Effect of Ethylene on Auxin Transport

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Summary. Ethylene was found to have no influence on auxin transport in hypocotyls of *Helianthus annuus* and *Phaseolus vulgaris;* coleoptiles of *Zea mays;* petiole sections of *Gossypium hirsutum, Phaseolus vulgaris,* and *Coleus blumei.* In the experiments described here, the tissues were treated with ethylene only during the 3 hours of polar transport. This short treatment is in contrast to the methods of others who found an effect of ethylene on auxin transport when plants grown in ethylene are used as experimental tissues.

Zimmerman and Wilcoxon (19) were the first to show that indoleacetic acid (IAA) stimulated ethylene production when they reported that a tomato plant treated with a lanolin paste containing IAA produced an emanation that caused an epinastic response in *Chenopodium album* plants. This observation has been confirmed recently by others (3, 6, 15), measuring ethylene production by gas chromatography and using a variety of plants as test material. In an earlier paper (2) we presented evidence that endogenous as well as externally supplied IAA may regulate ethylene production from plant tissue. Until recently (5, 6, 16), the reverse effect, that of ethylene on auxin metabolism and physiology, has been reported in relatively few papers (4, 10, 14, 18).

We are reinvestigating the effect of ethylene on auxin transport because others have used an inhibition (11, 16) or alteration (10) of auxin transport to explain the physiological effects of ethylene. For example, Hall and Morgan (11) found that greater amounts of IAA oxidase were extracted from ethylene-treated versus control plants. They postulated that the increased activity of the oxidase prevented the auxin from reaching the abscission zone and that lowered auxin levels in turn permitted leaf abscission. By measuring auxin transport through a variety of plant structures such as coleoptiles, hypocotyls, and petioles, it should be possible to determine if ethylene enhances oxidase levels in vivo. If increased levels of oxidase are acting in ethylene-treated sections, then less auxin should be collected from the bottom of treated sections. Inhibition of growth (5, 6) and geotropism (6, 8) by ethylene are other examples that implicate an effect of ethylene on auxin transport.

In this report we will present evidence that ethylene has no significant effect on auxin transport when tissue sections are not pretreated with ethylene.

Materials and Methods

Five plant species were chosen for the experiments, *Zea mays* L. var. Burpee's Snowcrop (corn), *Helianthus annuus* L. var. Mammoth Russian (sunflower), *Gossypium hirsutum* L. var. Acala 4–42 (cotton), *Phaseolus vulgaris* L. var. Red Kidney (bean), and *Coleus blumei* Benth. (coleus). The method of growing and collecting corn and sunflower was almost identical with those described by Gillespie and Thimann (8). Cotton and bean plants were grown in soil in 10-cm pots under 1200 ft-c of a combination of fluorescent and incandescent lights for 14 hours per day at 27°. Coleus plants were grown in the greenhouse in soil. Etiolated beans were grown on moist vermiculite at 25° and harvested after 7 days.

Petiole sections were collected from the middle of the petioles of A) the primary leaf of 2-week-old beans; B) the cotyledonary leaf of 3-week-old cotton plants; and C) the fourth leaf of coleus, leaf number one being the uppermost with a petiole of 5 mm or more. Hypocotyl tissue from etiolated beans was collected just below the crook.

The beta-indoleacetic-2 ^{14}C acid (^{14}C IAA) (specific activity 0.96 mc/mmole) was used as obtained from Baird Atomic Incorporated, Cambridge, Massachusetts. The purity of the compound was checked by paper chromatography (17) and the radioactivity of the spots was assayed with a strip scanner. Ninety-five percent of the activity was associated with a Salkowski-positive spot that had a R_F similar to that of pure IAA.

Agar disks (1.5%) containing ^{14}C IAA were prepared according to the method of McCready (13). Activity of the agar blocks and tissue was measured by a windowless flow counter. The method of preparing samples for counting followed essentially that of McCready (12).

