete within 24 hr. With decreasing ethylene concentrations the appearance of the first symptoms was delayed, and abscission occurred at a slower rate. Still, 5 μl/liter of ethylene produced nearly complete defoliation within 24 hr. Only the youngest leaves remained attached, but they were very chlorotic. When the plants were treated with 1 μl/liter ethylene, the first noticeable response was loss of nyctinasty of the leaves. Epinasty and subsequent defoliation followed, starting from the oldest leaves. The lowest level of ethylene tested, 0.5 μl/liter, caused mainly a loss of nyctinasty of all the leaves and induced epinasty of the older leaves. The action of ethylene was reversible in this case, if ethylene treatment was removed after 1 to 2 days.

Efflux of Electrolytes from Purslane Stem Segments. The observation that dicamba caused bending of the stems within 4 to 6 hr after treatment suggested changes in cell sizes, presumably due to changes in permeability and turgor. Therefore, the efflux of ions from the excised bending regions of the plants treated with 0.05 to 5 μg of dicamba was studied. The data in Table I suggest that the herbicide increased cell membrane permeability. The release of ions from the tissues treated with 5 μg of dicamba was twice that of control segments. The efflux of the electrolytes increased with the level of dicamba. The differences in the efflux of ions from the control and treated plant sections were not due to any significant difference in the total content of the electrolytes in these tissues (Table I).

Efflux of Rubidium from Purslane Leaves. Both dicamba and ethylene treatments caused epinasty of common purslane leaves and loss of nyctinasty, which also suggested changes in cell membrane permeability, especially in potassium fluxes (5, 22). Rubidium efflux was used to study permeability changes because this element is believed to be absorbed and released by the same mechanism which functions for potassium (12, 37, 40, 42).

The results of studies of rubidium efflux from leaves excised from purslane plants pretreated with 10 μg of dicamba per

Table I. *Efflux of Electrolytes from Purslane Stem Sections after Dicamba Treatment*

The foliage of 4-week-old plants was treated with dicamba. Six hours after treatment the efflux of electrolytes from excised basal portions of the stem was determined as described in "Materials and Methods." Total electrolyte content is expressed as specific conductance measured in a 25-ml volume of bathing solution.

Dicamba Treatment	Total Electrolyte Content	Efflux of Electrolytes
μg/plant	*μmho/cm·g fresh wt*	%
Control	1084 ± 116	12.2 a[1]
0.05	1002 ± 109	16.9 ab
0.5	971 ± 104	19.8 bc
5	1018 ± 64	25.9 c

[1] Means followed by the same letter do not differ significantly at the 5% level according to Duncan's multiple range test.

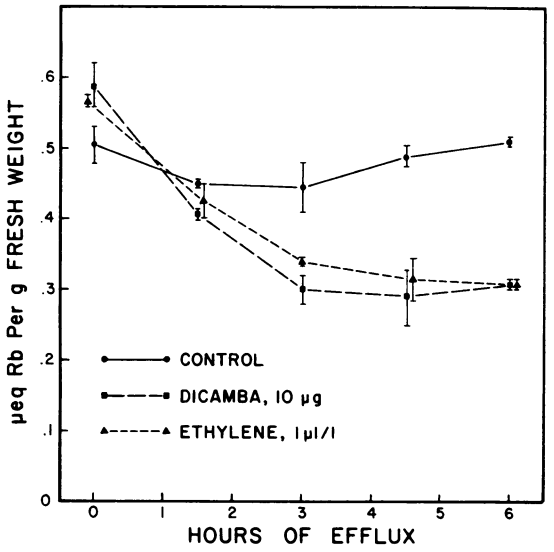

FIG. 1. Efflux of rubidium from purslane leaves after dicamba and ethylene treatment. Four-week-old purslane plants were treated foliarly with 10 μg of dicamba or fumigated with 1 μl/l ethylene. After 24 hr, duplicate samples of 5-g leaves were excised and immersed for 2 hr in 10 mM RbCl and then transferred to demineralized water. At 1.5-hr intervals Rb content in samples was determined as described under "Materials and Methods." Each symbol represents the average of six replications (three experiments with two replicates each time).

