Short Communication

Auxin Control for Orientation of Pea Roots Grown on a Clinostat or Exposed to Ethylene

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A lateral imbalance in auxin content has been shown to occur in the epinastic curvatures of branches (7) and leaves (8), and has been suggested as the cause of these as well as root (11) reorientation. There are differences of opinion about patterns of IAA transport in roots (3–5, 14), however, and even the participation of IAA in the growth of roots has been questioned (17). Evidence is lacking that the hormonal basis for plagiotropism in roots is similar to that for aerial organs.

Partial control of orientation of secondary roots by an epinastic factor, as defined by De Vries in 1872 (2) to oppose the effects of geotropism, first became known through increase in the liminal angles of plagiotropic roots grown on a horizontal clinostat (6). A strong effect of this epinastic factor appeared in the two seminal roots of young wheat seedlings grown in the NASA Biosatellite II experiment and its clinostat ground controls (9). When plagiotropic, adventitious roots or herbaceous stems are exposed to ethylene or acetylene, the orientation of the new growth has been reported to change to a right angle with the stem (18), as in the case of secondary roots formed on stems on a clinostat or exposed to ethylene (1).

The hormonal basis for the epinastic curvatures in leaves has been inferred to be the excess of IAA in the convex half of the growth zone (10). A similar imbalance in auxin supply to the upper and lower sides of a plagiotropic root is a reasonable hypothesis for any increase in its liminal angle, despite the uncertainty about the physiological function of auxin in roots. The present short communication offers support for this hypothesis and will suggest that the root epinasties on the clinostat and from ethylene are of similar origin.

MATERIALS AND METHODS

Roots grown on erect plants in darkness and moist air orient without effects of physical factors except gravity. This influence of gravity was eliminated by rotation of the plant at 1 rpm on a clinostat (9).

Young seedlings of a garden pea (*Pisum sativum* L. cv. Little Marvel) were used as a source of lateral roots large enough to bisect into upper and lower halves. Seeds were germinated in sets of 10 in pockets of special holders filled with wet vermiculite, as in Figures 1 and 2. Seedlings with straight primary roots were planted in the holders 2 days after initial soaking in water and allowed to grow another 2 days with their axes erect to gravity in darkness at 24 C.

Each seedling stem was then excised to leave a stump

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about 8 mm long. A short glass tube was fitted over each stump and filled with an aqueous solution of 1 μM IAA-5-^{3}H. Its radioactivity was 8.6×10^3 cpm per microliter. The amount of solution in a tube was about 20 μl. These tubes remained on the seedlings as lateral roots were produced during the following 3 days. The average amount of liquid taken into a seedling was about 10 μl and was available to lateral root growth by way of the primary root, as shown in Figures 1 and 2. When pea seedlings are supplied with IAA through apical buds, some of the auxin moves into lateral root primordia (13).

For growth of the root in the absence of an effective gravity vector, each sealed plastic cylinder with its set of 10 seedlings was attached at once to the axis of a horizontal clinostat. For a test of the effects of ethylene on root orientation, the seedlings were kept erect. The cylinder cover was removed while a desired concentration of the gas was allowed to diffuse in from the atmosphere of a closed chamber to which ethylene has been added in a suitable amount.

The concentration of ethylene around the seedlings was measured in 1-mm^3 samples from cylinders closed after 45-min exposure to the mixture of air and ethylene. Measurements of the gas content were made with a gas chromatograph as previously described for experiments with leaf epinasty (10).

After the later roots had grown for 3 days, either on a clinostat or on erect plants exposed to ethylene, the experiment was terminated by removal of the longest roots and measurement of auxin distribution between the upper and lower halves of the roots. This operation included manual, longitudinal bisection of each root, assembly of the two lots of half-roots, extraction of each lot by grinding it in a mortar with ethanol, and counting the radioactivity in solution and in the fibrous residue. A minimum of 100 roots was used for each experiment and care was taken to obtain equal weights of tissue in half-roots. Radioactivity was measured in a liquid scintillation counter.

Orientation of roots was described by their liminal angles, measured from enlarged wall projections of color transparencies taken at the close of each experiment. The value of each liminal angle was calculated to the nearest tenth of a degree with reference to the base of the root (11).

