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Regulation of Auxin Levels in *Coleus blumei* by Ethylene[1]

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ABSTRACT

An investigation of the effects of ethylene pretreatment on several facets of auxin metabolism in *Coleus blumei* Benth "Scarlet Rainbow" revealed a number of changes presumably induced by the gas. Transport of indoleacetic acid-1-^{14}C in excised segments of the uppermost internode was inhibited by about 50%. Decarboxylation of indoleacetic acid-1-^{14}C by enzyme breis was not affected by the pretreatment. Levels of extractable native auxin in upper leaf and apical bud tissue of the pretreated plants were approximately one-half of those present in untreated plants. The rate of formation of auxin from tryptophan by enzyme breis from pretreated plants was approximately one-half that occurring in incubation mixtures containing the enzyme system from untreated plants. The conjugation of indoleacetic acid-1-^{14}C in a form characterized chromatographically as indoleacetylaspartic acid was increased 2-fold in the upper stem region of plants pretreated with ethylene.

The effects of ethylene in lowering levels of auxin diffusing through plant tissues are well known (1, 6–8, 12, 16, 20, 22, 32). The manner in which the gas influences levels of diffusible auxin in plant cells is not entirely clear. In a brief report (32), the authors presented some evidence correlating low levels of diffusible auxin in ethylene-treated tissue with decreased auxin formation by enzyme breis from the same tissue. The studies have been expanded in an attempt to correlate several facets of auxin metabolism—auxin biosynthesis, conjugation, and destruction—with levels of extractable and diffusible auxin in the leaf and stem tissue.

MATERIALS AND METHODS

Plant Treatment. *Coleus* (*Coleus blumei* Benth "Scarlet Rainbow") plants derived from the University of Iowa clone (24) were grown in the greenhouse or in environmental chambers (Sherer Model CEL 25 - 7HL) or both. Plants grown in the growth chambers were exposed to a 16-hr photoperiod (light intensity, 1600 ft-c), 50 ± 5% relative humidity, and a temperature of 24 C during the light period and 20 C at night. Treatment with ethylene consisted of either placing the plants in polyethylene bags and injecting ethylene into the bags or providing ethylene in a continuous flow of air (30 liters/min) to the chambers. In early experiments, air flowing into the chambers was first passed through water and in later experiments through an ethylene scrubber. The scrubber consisted of a canister containing Purafil (pellets one-eighth inch in diameter composed of activated Al_2O_3 impregnated with $KMnO_4$), available from Marbon Division, Borg-Warner Corporation, Washington, West Virginia. The canister was constructed of degreased galvanized pipe (10 × 40 cm) with circles of screen wire in drilled pipe caps fitted with laboratory gas outlets. Teflon tape rather than oil was applied to the pipe threads to allow easy disassembly for refilling and to prevent contamination of the Purafil.

In the case of plants placed in plastic bags, temperatures inside the bags were maintained at 24 C during the light phase.

Unless indicated otherwise, plants were exposed to ethylene at concentrations of 25 or 100 μl/liter for an 18-hr period. The 18-hr period consisted of 6 hr of light, 8 hr of darkness, and light during the remainder of the pretreatment period.

Auxin Transport. Following procedures described earlier (31), we measured auxin transport through the uppermost internode of *Coleus* stems. Stem segments 5 mm in length were removed from the treated and untreated plants, coated with a ring of Vaseline to prevent surface transport, and placed on glass slides. One percent agarose cylinders of a volume of 23 μl containing IAA-1-^{14}C, 5 mg/liter (32,600 cpm average per block), served as auxin donors on the physiological apical end and similar cylinders without IAA served as receivers. During the transport period the sections were in a nearly vertical position with the donor above and the receiver below. After 5 hr in a moist chamber kept in the dark, the donor and receiver cylinders and the stem sections (macerated) were placed in counting vials separately or in groups of five and frozen. The radioactivity was assayed with a Nuclear-Chicago liquid scintillation counting system following procedures described in an earlier paper (33). A second counting medium which proved to be more convenient for counting the radioactivity in aqueous samples was used in later experiments. This medium consisted of Spectrograde toluene containing the fluors PPO and POPOP mixed with Triton X-100 in a ratio of 7:6. Distilled water was added to the counting vials containing the samples to be assayed. The counting mixture was then added in a ratio of 4:3. PPO and POPOP were present at final concentrations of 0.4 and 0.005% (w/v), respectively. Triton X-100 (alkyl phenoxy polyethoxy ethanol, Rohm and Haas, Philadelphia, Pa.) is available from Fisher Scientific Company as detergent Cutscum.