plant or fumigated with 1 μl/liter C_2H_4 for 24 hr are shown in Figure 1. The control leaves did not lose a significant amount of rubidium during a 6-hr incubation in demineralized water. The leaves of treated plants absorbed slightly more rubidium during a 2-hr incubation in 10 mM RbCl and subsequently lost half of their rubidium content during the first 3-hr incubation in water. The efflux of rubidium from the dicamba- and ethylene-treated leaves was very similar.

Studies of Protein Metabolism. The activity of nitrate reductase and the incorporation of ^{14}C-labeled L-phenylalanine into protein were used to determine the effects of dicamba and ethylene on protein synthesis in purslane leaves. Nitrate reductase was chosen because it is an inducible enzyme with a rapid rate of turnover (15). The rationale of the experiment was that such an enzyme would provide a sensitive index of any effect of the herbicide on protein metabolism (7).

Periodic assays of nitrate reductase from the leaves of purslane plants showed that the activity varied, depending upon the time of harvest of the tissues (Table II). In untreated plants, activity remained high during daylight hours but dropped temporarily at night and then returned again the next morning to the original level. Thus, the low level of activity observed 12 hr after initiation of the experiment with dicamba was associated with this diurnal fluctuation. Despite a strong epinastic response of the foliage to the dicamba treatment within 24 hr, nitrate reductase activity in the leaves of the treated plants did not change significantly relative to the control until 48 hr after treatment, when nearly 80% loss of activity was noted.

Ethylene fumigation of purslane plants caused a rapid decrease in nitrate reductase activity in the leaves (Table II). After 6 hr the change was detectable, and after 12 hr of fumigation (measured near the end of the light period) 80% inhibition was noted, comparable to the dicamba effect after 2 days.

The effect of dicamba on the incorporation of ^{14}C-phenylalanine into trichloroacetic acid-insoluble, alkali-soluble material was also studied as another index of the effect of the herbicide on protein synthesis. The uptake of radioactive amino acid was fairly constant during the course of the experiments (Table III). During the first 2 days after dicamba treatment the incorporation of L-phenylalanine-U-^{14}C into protein did not change significantly. However, incorporation decreased by half on the 3rd day, dropped to 31% of the control on the 4th day, and then continued to decrease very slowly. Thus the effect of dicamba on total protein synthesis was detectable much later than visible symptoms.

Other experiments included fumigation with 1 μl/liter ethylene or treatment with 50 μg per plant of cycloheximide, applied to the foliage (Table III). Cycloheximide at this rate did not cause any visible response in purslane plants during 24 hr, but within that time period it reduced phenylalanine incorporation by 70%, which was comparable to the dicamba effect after 4 days. At the same time ethylene decreased the incorporation by over 40%, similar to the dicamba effect after 3 days.

Effect of Dicamba on Ethylene Production. The similarity of common purslane response to dicamba and ethylene inevitably posed the question of whether or not the herbicide was effective by enhancing ethylene production. Normal ethyl-

Table II. *Nitrate Reductase Activity in Leaves of Purslane Plants Treated with Dicamba (10 μg) or Ethylene (1 μl/liter)*

Four-week-old purslane plants were treated with 10 μg of dicamba applied to the foliage or fumigated with 1 μl/liter C_2H_4. The reported values are averages of six samples each (two experiments with triplicate samples).