RESULTS

Clinostat Experiments. Lateral roots developed on a clinostat grow at right angles to the primary root. From measurements of 358 pea roots grown as in Figure 1, the mean liminal angle was found to be $88.8 \pm 0.8°$. A few of the longer roots grew at angles slightly greater than 90° (Fig. 1). As the corresponding mean liminal angle was $57.8 \pm 0.6°$ for 505 roots
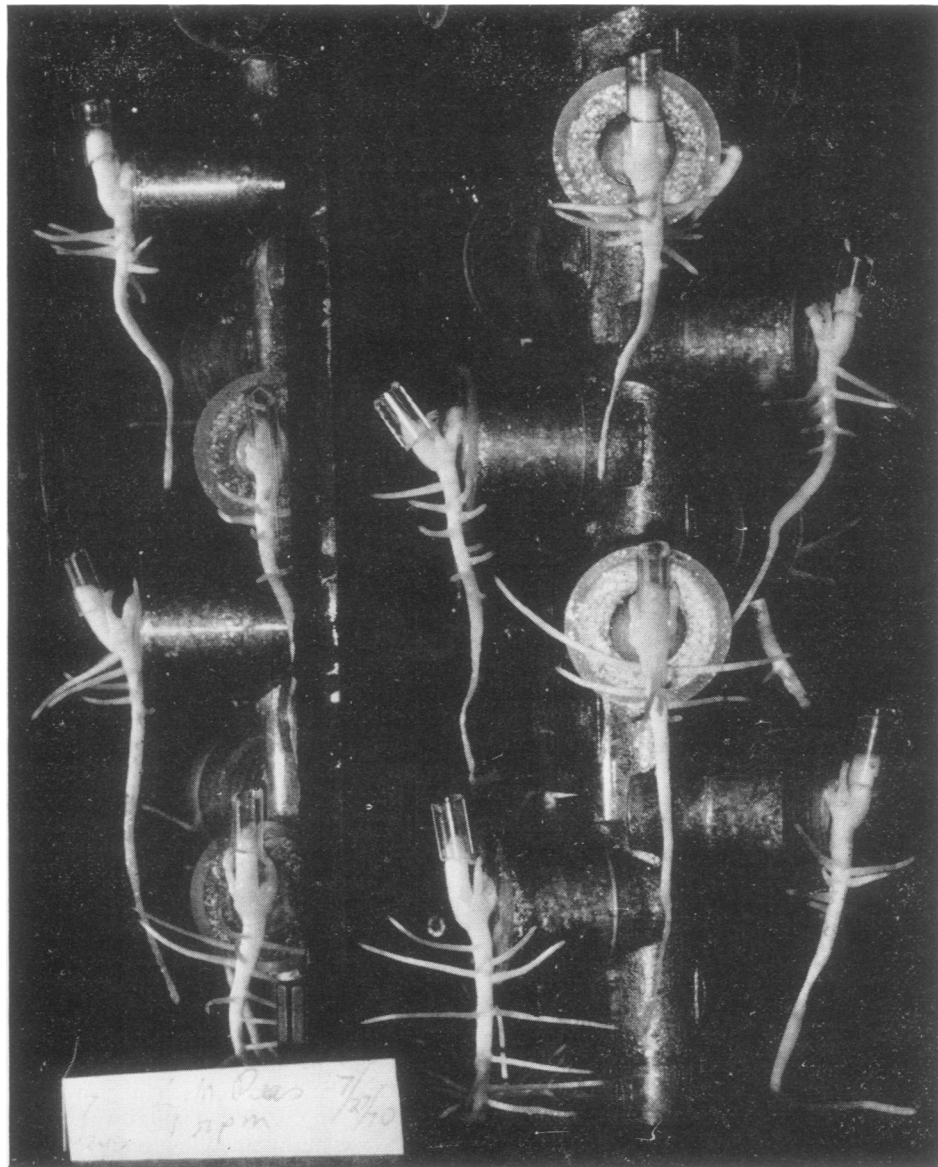
FIG. 1. Seedlings of garden pea grown 3 days on a clinostat after 4 days erect. Solution of IAA-5-³H was supplied from glass tubing during rotation on the clinostat. Mirror images at left show two side and two face views of four plants.

on seedlings erect to gravity (Figs. 2 and 3), the increase in the clinostat could represent a decrease in auxin transport, such as occurs in leaves and branches on the clinostat (7, 8).

An inhibition in the apipolar transport of auxin in roots grown on a clinostat is demonstrated by the data in Table I, columns 3 and 4. There is, roughly, a 3-fold decrease in the amount of auxin transported into the lateral root on the clinostat. A lateral redistribution of the auxin does take place, however, with 20.1% excess of IAA occurring in the upper half of the root. This extra IAA in the morphologically upper tissues of a root compares favorably with the 36.7% excess for tomato leaves and 22.3% for poinsettia leaves reported by Lyon in 1963 (8) from similar clinostat experiments. The control tests with erect seedlings (Table I) showed no excess of IAA in either side of the root.

Ethylene Tests. The effect of an agent like ethylene on a transport process will depend partly on the concentration available for entry and reaction with root tissues. Preparation of a dose-response curve was therefore the first step in deter-

mining if ethylene affects root orientation and auxin distribution. The results of 17 tests on the size of the liminal angle over a range of ethylene concentration are shown in Figure 3.