Auxin Destruction. Cell-free enzyme preparations of apical tissue from treated and untreated *Coleus* plants were prepared using a method similar to that previously described (30, 32). Apical buds of *Coleus* were surface sterilized with 1% calcium hypochlorite, rinsed with distilled water, and then ground in a chilled mortar with cold 0.1 M phosphate buffer, pH 7.0,

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containing 0.1 M sucrose. This material was then centrifuged at 17,300*g* for 30 min at 4 C to remove cell debris. Two milliliters of the supernatant enzyme brei were then incubated for 3 hr at 27 C in sealed flasks with 0.1 μc IAA-^{14}C (2 μM). Chloramphenicol was added as a safeguard against bacterial contamination in a final concentration of 50 μg/ml. The carbon dioxide released during the reaction was collected in 0.4 ml of 10% KOH in a well suspended over the mixture. At the end of the incubation period, trichloroacetic acid (0.5 ml of 5%) was added with a hypodermic syringe. After 20 min the KOH containing the $^{14}CO_2$ was transferred to counting vials and assayed as described above.

Auxin Extraction. After the 18-hr pretreatment period with ethylene, 10 plants were removed from each growth chamber and the apical portion of each stem including medium to large leaves (second internode from apical bud) was collected. The apical bud and leaf blades were excised from the stem, quickly weighed, and homogenized for 3 min in a blender in cold methanol. The cell debris was removed by centrifugation and re-extracted with methanol. The combined methanol extracts were evaporated *in vacuo*. A procedure reported earlier (17, 28, 29, 33), with slight modifications, was used to extract acidic indoles. The pH of the aqueous residue was first adjusted to 2.5 and extracted in a separatory funnel three times with methylene chloride. The combined organic extract was extracted three times with 50 mM potassium phosphate, pH 8.5. The combined aqueous fraction was then adjusted to pH 2.5 and was extracted with methylene chloride. The final extract was reduced to a small volume and separated by thin layer chromatography with cellulose as the matrix and isopropanol-1.1 M ammonia–water (10:1:1, v/v) as the solvent. The area of the chromatogram corresponding to authentic IAA (R_F 0.4–0.6) was scraped off and equilibrated with 25 agarose blocks in 0.2 ml of water. The auxin content was then measured by the *Avena* curvature bioassay. Auxin quantity was computed from the line (determined by the method of least squares) relating log IAA concentration to degrees curvature.

Auxin Formation. The effect of the ethylene pretreatment on auxin formation by apical bud tissue was studied following procedures reported earlier (30, 32). The enzyme breis were prepared as described in "Auxin Destruction." One milliliter of the enzyme brei was incubated with 1 ml of L-tryptophan (1.54 mg/ml) on a rotary shaker for 45 min at 27 C. Chloramphenicol at a final concentration of 50 μg/ml was included in the reaction mixtures. Agarose blocks (block size, 2.5 × 2.5 × 1.3 mm) were present in the reaction mixtures during the incubation period. These blocks were removed at the end of the incubation period and assayed for auxin with the *Avena* curvature bioassay.

IAA Conjugation. Eighteen hours after beginning the ethylene treatment, the plants were treated with auxin similarly to the method of Morris *et al.* (23). The apex of each of 10 *Coleus* plants was treated with 0.025 ml of a solution containing 0.5 μg of IAA-1-^{14}C (0.04 μc) and 0.05% Tween-20. After 4.5 hr (in the light under continuous exposure to ethylene) the apical portions of the stems were collected, the leaves were removed, and the remaining 1 to 2 cm of stem was rinsed to remove residual IAA. The stem tips were extracted overnight in a freezer with 85% (v/v) ethanol, ground in a blender, and then re-extracted twice with cold 85% ethanol. The combined extracts were evaporated to a small volume and separated on thin layer chromatography plates of cellulose in isopropanol-concentrated NH_4OH–water (8:1:1, v/v). Portions of the plate were scraped into counting vials and the radioactivity was counted as described above.

