SHORT-TERM GROWTH RESPONSE TO GROWTH REGULATORS IN ROOTS OF *ZEA MAYS*

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Summary

A study was made of the inhibition of root growth in *Z. mays* by 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), and indole-3-acetic acid (IAA) over a wide range of concentrations. IAA and 2,4-D caused identical responses whereas the response to 2,4,6-T was different. Application of 2,4-D or IAA at concentrations of $4 \cdot 5 \times 10^{-4}$M for 5 min resulted in an immediate reversible inhibition of root growth whereas long-term application gave an irreversible inhibition. The same concentration of 2,4,6-T caused a similar degree of inhibition of root growth, but only after an extended treatment, and the roots could then recover from the inhibition in the presence of the chemical.

Anatomical studies showed that 2,4-D inhibited cell maturation and stimulated cell division.

I. Introduction

Wedding and Black (1962) reported that 2,4-dichlorophenoxyacetic acid (2,4-D) acted as an uncoupling agent of oxidative phosphorylation. Oxidation was also inhibited. They subsequently showed that the inhibition of oxidation could be explained by the inhibition of malic dehydrogenase (Wedding and Black 1963). In experiments on the uncoupling action of 2,4-D, Wedding and Black made no attempt to determine whether the uncoupling was due to the growth-regulatory activity of 2,4-D or to side reactions. In order to clarify this point, the action of 2,4-D on plant growth and on isolated enzyme systems was compared with 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) [a compound of similar structure but lacking auxin activity (Ng and Audus 1964)], with the natural growth regulator indole-3-acetic acid (IAA), and with the classic uncoupling agent 2,4-dinitrophenol. This paper describes the effect of 2,4-D, 2,4,6-T, and IAA on the growth of the intact *Zea mays* plant.

Growth regulators are usually applied to intact plants by spraying the shoot; observations are then made on the aerial organs (Key, Hanson, and Bils 1960; Key and Hanson 1961; Key and Wold 1961; Shannon 1962; Shannon, Hanson, and Wilson 1964). Spraying, however, results in large variations in quantity of regulator received per shoot and, since it is virtually impossible to protect roots when spraying shoots, introduces the problem that observed effects on shoot growth may be an indirect effect, due to inhibition of root growth. Root irrigation lends itself to more uniform and reproducible application of the growth regulator to the plant and in the studies described below this method was used. Roots are normally

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much more sensitive to growth regulators than other organs (Thimann 1937), hence it was necessary to obtain some idea of the response of the intact tissue to the inhibitors.

II. Materials and Methods

Plants

In all experiments etiolated corn seedlings, *Z. mays* cv. WF9 × M14, were used. Seeds were dusted with Spergon fungicide (Science Products, Inc.) and placed, germ downwards, on four layers of paper towelling saturated with 10^{-4}M calcium chloride solution in a 34 by 22 cm glass planting tray. The trays, each containing 180 seeds, were covered with Saran Wrap (Dow Chemical Company, Midland, Michigan), and cuts were made for ventilation. The seeds were germinated in a growth chamber at 28°C in the dark. The seedlings were watered daily with 100 ml 10^{-4}M calcium chloride solution.