One notable effect of the gas was its action on organ orientation at concentrations about a tenth of those effective in producing leaf epinasty (10). Roots failed to grow well in air with more than 22 μl ethylene/liter. At this concentration of gas the mean liminal angle had increased to about 88°, but an orientation of about 85° appeared with an ethylene concentration of only 5 μl/liter.

Throughout this range of concentrations the orientation pattern of the roots was very similar to that shown in Figure 1 for growth on a clinostat. To determine if the cause for the large liminal angles was inhibition of the transport of IAA, as in leaf epinasty, the auxin distribution in roots with large liminal angles was measured as for roots grown on a clinostat. The results are shown in Table I. The concentration of ethylene for these four tests varied from 6.4 to 9.1 μl/liter. The roots were shorter by partial inhibition of elongation, but
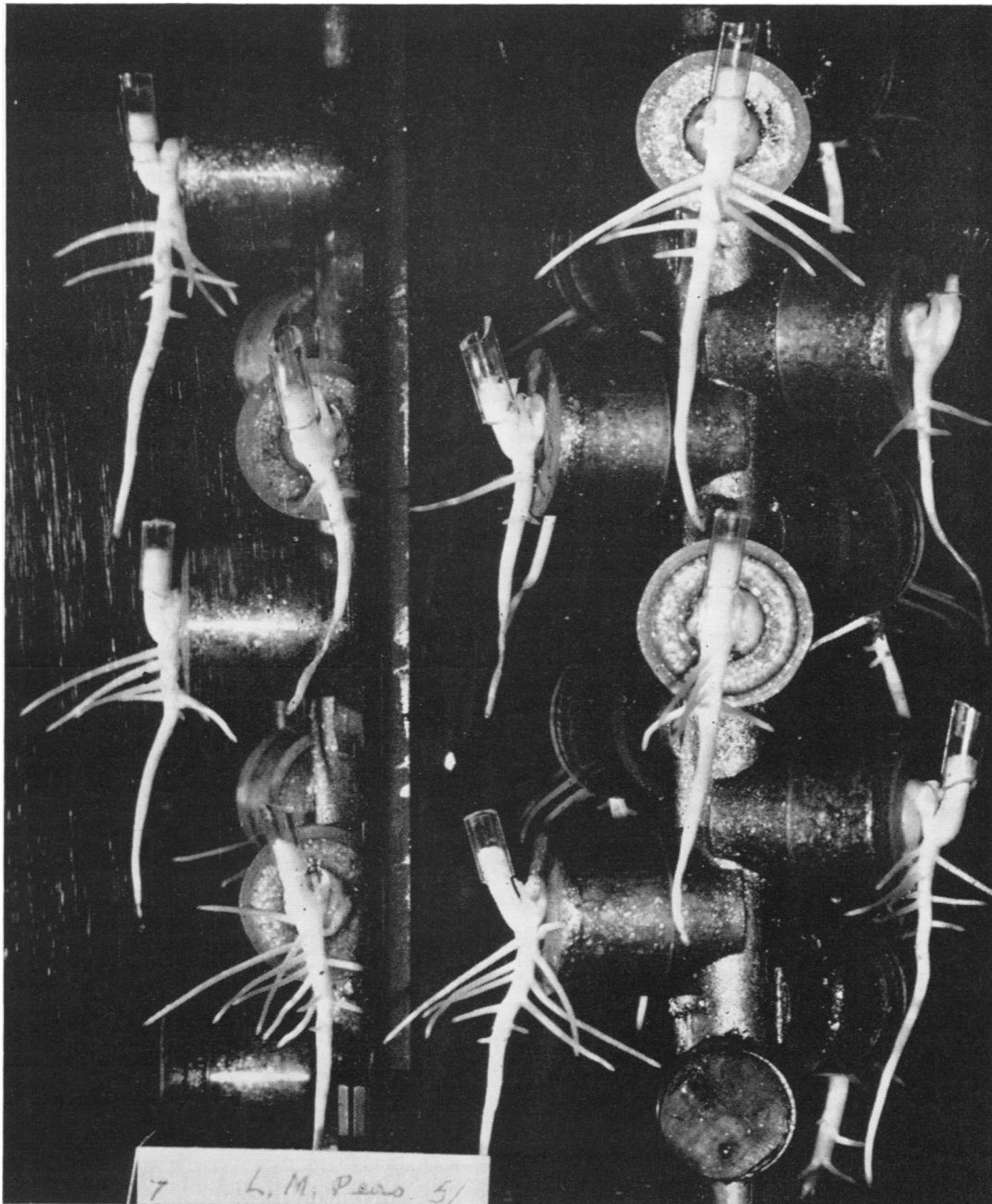
FIG. 2. Erect seedlings of garden pea grown 3 days with supply of IAA-5-^{3}H in solution from glass tubes after 4 days with only endogenous auxin. Mirror images at left show two side and two face views of four plants.

other aspects of growth remained unchanged. As with the clinostat, there was about a 3-fold reduction in amount of auxin transported into the roots and a 20% excess of auxin in the upper halves. In the three treatments—clinostat, ethylene, and control—there was no significant correlation between weight of tissue and total radioactivity and lateral distribution of activity.