Carboxyl-labeled IAA-^{14}C (IAA-1-^{14}C) with a specific activity of 10 mc/mmole was obtained from New England Nuclear. Indoleacetylaspartic acid was obtained from Calbiochem.

RESULTS

The effect of ethylene pretreatment on the capacity of *Coleus* stem tissue to transport IAA-1-^{14}C is shown in Table I. The amount of labeled auxin delivered to the receiver blocks during the 5-hr diffusion period is decreased by nearly 50%. There appear to be small increases in the uptake of auxin from the donor blocks by the stem segments, as well as in the amount of radioactivity present in the tissue at the end of the transport period.

An investigation of the effect of ethylene on decarboxylation of IAA-1-^{14}C by leaf and apical bud tissue shows that the gas has no effect on the breakdown of the auxin (Table II). Earlier reports on the breakdown of auxin by *Coleus* tissue are in agreement with this conclusion (10, 32).

The results of investigations of the effects of ethylene on levels of extractable auxin from *Coleus* plants are shown in Figure 1 and Table III. An IBM computer, Model 1130, was used to compute and plot best fit straight lines for the curvature obtained in the *Avena* curvature test from different concentrations of authentic IAA (Fig. 1). The auxin activity was determined from the computed straight line. In all experiments except one, results of tests show that there is a significantly lower level of extractable auxin in ethylene-treated plants. The significant difference is at a confidence level of 99% or higher.

Table IV shows the results of a representative experiment in which the capacity of apical bud tissue to form auxin is compared with levels of extractable auxin present in the tissue. The rate of auxin formation by enzyme breis from ethylene-treated plants is roughly one-half of that occurring in incubation mixtures containing the enzyme system from untreated plants. The amount of auxin is also about one-half of the amount of extractable auxin present in untreated plants. The levels of auxin diffusing from apical buds removed from ethylene-treated and untreated plants are also significantly lower in the treated plants.

Figure 2 represents chromatograms of stem tissue extracts

Table I. *Effect of Ethylene on Basipetal Transport of IAA-1-^{14}C*

Donor blocks containing an average of 32,600 cpm were applied to the apical end of segments 5 mm in length removed from the uppermost internode of the stem.

Treatment	Radioactivity		
	Receiver block	Uptake from donor block	Tissue segment
	cpm		
Control	440	13,215	5,590
Ethylene, 100 μl/liter	230	15,245	6,260

Table II. *Effect of Ethylene on Decarboxylation of IAA-1-^{14}C by Apical Bud Tissue*

The amount of radioactivity provided in the medium was 140,000 cpm.

Treatment	$^{14}CO_2$	
	cpm observed	cpm/g fresh wt·hr
Control	8485	7070
Ethylene, 100 μl/liter	8450	7040

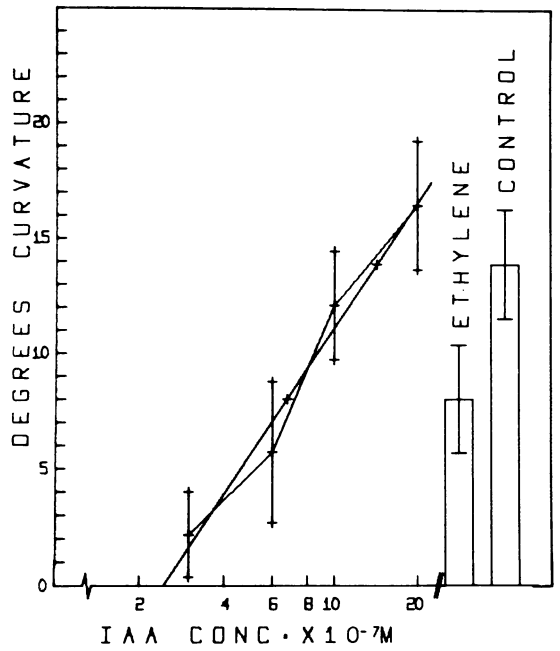

FIG. 1. Standard curve for the *Avena* curvature bioassay for a representative auxin extraction experiment. The response to authentic IAA with the computed best fit straight line is shown on the left. The bars on the right represent the mean curvature of 20 coleoptiles receiving agarose blocks which had been equilibrated in extracts from ethylene (100 μl/liter)-treated and untreated *Coleus* plants. The 95% confidence interval for each mean is represented by the vertical lines superimposed on each bar.