Duration of Treatment	Nitrate Reductase Activity		Inhibition
	Control	Dicamba treated	
	μmoles NO_2^-/mg protein·hr		%
0	0.154 ± 0.013		0
6 hr	0.174 ± 0.012	0.154 ± 0.011	9
12 hr	0.080 ± 0.010	0.076 ± 0.001	5
1 day	0.225 ± 0.011	0.214 ± 0.010	16
2 days	0.165 ± 0.009	0.038 ± 0.007	77
	Control	Ethylene-treated	
	μmoles NO_2^-/mg protein·hr		
6 hr	0.270 ± 0.020	0.200 ± 0.015	26
12 hr	0.260 ± 0.014	0.050 ± 0.013	81

Table III. *Uptake and Incorporation of L-phenylalanine-U-^{14}C into Protein in Leaves of Purslane Plants Treated with Dicamba, Ethylene, or Cycloheximide*

Four-week-old plants were treated with 10 μg of foliar-applied dicamba. At the same time each day triplicate samples of leaves (1 g) were excised and incubated with 0.05 μc of ^{14}C-labeled phenylalanine (specific radioactivity 224 mc/mmole) in buffer solution for 2 hr (upper part of the table). In other experiments (lower part of table) 4-week-old plants were treated with 50 μg of cycloheximide applied to the foliage or fumigated with 1 μl/liter ethylene. After 24 hr of treatment triplicate samples of leaves (1 g) were excised and incubated as above. After the incubation the leaves were ground in 9 ml of 10 mM $HgCl_2$, and aliquots of the homogenate were assayed to calculate total uptake of phenylalanine. Protein was extracted from the samples, purified as described in "Materials and Methods," weighed, and counted. The data are the means of six replications (two experiments with triplicate samples).

Time after Treatment	Phenylalanine Uptake		Phenylalanine Incorporation		
	Control	Treated with 10 μg of dicamba	Control	Treated with 10 μg of dicamba	Inhibition
days	*cpm/g fresh wt*		*cpm/mg protein*		%
1	5253 ± 314	4726 ± 643	156	167	0
2	5301 ± 559	5694 ± 900	154	165	0
3	4839 ± 120	5625 ± 710	152	82	46
4	5607 ± 867	4639 ± 589	155	48	69
5	4821 ± 315	4905 ± 451	150	47	69
6	5238 ± 602	4496 ± 504	153	43	72
LSD[1]				14	

	cpm/g fresh wt	*cpm/mg protein*	%
Control	4257 ± 157	118	
Ethylene, 1 μl/liter	4890 ± 558	69	42
Cycloheximide, 50 μg	4693 ± 600	35	70
LSD		17	

[1] LSD = the least significant difference at the 5% probability level.

ene production of untreated purslane plants was so low that three 4-week-old plants enclosed in a 10-liter desiccator produced barely detectable amounts of ethylene in a 48-hr period (Table IV). Nevertheless, these minute amounts of ethylene building up to 1 μl/liter in the closed jar during 1 week produced ethylene responses, and some defoliation occurred. This suggests a greater sensitivity of common purslane to ethylene as compared to beans which did not defoliate even at 8 μl/liter ethylene. When a comparatively large dose (100 μg) of dicamba was applied to the roots of purslane and bean plants, the ethylene levels in the desiccators rose greatly (Table IV). The gas chromatograph available for use was not sensitive enough to record any differences in ethylene concentration after application of 1, 5, and 10 μg of dicamba to whole plants. In order to study the effects of the lower levels of dicamba which were used throughout this work, detached purslane leaves were enclosed in 50-ml flasks, which permitted a more reliable determination of changes in ethylene evolution. Evolution of ethylene was followed for 2 weeks. Control leaves were not stimulated, by excision, to produce a large amount of ethylene. Dicamba caused a great increase in ethylene production by purslane leaves, proportional to the applied rate of the herbicide within the investigated range (Fig. 2). The ethylene response was very rapid, and differences were noted 4 hr after the dicamba application. The duration of the elevated ethylene evolution was also a function of dicamba concentration with the longest period occurring at 10 μg of the herbicide (data not shown). With lower doses ethylene production eventually dropped to the control level, while 60 μg hastened the senescence and death of the leaves with complete loss of ethylene production within 6 days. As Abeles and Abeles (1) pointed out, the stress-induced ethylene is a product of living tissue, since it ceases when damage is severe enough to kill.