DISCUSSION

The growth and orientation of these roots in intact seedlings are the same with or without supplement with labeled IAA. Yet it is clear that both the clinostat and ethylene reduce by about two-thirds the apipolar transport of IAA (Table I). The impairment is greater in the lower side than in the upper, to about the same degree with the clinostat as with the ethylene treatment (Table I). As a result the upper tissues have more auxin than the lower. Presumably, auxin is at a concentration in these root tissues that is growth inhibitory (16). The liminal angle therefore increases; the orientation of the lateral roots toward the primary root changes from 55°–60° (Figs. 2, 3) to approximately a right angle (Figs. 1, 3).

The hormonal basis for plagiotropic roots thus becomes the same as that for leaves and branches except for the origin of the IAA and the direction of movement along the organ. For lateral roots the auxin enters from the primary root and moves in an acropetal flow. In leaves and branches the auxin
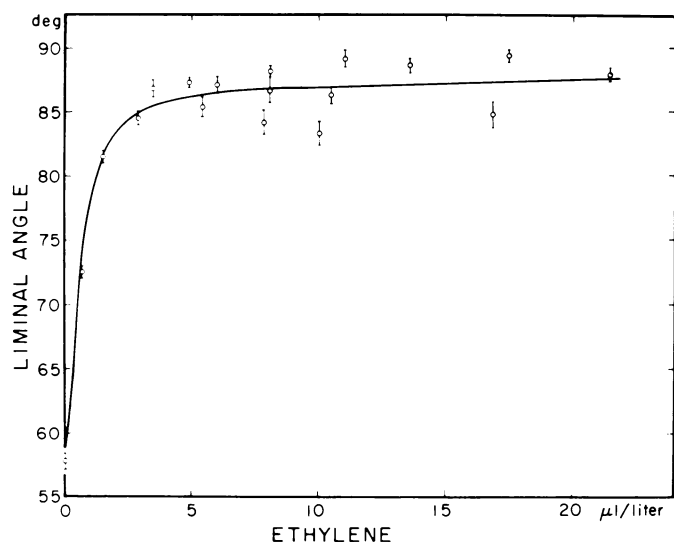
FIG. 3. Dose-response curve for liminal angles of lateral roots of garden pea developed on erect seedlings while exposed to ethylene gas in moist atmosphere of a closed chamber. Vertical bars at data points show the standard errors of the mean.

Table I. *Distribution of Auxin in Lateral Roots of Peas Measured by Radioactivity from IAA-5-^{3}H*

Experiment	Dry Wt of tissue	Total Radioactivity		Excess Auxin in Upper Side
		Upper side	Lower side	
	mg	*cpm*		*%*
Clinostat				
1	8.9	272	194	40.3
2	8.0	782	702	11.1
3	15.0	996	863	15.4
4	15.0	1150	1020	13.6
Mean		800	695	20.1 ± 6.8
Ethylene				
1	12.0	591	479	23.5
2	9.0	1060	965	10.2
3	7.0	1150	1010	14.1
4	5.0	648	490	32.2
Mean		862	736	20.0 ± 4.9
Controls				
1	13.5	1990	2010	−0.9
2	14.0	2950	2970	−0.7
3	11.6	2640	2630	1.8
Mean		2530	2540	

is formed primarily in green tissues and is transported in a strong basipetal flux.

True nastic curvatures developed in some lateral roots of Figure 1. They can also be produced by allowing the plant to grow alternately on a clinostat or erect. Evidence for such changes in root orientation was presented by Lyon and Yokoyama (11) for lateral roots of wheat seedlings grown in holders like those used for our experiments with peas.

The importance of auxin in the regulation of root orientation is suggested by the inhibition of its acropetal transport through the action of a low concentration of ethylene (cf. also 12). The inhibitory effect was equal to that induced by elimination of the gravity vector on the clinostat, whether measured by liminal angle or by the lateral difference in transport of IAA. From these observations, we raise the possibility that the effect of the clinostat is mediated by the production of ethylene. The clinostat and ethylene are identical in their effects on auxin transport and lateral distribution. Furthermore, it was shown that 40 C for 4 hr made tomato seedlings unable to produce ethylene or to become epinastic when treated with IAA in a way that produced epinasty in control plants at 25 C (15). The auxin treatment was shown to stimulate ethylene synthesis in the control plants. When ethylene was applied to heat-treated or control plants, both became epinastic. Thus, the critical factors for epinasty is the capacity to produce ethylene. It would be of interest to determine if horizontal rotation on the clinostat raises the production of ethylene.

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