Sections of tissue 5.7 mm long, apical or distal end upward, were set on plain agar disks (height 1.5 mm, diameter 5 mm). Another agar disk of similar dimensions that contained 6×10^{-10} moles of ^{14}C IAA was set on top of the tissue sections. This concentration of ^{14}C IAA was the lowest that would give significant counts in the receiver blocks. Higher concentrations were not used because they might interfere with endogenous transport or give rise to a stimulation of endogenous ethylene evolution. Others (8) have shown that the concentration of ^{14}C IAA used here does not cause a saturation of the polar transport system and probably is within the physio-

logical range for transport experiments. During the time allotted for these experiments no ethylene was observed in the gas phase above the control sections. The gas-chromatographic technique used to measure ethylene is capable of measuring levels as low as 0.05 ppm (0.05 nl C_2H_4/ml gas phase).

Ten sections with donor and receiver blocks were set in a glass container that was sealed with a rubber vaccine cap. When required, 1 ppm ethylene was injected into the bottles after the last section was set up. This concentration is maximal for most effects of ethylene on physiological processes such as growth, abscission, and fruit ripening (2, 5). Higher concentrations usually do not have any additional effect. Moist filter paper was placed inside the bottles to prevent the agar blocks and tissue from drying out. After 3 hours, donor blocks, receiver blocks, and tissue sections were collected and pooled into 3 separate planchets and counted. Evidence that the radioactivity is associated with the IAA in the receiver blocks has been discussed by Goldsmith and Wilkins (9). Each experiment with 3 controls and 3 ethylene-treated containers was repeated 3 times except those with coleus, which were repeated twice. Data are presented as the mean of each group, plus or minus the standard deviation.

Results

Table I summarizes a series of experiments designed to measure the effect of ethylene on polar auxin transport. There was no observable difference between treated and control sections using a variety of plants. In addition, ethylene had no effect on the amount of activity left in the donor block or incorporated in the tissue sections.

Some experiments were performed measuring the effect of ethylene on nonpolar transport (donor block basal) on corn coleoptiles and bean hypocotyls. Little transport was observed (ca. 1-2 %) under these conditions, and ethylene had no effect on the amount of activity observed.

Discussion

Van der Laan (18) found that 5 to 50 ppm ethylene had no effect on auxin-a transport through *Avena* coleoptiles. However, since his experiments were not performed with IAA, comparison with more recent experiments is not possible.

Michener (14) reported that IAA transport through oat coleoptile sections cut from ethylene-treated and normal plants was the same. In another experiment he found that 1000 ppm ethylene reduced auxin transport through pea hypocotyls but he interpreted this as due to enhanced destruction of auxin by ethylene-treated hypocotyls. However it is difficult to compare his experiments on auxin destruction and transport because he used intact plants in the destruction experiments and decapitated plants in the transport experiments. Recently, Burg and Burg

Table I. *Effect of Ethylene on Auxin Transport β-Indoleacetic-2-^{14}C Acid; 0.96 mc/mmole, 6 × 10^{-10} mole in 60 mm³ 1.5 % Agar Disk (10^{-5} M).*

Ethylene; 1 nl/ml gas phase, total gas phase 41 ml. Duration of experiment, 3 hours. Temperature 25°.

Plant material	% Total initial radioactivity in receiver blocks*	
	Control	Ethylene
Zea mays coleoptiles	26.6 ± 4.4	31.2 ± 7.9
	23.8 ± 0.2	26.0 ± 5.8
	18.6 ± 4.1	18.3 ± 2.6
Helianthus annuus hypocotyl	7.8 ± 1.2	3.8 ± 1.4
	3.8 ± 0.2	3.4 ± 0.3
	4.8 ± 1.5	5.8 ± 3.0
Phaseolus vulgaris hypocotyls	15.9 ± 7.2	19.6 ± 8.4
	16.8 ± 5.9	12.6 ± 4.5
	16.7 ± 6.0	15.6 ± 0.6
Gossypium hirsutum petioles	3.5 ± 2.5	4.1 ± 1.2
	2.9 ± 0.9	3.4 ± 1.0
	2.5 ± 1.0	3.0 ± 1.4
Phaseolus vulgaris petioles	2.6 ± 0.2	2.6 ± 0.8
	3.0 ± 1.0	1.8 ± 0.1
	2.0 ± 1.2	1.3 ± 0.6
Coleus blumei petioles	2.8 ± 1.0	2.8 ± 1.6
	1.3 ± 1.1	1.1 ± 0.2

* Plus or minus standard deviation.