DISCUSSION

Common purslane is quite sensitive to dicamba treatment and exhibits characteristic responses to the herbicide. Many of these responses resemble typical ethylene effects (35), such as enhanced stem swelling, epinasty, defoliation, loss of apical dominance, inhibition of elongation, and loss of nyctinasty. The fumigation of purslane with low levels of ethylene also produced loss of nyctinasty, pronounced epinasty, and defoliation. The difference in the sequence of epinasty, which proceeded from the oldest to the immature leaves in the case of ethylene, and from the youngest leaves to the mature ones in

Table IV. *Ethylene Production by Purslane and Bean Plants after Dicamba Application*

Three 4-week-old purslane plants growing in one pot and three 1-week-old bean plants were treated with 100 μg of dicamba per pot and enclosed in 10-liter desiccators with CO_2 traps. Samples of air were periodically withdrawn from the desiccators and assayed for ethylene as described in "Materials and Methods." Control desiccators contained untreated plants. The data show ethylene concentration in desiccators at a given time.

Time after Treatment	Common Purslane		Beans	
	Control	Treated	Control	Treated
hr	*μl/liter*			
24	0.05	0.5	0.1	2.0
48	0.1	1.0	0.2	8.0

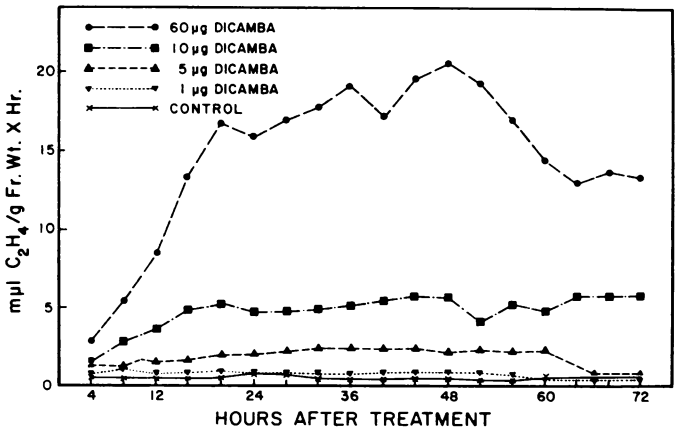

FIG. 2. Ethylene production in detached purslane leaves at various rates of dicamba treatment. Each symbol represents the average of six replications (three experiments with two samples each time).

dicamba treatment, could be explained by the fact that the herbicide is initially translocated mainly to the youngest parts of the treated purslane plants (41). However, the oldest leaves are most sensitive to ethylene (4, 8), and under conditions of uniform fumigation level they react first.

The early curvature of the stem produced by dicamba was not mimicked by ethylene. Such a sudden change in stem position could be explained on the basis of a differential change in cell volumes in the bending zone of the stem, possibly due to the loss of turgor.

Rapid changes in cell sizes could be brought about by changes in membrane permeability and efflux of ions (22). The investigation of electrolyte efflux from purslane stem segments (Table I) reveals that even small doses of dicamba increase the ion release from the tissues. The permeability of cell membranes in stems seems to depend on the amount of herbicide applied and translocated to the bending zone because the plants treated with lower doses of dicamba bent later, if at all. However, once the permeability change in a predisposed region of the stem reached a certain threshold, the efflux of ions could cause such turgor changes that suddenly the whole stem changed its position (an "all or none" response).