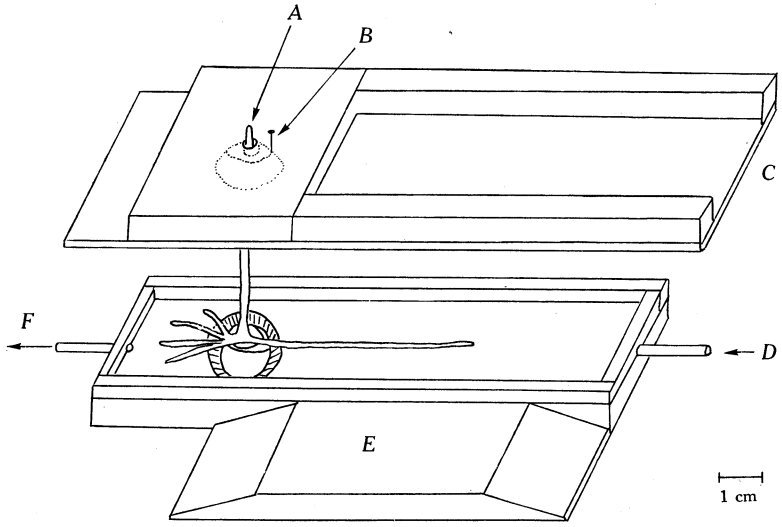


Fig. 1.—Growth chamber used in short-term growth studies. *A*, plant; *B*, pin; *C*, lid; *D*, aerated solution; *E*, fitting for mechanical stage on microscope; *F*, to suction pump.

Inhibitors

2,4-D was purified by dissolving commercial 2,4-D in water as the potassium salt, precipitating it as the free acid, and washing the free acid with water. After five cycles of this procedure the free acid was dried over fused calcium chloride and stored at −15°C.

Alkaline solutions of the potassium salts of 2,4-D, 2,4,6-T, and IAA were adjusted to pH 7·0 with hydrochloric acid. The stock solutions (approx. 10^{-2}M) were stored at −15°C and diluted as required.

Plant Treatment

Plants 2–2·5 days old were transferred to planting trays with different inhibitor concentrations and the lengths of the primary and lateral seminal roots [the nomenclature of Kiesselbach (1949) is used], and shoot of each plant measured at intervals. Serial segments of the primary seminal root and shoot were fixed in a solution containing 5 ml formaldehyde, 5 ml glacial acetic acid, and 90 ml 50% ethanol according to Johansen (1940), and studied microscopically for anatomical changes.

Short-term growth changes of the primary seminal root were observed using the growth chamber (Fig. 1) mounted on the mechanical stage of the microscope. Growth was followed using an ocular micrometer. After an equilibration period the growth regulator was passed through the chamber.

2,4-D Uptake

Rate of 2,4-D uptake was followed with [1-^{14}C]2,4-D. The 3-day-old plants were transferred to $4 \cdot 5 \times 10^{-4}$M 2,4-D (53,400 counts/min/ml) in a planting tray and agitated to maintain aeration. At intervals plants were removed, rinsed in distilled water, cut into sections, and homogenized in a VirTis "45" tissue homogenizer (VirTis Company, Inc.) in 0·01M Tris–HCl buffer at pH 7·5. The homogenates were then centrifuged at 3000 *g* for 10 min at 0°C to clear the the debris and 1-ml aliquots of the supernatants, which were found to contain all the radioactivity from the sections, were plated in duplicate, dried at room temperature, and the radioactivity counted.

The concentration of the 2,4-D in the tissue was determined by sectioning identical material after freezing in a dry ice–acetone bath and determining the fresh and dry weight of the sections. The concentration is therefore the average concentration in the tissue and takes no account of the intracellular location of the 2,4-D.

III. Results

(*a*) *Morphology and Anatomy*

The appearance of plants treated for 63 hr with a range of 2,4-D concentrations is shown in Figure 2.