(6) and Morgan and Gausman (16) reported that ethylene did not stimulate the destruction of ^{14}C IAA in pea and cotton sections, respectively.

Although no evidence was presented, Borgström (4) advanced the idea that "under the influence of ethylene, auxins are assumed to be spread laterally throughout the tissue, which implied that the phloem loses the capacity for retaining the growth hormone." Guttenberg and Steinmetz (10) failed to substantiate Borgström's theory as they were unable to find any difference in the inward spread of auxin applied on the surface of ethylene-treated and control oat coleoptile sections. Guttenberg and Steinmetz (10) also measured the effect of ethylene on auxin transport through 2-mm portions of *Avena* coleoptiles using the auxin diffusing from coleoptile tips as the source of hormone. In contrast to the results published by Michener (14) they found that coleoptile sections cut from ethylene-treated plants inhibited the flow of auxin into agar receiver blocks. They obtained similar results with *Helianthus annuus* and *Phaseolus vulgaris*.

More recently Morgan and Gausman (16) confirmed Guttenberg and Steinmetz (10) observations. They found that auxin transport through sections of cotton and cowpea (*Vigna sinensis* Endl.) stems cut from ethylene-treated plants was reduced when compared to controls.

In experiments similar to those reported here, Burg and Burg (5, 6) found no effect of ethylene on auxin transport through pea, corn, or sunflower sections in a 3-hour period. Repeating Morgan and Gausman's (16) experiments they found that the inhibition of auxin transport first became noticeable

after 4 to 5 hours of ethylene fumigation and was almost complete after 18 hours.

An inhibition of auxin transport through sections cut from ethylene-treated plants has been observed by all workers (6, 10, 16) except Michener (14). However, Michener's (14) experiments are difficult to interpret because he did not indicate the length of time he incubated his oat coleoptiles in ethylene.

Results presented here, and those of Burg and Burg (5, 6) indicate that there is no effect of ethylene on auxin transport when the gas is supplied during the transport experiment.

In summary, most workers have found that when plants are grown in ethylene their auxin transport system is altered. On the other hand ethylene has no effect on 3-hour auxin transport experiments with normal tissues. It seems reasonable that an ethylene fumigation that inhibits growth would be difficult to analyze in terms of subsequent effects on auxin transport; that is, it is hard to distinguish between auxin transport in tissue whose normal growth has been inhibited and any special effect of ethylene on the transport system itself.

The best example of an experiment that shows that the physiological response of plant tissue to ethylene is due to an interference of auxin movement has been presented by Burg and Burg (6). They found that ethylene inhibited the geotropic curvature of pea sections within an hour after the presentation of a geotropic stimulus and that ethylene almost immediately inhibited lateral transport of ^{14}C IAA in this tissue. They also reported that ethylene would inhibit the growth of pea-stem sections after a 3-hour lag. If the inhibition of growth was due to an inhibition of IAA transport, then it should have been observable within the time alloted to these experiments. It is apparent that some explanation other than an inhibition of auxin transport will have to account for the inhibiting effect of ethylene on pea-stem growth.

Morgan and Gausman (16) cite their experiments as supporting the idea that an inhibition of auxin transport by ethylene accounts for the action of ethylene on abscission as well as other formative effects of the gas. It is well known that IAA prevents abscission. Presumably then, restriction of auxin transport by ethylene that in turn reduces auxin levels in the petiole could account for the abscission-stimulating effect of ethylene. However, ethylene had no effect on the auxin transport experiments with bean, cotton, and *Coleus* petiole sections reported here. Recent experiments (2) suggest an alternative hypothesis as to how ethylene stimulates abscission. We have found that inhibitors of protein synthesis such as actinomycin D and cycloheximide prevent abscission and that ethylene is capable of stimulating RNA and protein synthesis in the separation layer of bean explants. These results suggest that the role of ethylene, in abscission at least, is not to prevent auxin transport, but to act as an effector substance in a specially sensitive region of the plant leading to the formation of protein specifically required for the abscission process.

Acknowledgments

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