The similarity of other responses of common purslane to dicamba and ethylene suggested that dicamba might trigger an increased ethylene production in plants. Other systemic herbicides (2,4-D, 2,4,5-T, picloram) were found to act in such a way (2, 3, 16, 28, 32). Our investigations with kidney bean plants and common purslane (Table IV) as well as with detached purslane leaves (Fig. 2) support this hypothesis. Upon dicamba application the production of ethylene rose within 4 hr and was maintained for a long time on a fairly stable level, depending on the concentration of the herbicide. Ten micrograms of dicamba caused a steady rate of 5 mμl C_2H_4/g fresh wt·hr. Using Burg's (9, 10) factor of 0.2 μl/liter C_2H_4 as internal concentration when plants produce ethylene at the rate of 1 μl/kg fresh wt·hr, the excised leaves of purslane could contain 1 μl/liter ethylene after 24 hr following the application of 10 μg of dicamba (Fig. 2). One microliter per liter ethylene is saturating for many physiological responses in various plants (8, 10).

These considerations could explain similar changes in cell membrane permeability toward rubidium in purslane leaves after dicamba (10 μg per plant) and ethylene (1 μl/liter) treatment (Fig. 1). Nastic responses of *Mimosa pudica* leaves were connected with reversible fluxes of potassium ions in the cells of the pulvinus (5). It could be imagined that if cell membrane permeability changes irreversibly the nastic movements would be arrested. The results of our experiments indicated that rubidium was lost from the leaves treated with dicamba and ethylene much faster than from control leaves, which did not lose significant amounts of rubidium during the first 6 hr. This observation is in agreement with many reports of rubidium and potassium efflux (36, 37) from plant tissues. Rubidium is presumably absorbed by a mechanism normally operating for potassium (12, 37, 40, 42). The fact that the efflux of rubidium from treated leaves stopped after 3 hr suggests that the treatment affected only certain tissues or certain compartments within leaf cells, leaving other pools of Rb^+ ions undisturbed. It is reasonable to assume that if the bulliform cells, suspected of participating in purslane leaf movement (13), irreversibly lost potassium due to dicamba and ethylene action on cell membranes, they could not resume their rhythmical activity and the leaves would remain open during the night. The natural nyctinasty of leaves of mesquite and huisache is also inhibited by ethylene treatment or by doses of picloram which trigger an increased ethylene production (32).

A decrease in the permeability of cell membranes of nuts-edge leaves after dicamba treatment (26) was recorded 5 days after treatment with 10 to 0.1 mM dicamba spray to run-off. This discrepancy with our results could be explained by the fact that low concentrations of plant growth hormones (0.1 to 10 μM) increase cell membrane permeability (30) while high concentrations (10 μM to 1 mM) tend to decrease permeability (17, 18).

The delayed decrease in protein synthesis of purslane leaves was also comparable in the effects of 10 μg dicamba and 1 μl/liter ethylene. The experiments aimed at determination of the effects of dicamba on nitrate reductase indicated that the enzyme activity was not affected until 2 days after the treatment (Table II). Ethylene fumigation at 1 μl/liter caused 80% inhibition within 12 hr. The effect of ethylene seems to be more immediate than that of dicamba. Besides, the slow rate of translocation of the herbicide to the mature leaves (41) must have been a limiting factor as opposed to rapid penetration of ethylene in fumigation experiments. Once dicamba accumulated in leaf tissues, it could influence the rate of ethylene evolution. Increased ethylene concentration could affect compartmentation and release of toxic materials from vacuoles, *e.g.*, phenols, which could affect extractable activity of nitrate reductase.

Total protein synthesis in purslane leaves, as reflected by phenylalanine-^{14}C incorporation into protein, was 70% inhibited by dicamba on the 4th day after treatment (Table III). Similar inhibition was achieved with 24 hr of ethylene fumigation at 1 μl/liter. The discrepancy in time could again be explained by the difference in the rate of translocation of the gas and the herbicide (41). Most reports (14, 31) do not reveal a strong inhibition of protein synthesis by rather high rates of dicamba, but the duration of treatments may have been too short for the slowly developing response. Possibly, the observed inhibition of protein synthesis in purslane leaves after dicamba application is mediated by a rise in ethylene production. It seems most reasonable that the disruption of protein metabolism would contribute to the death of the leaves.

In conclusion, the action of dicamba upon common purslane involves a promotion of ethylene production, and this is at least partly connected with effects of this herbicide.

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