Timing of growth regulator responses in peas [1]

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SUMMARY

Using an electric position-sensing device, the latent times for growth responses of individual pea plants to auxin, cytokinin, gibberellin, ethylene and abscisic acid were found to be within a range of 5 to 23 min. These short latent periods for growth regulations as well as the short after-effect following removal of ethylene (16 to 21 min.) suggest direct enzymatic controls by the entire set of known growth regulators in the cell. The latent periods resemble that of a metabolic inhibitor (dinitrophenol, 11 min.), and are much shorter than that of a nucleic acid inhibitor (Actinomycin D, 110 min.).

MATERIAL AND METHODS

Using a position-sensing transducer (Brush Instruments Division of Clevite Corporation, Cleveland, Ohio), with a 2 cm needle through the axis and resting on the tip of an etiolated pea plant, we can measure the growth of the plant with a sensitivity of 1 μ/min, read at 2 min intervals. Rotation of the axis of the transducer alters the balance of current output by two opposing coils and con-

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verts 1 degree of rotation into a 100 mv change in electrical output, which is recorded on a Sargent recorder. The recorder scale can be calibrated into units using a microscopic measurement of needle displacement. Alaska pea seedlings were utilized, germinating the seeds at 24° in the dark for 4 days. The growth regulators were applied to the seedling by dripping the solutions from a burette over the sensing needle and the decapitated seedling at a constant rate. Ethylene was applied by enclosing the intact seedling and needle components in a plexiglass box (850 ml volume) into which ethylene was injected or removed by flushing with moist air.

RESULTS

The latent time for auxin (indoleacetic acid) was determined as illustrated in Fig. 1, from which it can be seen that about 11 min after the auxin had been applied the growth rate began to increase, and a new elevated rate was achieved at about 25 min. This is similar to the 10 min latent time recorded for the auxin response of Avena coleoptile sections by Evans and Ray (1).

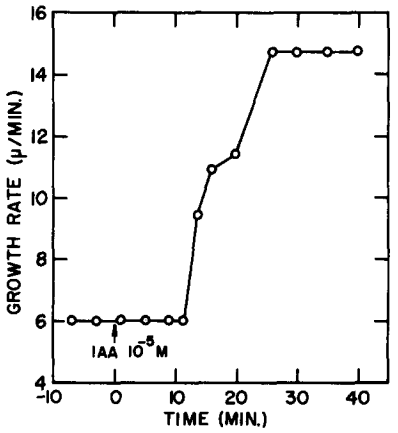

Fig. 1 - The time course of changes in growth rate of etiolated peas following application of indoleacetic acid. The time of auxin application (arrow) was 30 min after decapitation.

Exposure of a seedling to 10 ppm ethylene resulted in a depression of growth rate from about 7.5 μ/min to about 3 μ/min, the depression of growth rate beginning

in about 10 min as shown in Fig. 2; removal of the ethylene from the ambient atmosphere resulted in a restoration of growth rate beginning at about 20 min after the gas had been flushed out (Fig. 2). Average values of latent times for 6 plants are presented in Table 1 as 6.4 for the onset of ethylene-induced inhibition and 20.9 for the onset of restoration of growth rate. If a lower concentration of ethylene were utilized (5 ppm), the latent time for onset of inhibition was lengthened (17.8) min) and the after-effect before restoration began was shortened (16.1 min).

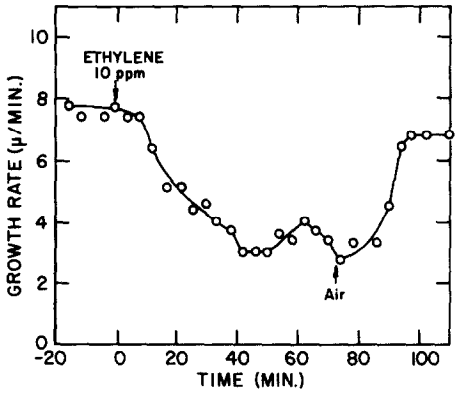

Fig. 2 - The time course of growth rates of intact pea following application of ethylene at time 0, and the subsequent removal of the treatment 72 min later.

A summary of latent times for responses to an auxin, a cytokinin, abscisic acid, gibberellic acid and ethylene are presented in Table 1, and in each instance the values obtained were between 5 and 23 min. The relatively long latent time recorded for gibberellin was associated with the fact that the pea plants had been pretreated with 12 hrs. of red light in order to enhance the responsiveness to applied gibberellin; this treatment greatly retarded the growth rate to about 1 μ/min, and may well have reduced the sensitivity of the timing measurements.