Table III. *Effect of Ethylene on Levels of Extractable Auxin in Apical Bud and Leaf Tissue*

Plants used in the experiments conducted in August were exposed to a continuous flow of air with or without ethylene (25 and 100 μl liter, respectively). Plants used in the remaining experiments were placed in polyethylene bags, treated with ethylene (100 μl/liter), and placed in a growth chamber.

Time of Year	Curvature		IAA Equivalents		*t* Values
	Control	Ethylene	Control	Ethylene	
	degrees ± SE		*ng/g fresh wt*		
August	19.3 ± 1.5	9.5 ± 1.3	10.1	4.2	4.8
August	14.9 ± 0.9	10.2 ± 1.1	6.9	4.4	3.3
January	18.3 ± 1.2	3.2 ± 0.6	12.1	1.7	11.1
January	13.9 ± 1.2	8.0 ± 1.2	11.1	5.3	3.4
February	18.7 ± 1.5	15.8 ± 1.2	11.0	9.9	1.5
February	11.9 ± 0.9	7.2 ± 1.0	8.4	4.4	3.4
March	8.4 ± 0.7	5.2 ± 0.8	3.8	2.8	2.8
May	7.1 ± 1.0	2.5 ± 0.7	3.1	1.9	3.7
Average	14.1	7.7	8.3	4.3	

taken from plants treated with IAA-1-^{14}C. As seen in the figure, extracts of tissue contain substances with R_F values identical to authentic IAA and IAAsp[3] (indoleacetylaspartic acid). In tissue from plants pretreated with ethylene the level of radioactivity appearing at the R_F for IAAsp is approximately twice that extracted from plants not receiving the ethylene pretreatment. The level of radioactivity appearing at the R_F for IAA is the same in extracts from ethylene-pretreated plants and from those not receiving the pretreatment.

In the experiments reported here, treatment of the plants with the higher concentration of ethylene often induced an epinastic response by the leaf tissue. This was observed only in a few experiments in the case of the 25-μl/liter concentration. No significant differences in fresh weights of either leaf or stem tissue at the end of the pretreatment period were observed.

DISCUSSION

The effect of ethylene in inhibiting transport of IAA in the stem tissue of *Coleus* is similar to that reported for many other plant species. The effect of ethylene on transport apparently cannot be explained in terms of increased auxin destruction, because as seen in this report and in a previous one (32) ethylene does not enhance decarboxylation of the hormone by enzyme breis from the apical buds of the treated plants. The action of ethylene in inhibiting polar transport does not appear to be through an effect of the gas on the decarboxylation of IAA (3, 5, 8). In the experiments reported here, enzyme breis of the apical buds of ethylene-treated plants did not decarboxylate IAA at rates significantly different from those of breis of untreated plants. In studies (unpublished data) where excised apical buds from ethylene-treated *Coleus* plants were incubated in a medium containing IAA-1-^{14}C, small increases in decarboxylation of the auxin were observed. However, these increases may be due to increased uptake of IAA by the tissue. As is shown in this report (Table I), ethylene did increase uptake of auxin by segments of stem tissue. Other investigators have also reported such an effect by ethylene in other plant species (3, 21).

If ethylene is acting at sites for auxin transport within the tissue, an accumulation of auxin should occur at regions of synthesis, *i.e.*, the leaf tissues of the upper region of the plant. This does not happen in *Coleus* although it appears to occur in the apices of etiolated pea seedlings (S. Burg, personal communication). Why these discrepancies exist between *Coleus* and pea is not known. Earlier measurements of extractable auxin in ethylene-treated bean plants have shown that there is less auxin than in untreated plants (12). There is also less extractable auxin in ethylene-treated *Avena* coleoptile tissue than in untreated tissue (J. C. Botjes, cited in Ref. 12).

