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Failure of Ethylene to Change the Distribution of Indoleacetic Acid in the Petiole of *Coleus blumei* X *frederici* during Epinasty[1]

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ABSTRACT

The effect of ethylene on the distribution of applied indoleacetic acid in the petiole of *Coleus blumei* Benth. X *C. frederici* G. Taylor has been investigated during the development of epinastic curvature. Using intact plants, ^{14}C-IAA was applied to the distal region of the leaf lamina and the accumulation of label in the abaxial and adaxial halves of 5 mm petiole sections was determined after 1.5, 3, and 6 hours. Over this period the label was transported out of the lamina into the petiole at a rate of at least 66 mm hr^{-1}. Of the total amount of label in the petiole sections, 24 to 30% was located in the adaxial half and this distribution was not altered significantly by exposing plants to an atmosphere containing 50 μl/l ethylene. Thus when epinastic curvature is induced by ethylene there is no associated increase in the IAA content of the expanding adaxial half. The role of endogenous IAA in petiole epinasty was studied by restricting its movement with DPX 1840 (3,3a-dihydro-2-[*p*-methoxyphenyl]-8H-pyrozolo{5,1-a}isoindol-8-one). The leaf petioles still showed an initial epinastic response to ethylene. It is concluded that ethylene-induced epinasty is not dependent upon either any change in the transport of IAA or its redistribution within the petiole.

The epinastic curvature of the leaf petiole that is induced by ethylene in many dicotyledonous plants results from the stimulation of elongation growth in the adaxial (upper) half of the petiole, while elongation in the abaxial (lower) half is suppressed (8). Epinasty can also be induced by the application of IAA, 2,4-D, naphthaleneacetic acid, or the rotation of the plant on a horizontal clinostat, but in these cases there is evidence that the causal agent is endogenously produced ethylene (4, 9). This raises the possibility that in some plant organs, ethylene is able to stimulate cell expansion directly. Lyon (5), however, proposed that ethylene-induced leaf epinasty is an auxin effect, resulting from the accumulation of lamina produced auxin in the upper half of the petiole with the consequent stimulation of growth there. His theory was supported by experiments with tomato and pepper leaves, in which ^{14}C-IAA was applied to the lamina in the presence of ethylene and its subsequent distribution in the petiole determined. This was done by solvent extraction of bulked tissue cut from the upper and lower halves of the petiole and the measurement of radioactivity in the concentrated extracts. The values obtained were used to estimate the concentration of ^{14}C-IAA in each half of the petiole.

Lyon's experimental procedure appears now to be unsatisfactory in a number of respects. Ethylene initiates epinasty in tomato and pepper plants within 2 or 3 hr, but Lyon did not determine the distribution of ^{14}C in the upper and lower halves of the petiole tissue until 24 hr after the application of the label to the lamina surface, so that the reported preferential accumulation in the upper half, in the ratio of 3:2, could have occurred after the completion of the epinastic curvature. In addition, he did not establish that the ^{14}C extracted from the petiole tissues was still labeling IAA. Lyon performed 17 experiments with tomato and pepper, but only 6 included control plants which had not been exposed to ethylene. In four of these controlled experiments, ethylene increased the amount of ^{14}C extracted from both halves of the petiole, although ethylene is generally known to reduce polar transport of IAA in intact plants (6). In *Ranunculus sceleratus*, however, where ethylene promotes petiole elongation, it has been reported that the capacity of petiole sections to transport IAA by polar transport is increased by ethylene (7).

This paper describes experiments in which Lyon's hypothesis has been re-examined. A measured amount of ^{14}C-IAA was applied to one part of the leaf lamina in either the presence or absence of ethylene. The ^{14}C-IAA content of individual petiole half-sections was then determined directly by scintillation spectrometry. This was done during the first 7 hr, following exposure to ethylene, while the epinastic reaction was still actively developing. Lyon's assumption that the basic cause of leaf epinasty is the lateral displacement of lamina-synthesized auxin, as it moves through the petiole by polar transport, was tested by means of the synthetic plant growth regulator, DPX 1840, (3,3a-dihydro-2-[*p*-methoxyphenyl]-8H-pyrazolo{5,1-a}isoindol-8-one). This compound restricts both the polar transport and lateral redistribution of IAA in plant shoots (1). It is also reported to induce epinasty in stems and leaves (1), but the occurrence of rapidly developing dorso-convex curvature in leaves, that characterizes true epinasty, has not been described. The development of downwardly directed growth curvatures in stems and leaves following the application of DPX 1840, probably results from its ability to inactivate the orienting processes of geotropism.

MATERIALS AND METHODS

The plant material was a hybrid between *Coleus blumei* Benth. and *C. frederici* G. Taylor. This hybrid possesses leaf petioles which are almost square in cross-section, facilitating their bisection into upper and lower halves. The actively elongating petioles of the 3rd and 4th pair of leaves from the terminal bud were used as experimental material. The plants were grown under glasshouse conditions and for 12 hr before use, they were kept under continuous fluorescent illumination at 2.60 w m^2 intensity, at an air temperature of 25 C and 75% relative humidity. This pretreatment of the plants reduced variability in the results, both between plants and between replicates. The experiments were performed in gas-tight glass cabinets at 25 C and 444 mw·m^2 light intensity provided by incandescent illumination.