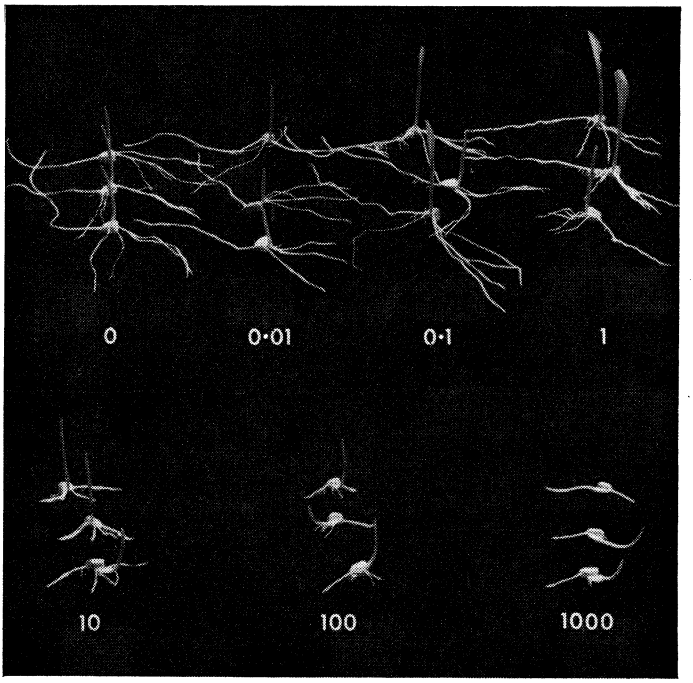
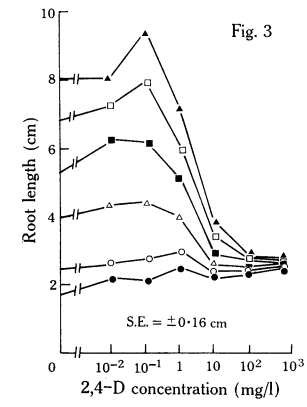
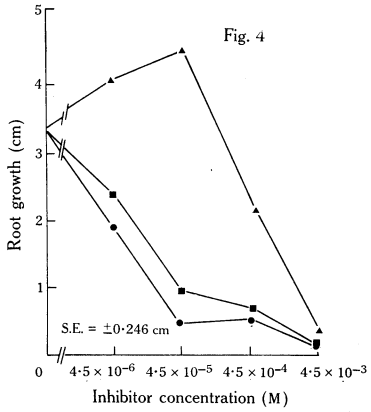


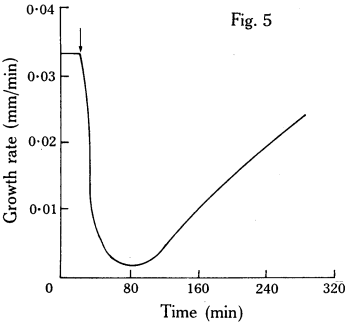
Fig. 2.—Effect of different concentrations (mg/l) of 2,4-D on seedling growth after treatment for 63 hr. 2-day-old seedlings were treated by root irrigation in the dark at 28°C.

The rate of plumule emergence was increased with increasing concentration up to 1 mg/l and thereafter was decreased. In concentrations of 100 and 1000 mg/l

the mesocotyl was observed to be swollen after 26 hr. The swelling continued and after 60 hr adventitious roots appeared in the swollen areas. These roots also formed in plants treated with lower concentrations of 2,4-D and generally the higher the 2,4-D concentration, the greater were the number of adventitious roots. At a concentration of 1000 mg/l the adventitious roots were fasciated and also the shoot showed no geotropic response.







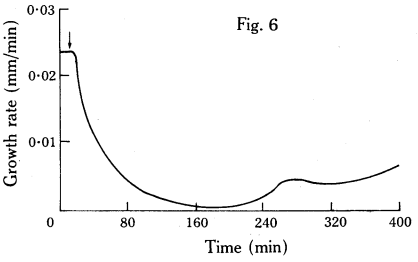


Fig. 3.—Effect of 2,4-D with time on growth of primary seminal roots. Measurements were made on 2-day-old seedlings incubated with 2,4-D for 0 (●), 4(○), 14 (△), 24 (■), 36 (□), and 48 hr (▲) at 28°C in the dark.

Fig. 4.—Comparison of the effect of 2,4-D (●), 2,4,6-T (▲), and IAA (■) on growth of primary seminal roots. 2-day-old seedlings were incubated with inhibitor for 24 hr at 28°C in the dark.

Fig. 5.—Short-term study of inhibition of growth by 2,4-D. 2-day-old seedlings were treated with 100 mg/l 2,4-D for 5 min (arrow) and the rate of growth of the primary seminal root followed at short intervals.

Fig. 6.—Short-term study of inhibition of growth by 2,4,6-T. 2-day-old seedlings were treated with $4{\cdot}5 \times 10^{-4}$M 2,4,6-T continuously (arrow) and the rate of growth of the primary seminal root followed at short intervals.