For comparison of the latent times for the various growth regulators with those of a metabolic poison and a nucleic acid poison, measurements were also made of growth rate responses to dinitrophenol (DNP) and Actinomycin D (Act D). A solution of 10^{-4} M DNP brought about an initial inhibition of growth in 11.2

Table 1

Latent Times of Various Growth Regulators and Inhibitors when Applied to Decapitated Etiolated Pea Seedlings

Experiments were started 30 min after decapitation and run in dim green light at 24°C. Each datum represents an average of 4 to 6 plants.

Treatment	Concentration	Latent Time (min)
Indoleacetic Acid	10^{-5} M	9.3 ± 0.8
Benzyladenine	10^{-5} M	11.7 ± 1.7
Abscisic Acid	10^{-5} M	5.1 ± 0.3
Gibberellic Acid	10^{-4} M	23.7 ± 8.8
Ethylene	10 ppm	6.4 ± 2.3
	5 ppm	17.8 ± 5.0
Ethylene Removal	10 ppm	20.9 ± 3.0
	5 ppm	16.1 ± 3.0
Actinomycin D	20 ug/ml	110 ± 44
Dinitrophenol	10^{-4} M	11.2 ± 1.2

min. A solution of 20 mg/l of Act D produced quite variable results, but an average of 6 experiments gave a latent time of 110 minutes for the initial inhibition of growth. In order to be sure that the Act D was in fact depressing RNA synthesis, incorporation of P^{32} into phenol extractable RNA was measured at time intervals of 30 to 180 minutes after application of the inhibitor (method of Cherry et al., 2). In each of the four time intervals, Act D treatment depressed the RNA synthesis (cpm/OD unit of RNA) by 30 to 40%.

DISCUSSION

Our experiments indicate that representatives of each of the known classes

of natural plant growth regulators are capable of altering growth rates of pea in periods of time ranging from 5 to 25 min. Nissl and Zenk (3) have asserted that the latent time for auxin stimulation of growth was mainly a function of the uptake of auxin and its equilibration into the growing cells . The dependence of the latent time for ethylene response upon the applied concentration (Table 1) likewise suggests that a substantial part of the latent time is due to the time requirement for entry of the ethylene.

The evidences that some plant responses to auxin are instituted almost at once upon application of auxin have been reviewed by Evans and Ray (1). They have also discussed the difficulties in interpreting the short latent time for auxin within the concept of an initial action involving an alteration of nucleic acid and protein synthesis. Most experiments on this subject have of course been carried out using exogenous auxins; a natural redistribution of endogenous auxin in geotropism can occur with latent times as short as 8 or 10 min (4).

The shortest latent time previously reported for an ethylene response was 1 hr. (5). The shortest latent time previously reported for a gibberellin response was in the range of 2 to 8 hrs. (6). To the best of our knowledge no critical timing experiments have been previously published for a cytokinin or abscisic acid effect.

Attempts to find the earliest detectable change in nucleic acid synthesis after the application of auxin led Masuda and Kamisaka (7) to suggest that increased synthesis may occur in 10 min after auxin application, but their variability appears to be as large as the reported difference in synthesis (standard errors of 25-30%, differences between treatments of 25%). Some of the difficulties of doing such experiments have been discussed by Nooden (8). Pollard and Singh (6) have attempted to find the earliest time of altered nucleic acid synthesis following gibberellin treatment of barley, and found the first detectable alteration at 8 hrs. Some evidences have previously been reported to indicate that at least some plant responses to auxin and ethylene are independent of RNA and protein synthesis, on the basis that the responses were not inhibited by Act D, cycloheximide, or 5-fluorouracil (9,10).

Our experiments show that the regulation of pea growth rates by the various classes of known plant growth regulators is instated within minutes of the application of the regulator (5 to 23 min). We have shown that the removal of ethylene treatment likewise results in a very brief after-effect before the growth rate begins to return to its endogenous rate (16 min or more). These characteristics more closely resemble alterations of enzyme actions than they do the alterations of nucleic acid and protein synthesis, suggesting that the various plant growth regulators may act initially through facile association with some growth-limiting enzyme systems in the cell. The rapid onset of the decay of regulation upon removal of ethylene, like that of auxin, suggests an easy disassociation of the regulator from the site of action as well.

References and Notes

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