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Relative humidity ranged from 85 to 95%. Control plants were kept in laboratory air. In the ethylene treatment, plants were exposed to an ethylene-air mixture of 50 μl/l, commencing 1 hr prior to the application of the label and continuing for 6 hr thereafter. With this concentration of ethylene, epinastic curvature of the leaf petiole became apparent after 2 hr and continued to develop actively for about 12 hr (see ethylene treatment, Fig. 1).

IAA-1-^{14}C (55 mCi/mmol), in aqueous solution, was applied to the distal region of the leaf lamina in the form of 30 equally spaced droplets, so that each leaf received a dose of 0.12 to 0.14 μCi ^{14}C in 10μl of solution. The region of application was 100 to 110 mm from the test zone in the petiole. A smear of grease was applied to the base of the lamina to prevent the possibility of surface migration of radioactivity to the test zone. The distal region of the lamina was used as the application site following preliminary experiments which showed that applications of ^{14}C-IAA to the basal region resulted in relatively more label appearing in the upper half of the petiole, whereas applying the label to the distal part of the lamina gave more ^{14}C counts in the lower half of the petiole. A similar relationship between the lamina and petiole tissue has been found for *Fatsia japonica* (10) and *Carica papaya* (11). Since a distal application provides a good case for testing the ethylene effect, ^{14}C-IAA was applied to the distal part of the lamina in every experiment.

In each treatment, 6 to 10 leaves were used and the vertical distribution of ^{14}C-IAA in each petiole was determined in five 5 mm sections cut from the distal region and then bisected into an upper and lower half. Each half was placed in a scintillation vial, covered with scintillation fluid composed of 75% toluol and 25% ethanol (v/v) with added fluors. After standing for 24 hr the vials were counted three times for ^{14}C in a scintillation spectrometer and the mean dpm for the upper and the lower half-section from each petiole was then determined. The labeled substances extracted by the scintillation fluid were separated using TLC and two solvent systems, either ethyl acetate-isopropanol-ammonia (90:75:35) or chloroform-ethyl acetate-formic acid (50:40:10). In each system 89 to 95% of the total radioactivity appeared in the same R_F zone as unlabeled IAA, identified by UV fluorescence, leading to the conclusion that the ^{14}C eluted from the half-sections was predominantly with IAA. The ^{14}C remaining fixed in the half-section did not comprise more than 3% of that eluted by the scintillation fluid. DPX 1840 (0.53 mg/ml) was applied to the test leaf in aqueous solution. This was sprayed onto both lamina surfaces and onto the petiole, 12 hr before experiments were commenced. Its uptake by the leaf was demonstrated by the gradual loss of normal orientation. Epinasty was recorded by measuring the radius of curvature of the leaf petiole in mm and expressed as the reciprocal of the radius $\times 10^2$ (9).

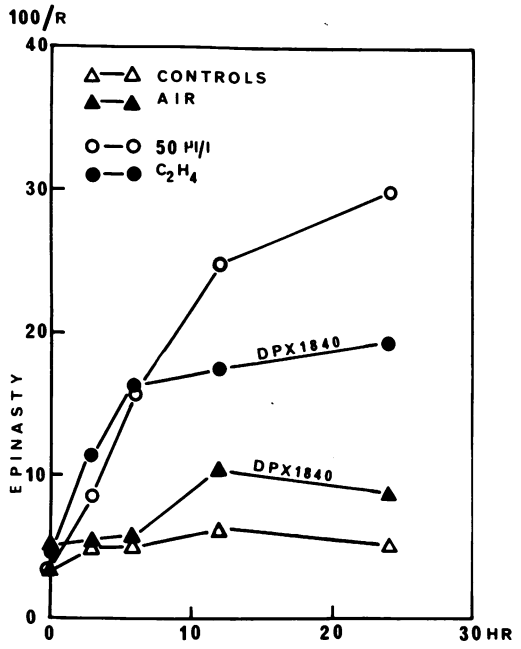

FIG. 1. Effect of DPX 1840 on the epinastic response of leaf petioles to ethylene. Values are means for 28 petioles and 3 replicates.

RESULTS AND DISCUSSION

After 1.5 hr from its application to the lamina, ^{14}C-IAA was detected in the upper and lower half-sections of the petiole in both the control and ethylene treatments (Table I). From this the velocity of IAA transport into the petiole was calculated to be greater than 66 mm hr^{-1}. Since polar transport moves IAA through stem and petiole tissues at only 3 to 6 mm hr (3) this mechanism could not have been the means of transport. Goldsmith *et al.* (2) have shown that fast transport of ^{14}C-IAA can occur in the vascular tissues of intact *Coleus* plants at a velocity of 100 to 200 mm hr^{-1}, which is greater than the rate found in these experiments. This discrepancy may have been due to the time taken for the applied IAA to move from the lamina surface into the leaf veins.