In experiments where both auxin formation and the levels of extractable auxins were examined, there was less auxin for-

Table IV. *Effects of Ethylene on Auxin Formation, on Levels of Extractable Auxin in Apical Bud and Leaf Tissue, and on Levels of Auxin Diffusing from Apical Buds*

Diffusible auxin was collected in agarose blocks placed on the cut surfaces of the apical buds during a 2-hr diffusion period.

Auxin	Curvature		IAA Equivalents		*t* Values
	Control	Ethylene, 25 μl/liter	Control	Ethylene	
	degrees ± SE		*ng/g fresh wt·hr*		
Formed from L-tryptophan	10.5 ± 0.9	5.5 ± 0.9	1062	685	3.8[1]
Extractable	18.9 ± 0.9	9.7 ± 0.8	11.1	5.1	7.5[1]
			ng/apical bud·hr		
Diffusible	5.7 ± 1.1	2.3 ± 0.8	0.31	0.23	2.4[2]

[1] Significant at the 99% probability level.

[2] Significant at the 95% probability level.

[3] Abbreviation: IAAsp: indoleacetylaspartic acid.

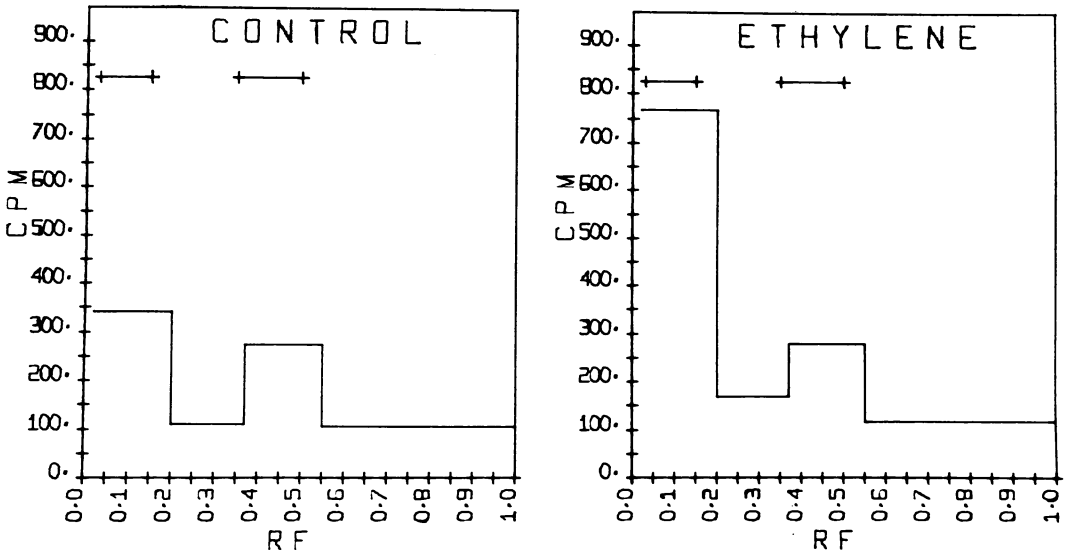

FIG. 2. Effect of ethylene (100 μl/liter) on the metabolism of IAA-1-^{14}C in stem tissue of *Coleus*. The histograms represent radiolabeled sub stances separated by thin layer chromatography of extracts taken from stem sections subtending the stem apex. IAA-1-^{14}C (0.04 μc), 0.5 μg, was applied to the apex of each plant. Uptake by each plant, as calculated by washings of the residual IAA-1-^{14}C, averaged 25,000 cpm. Extracts were prepared from stem segments (total fresh weight, 1.0 g) taken from 10 plants pretreated with ethylene and from 10 plants receiving no ethylene. The radioactivity distribution shown in each histogram is from one-half of the chromatogram. Markers in the upper part of the figure indicate the R_F values of authentic IAA (0.45) and indoleacetylaspartic acid (0.1).

mation and less extractable auxin in ethylene-treated *Coleus* plants. As is shown here and in a short communication published earlier (32), a similar correlation also exists between less auxin formation and lower levels of diffusible auxin in ethylene-treated *Coleus* plants.