Lyon's hypothesis can be applied to a fast transport system if it is assumed that ^{14}C-IAA can leak from the vascular tissue into the surrounding parenchyma (2), and is then moved in a basipetal direction by polar transport, while at the same time being deflected into the upper half of the petiole, under the influence of ethylene, in the same way that gravity deflects auxin toward the lower half of a horizontally placed stem or coleoptile. However, Table I does not provide any evidence that such a mechanism was operating during the period from 1.5 to 6 hr, when ^{14}C-IAA was present in the petiole and the epinastic reaction was developing actively. In the ethylene treatments, and in the controls, the label occurred predominantly in the lower half, and there was no statistically significant increase in the amounts of ^{14}C extracted from the upper half-sections in the presence of

Table I. *^{14}C Content of Petiole Half-sections*

The 5 mm half-sections were analyzed following application of 0.12 to 0.14 μCi IAA-1-^{14}C to the distal part of the lamina in the presence or absence of ethylene.

Transport time hr	Applied ^{14}C-IAA dpm X 10^{-3}	^{14}C extracted from half-section Lower dpm	Upper dpm	dpm in upper-half %	Replicates No	Half-sections
A. Air						
1.5	129	24.72 ± 1.15	7.90 ± 0.53	24	2	60
3.0	138	32.38 ± 6.64	13.77 ± 3.51	30	3	100
6.0	120	174.30 ± 16.80	71.55 ± 6.40	29	6	190
B. Ethylene-air						
1.5	129	26.10 ± 3.66	5.40 ± 1.15	17		
3.0	138	34.08 ± 7.62	13.19 ± 2.82	28		
6.0	120	151.60 ± 16.50	70.70 ± 9.50	32		

ethylene during the 6 hr period of the experiment. The percentage of total counts in the upper half increased from 17 to 32% in the ethylene treatment, but this is not significantly different from the increase of from 24 to 29% that occurred in the control half-sections. As in other plants, the elongation rate of the upper half of the petiole increases markedly during the epinastic response, so that these results do not support the theory of Musgrave and Walters (7) that in systems where ethylene promotes tissue elongation, the capacity of the system for IAA transport is also enhanced.

Figure 1 shows that the initial epinastic response to ethylene still occurred in leaf petioles that had been pretreated with DPX 1840 to arrest redistribution of endogenous IAA. The decline in the rate of development of epinasty in DPX 1840-treated leaves after 6 hr, suggests that lamina IAA may be a co-factor for ethylene action in *Coleus*. This contrasts with *Helianthus annuus* where ethylene-induced petiole epinasty is independent of lamina factors (8).

No evidence has been found that mobile IAA is involved in ethylene-induced epinastic petiole curvature in *Coleus blumei* X *C. frederici*. In this plant the curvature is not caused either by the accelerated transport of lamina IAA into the upper half of the petiole, its enhanced accumulation there or by its redistribution within the petiole. These findings support the hypothesis that in some circumstances, ethylene may directly promote elongation growth of plant tissues (8).

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LITERATURE CITED

1. Beyer, E. M. 1972. Auxin transport: a new synthetic inhibitor. Plant Physiol. 50: 322-327.
2. Goldsmith, M. H., D. A. Cataldo, J. Karn, T. Brenneman, and P. Trip. 1974. The rapid nonpolar transport of auxin in the phloem of intact *Coleus* plants. Planta 116: 301-317.
3. Jacobs, W. P. 1967. Comparison of the movement and vascular differentiation effects of the endogenous auxins and of phenoxyacetic weed killers in stems and petioles of *Coleus* and *Phaseolus*. Ann. N. Y. Acad. Sci. 144: 102-117.
4. Leather, G. R., L. E. Forrence, and F. B. Abeles. 1972. Increased ethylene production during clinostat experiments may cause leaf epinasty. Plant Physiol. 49: 183-186.
5. Lyon, C. J. 1970. Ethylene inhibition of auxin transport by gravity in leaves. Plant Physiol. 45: 644-646.
6. Morgan, P. W. and H. W. Gausman. 1966. Effects of ethylene on auxin transport. Plant Physiol. 41: 45-52.
7. Musgrave, A. and J. Walters. 1973. Ethylene-stimulated growth and auxin transport in *Ranunculus sceleratus* petioles. New Phytol. 72: 783-789.
8. Palmer, J. H. 1972. Roles of ethylene and indole-3-acetic acid in petiole epinasty in *Helianthus annuus* and the modifying influence of gibberellic acid. J. Exp. Bot. 23: 733-743.
9. Palmer, J. H. 1973. Ethylene as a cause of transient petiole epinasty in *Helianthus annuus* during clinostat experiments. Physiol. Plant. 28: 188-193.
10. Yamane, G. 1941. Untersuchungen über die vertikalen phototropischen Bewegungen der Laubblätter von *Fatsia japonica* vom Standpunkt der Wuchsstofflehre. Jap. J. Bot. 11: 305-326.
11. Yin, H. C. 1941. Studies on the nyctinastic movement of the leaves of *Carica papaya*. Am. J. Bot. 28: 250-261.