In studies of the conjugation of IAA-1-^{14}C, it was found that ethylene pretreatment of *Coleus* plants increased the level of a substance in stem tissue which was characterized as IAAsp. This substance is a known conjugate of auxin in plants (2, 11). Rechromatographing by thin layer chromatography of the substance in *tert*-butanol-acetic acid-water (3:1:1, v/v) also showed the material to have the same R_F as IAAsp. The amount of radioactivity chromatographing as IAA-1-^{14}C, which was extracted from the stem tissue of ethylene-treated plants, was not significantly different from the amount extracted from untreated plants. Thus, the extent to which destruction of auxin is increased in the uppermost regions of the stem by pretreating the plants with ethylene appears to be minimal. The influence of ethylene on auxin conjugation as observed here indicates that the gas enhances auxin conjugation.

In a recent paper, Beyer and Morgan (5) attributed the increased conjugation of auxin in ethylene-treated cotton plants to higher levels of auxin present in the tissue. The higher levels of auxin were considered to be a result of ethylene inhibition of polar transport. However, in the studies reported here, ethylene treatment of *Coleus* plants did not appear to cause a build-up of auxin in the tissue.

The effect that increased conjugation of auxin may have on diffusible auxin levels would appear to be one of lowering levels of auxin moving through the tissue. However, in the paper cited above (5), it was reported that, whereas the transport of naphthaleneacetic acid-1-^{14}C in stem sections removed from ethylene-treated cotton plants was greatly reduced, no increased conjugation of the synthetic auxin was observed. It is not clear, therefore, as to what relationship may exist between auxin conjugation and auxin transport in *Coleus* plants insofar as ethylene pretreatment is concerned.

Beyer and Morgan (4) have also reported that ethylene pretreatment periods as short as 1.5 to 3 hr increase the auxin loss rate from a pulse of auxin in transit through stem tissue of cotton plants. The reports cited above (4, 5) indicate that ethylene acts more or less directly on the auxin transport system in cotton plants. However, it should be noted that in the case of ethylene modification of the auxin pulse (4) the experiments were conducted with excised stem sections exposed to 1 ml of ethylene per liter of air at 30 C. Exposure of excised tissue to a high concentration of ethylene could induce abnormal physiological responses by the tissue. The authors (4) were careful, however, to point out that the experiments were designed to relate modification of the transport of auxin inside the plant by exogenous treatments of ethylene. In electron microscope studies of stem tissue of pea seedlings treated with 500 μl of ethylene per liter of air, significant changes in cellular structure have been reported (27). However, in experiments conducted in this laboratory (unpublished data) treatment of the same variety of pea with 25 μl of ethylene per liter of air did not result in changes in the fine structure of the stem tissue. It has also been reported that treatment of plant tissues with ethylene alters membrane permeability and induces the swelling of mitochondria (13, 18, 25, 26). However, more recent studies (15, 19) indicate that this occurs in the presence of higher concentrations of ethylene (100 μl/liter and above) and that other aliphatic gases, including saturated ones, which have no physiological activity have similar effects on mitochondria.

In the experiments reported here, treatment of the plant tissue with a 100-μl/liter concentration of ethylene often resulted in epinasty of the tissue. This phenomenon was observed only in a few instances in the case of the 25-μl/liter concentration. Since the latter concentration is just above the saturation dose (10 μl/liter) required for many physiological responses (9), the influences of ethylene on auxin metabolism as reported here may indicate similar functions for endogenous ethylene.

Application of the *n*-butyl ester of 2,4-D or of 2,4,5-T to the leaf blades of *Euonymus japonica* results in the yellowing of areas of leaf tissue surrounding the areas of application (14). The senescence of the tissue is considered to be induced by ethylene which is produced in the treated regions of the leaf (14). The treated areas remain green presumably due to the

high auxin content of those areas. An effect of ethylene in inducing senescence may be through an influence of the gas on auxin metabolism. Experiments are in progress to determine what effect, if any, ethylene may have on auxin metabolism at concentration levels more comparable to those occurring naturally within the plant.

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