The number of primary and lateral seminal roots was not affected for up to 48 hr, although changes in the pattern of development were observed before this time. The primary seminal roots showed pronounced root hair development after

26 hr of treatment with 1 and 10 mg/l of 2,4-D whereas the root tip was swollen and without root hairs at higher concentrations. As with the mesocotyl, the swelling continued and after 60 hr secondary lateral seminal roots appeared in the swollen areas, being fasciated at the highest concentration used. The swelling was found to be due to secondary thickening of the tissue in the region of the pericycle, together with secondary lateral root formation.

(*b*) *Growth Responses*

Growth responses of the primary seminal roots to a range of 2,4-D concentrations are shown in Figure 3. Low concentrations stimulated growth whereas high concentrations inhibited it after short periods of treatment. The response of the shoots to the range of 2,4-D concentrations was similar to that of the roots except that the growth of the shoots was increased by high 2,4-D concentrations for up to 14 hr of treatment but thereafter was decreased.

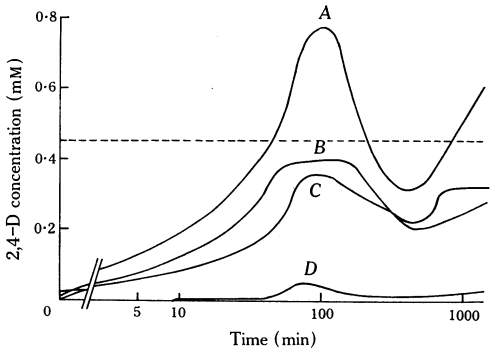


Fig. 7.—Rate of 2,4-D uptake by 3-day-old seedlings incubated in [1-^{14}C]2,4-D (100 mg/l), removed at various times, and cut into the following sections for ^{14}C analysis: *A*, 0–1 cm from tip; *B*, 1–2·5 cm from root tip; *C*, remainder of root; *D*, shoot. ---- Initial concentration of 2,4-D in external solution.

This study was primarily concerned with herbicidal concentrations of inhibitor and it is clear from Figure 4 that the plant response to 2,4-D and IAA is identical, while the response to 2,4,6-T is markedly different.

A concentration of 100 mg/l ($4{\cdot}5\times10^{-4}$M) 2,4-D was selected for short-term studies on inhibition of growth. This was the minimum concentration which constantly produced a maximum inhibition of root growth. Treatment with $4{\cdot}5\times10^{-4}$M IAA or 2,4-D for 5 min caused an immediate decrease in the rate of primary seminal root growth (Fig. 5). The inhibition was not permanent for the root was able to return to its original growth rate. There is no such recovery with continued 2,4-D or IAA treatment at this concentration. Treatment with $4{\cdot}5\times10^{-4}$M 2,4,6-T for 5 min did not inhibit root growth. However, with continuous 2,4,6-T treatment root growth was slowly inhibited, with a considerable recovery after 8 hr (Fig. 6). Since root growth was not greatly inhibited after treatment for 24 hr with this concentration (Fig. 4), recovery from the inhibition must continue beyond that recorded at 8 hr.

(*c*) *2,4-D Uptake*

When the roots were treated with $4{\cdot}5\times10^{-4}$M (100 mg/l) 2,4-D the rate of uptake, initially rapid, decreased and then increased again (Fig. 7). Uptake was greatest in the most densely cytoplasmic region of the tissue (0–1-cm section) where

a concentration 1·7 times that of the external solution was attained after 2 hr. Accumulation of 2,4-D in the shoot was much slower than in the roots.

IV. Discussion

Z. mays plants show the typical response to auxins, growth being stimulated by low and inhibited by high concentrations (Fig. 3). Herbicidal concentrations produced measurable inhibition of growth of the primary seminal root within 4 hr of commencing treatment. Shoot growth was initially stimulated by high concentrations of auxin but the stimulation decreased with time. Since root growth was inhibited at the same concentration of 2,4-D, the stimulation in shoot growth could be due to a redistribution of materials for growth from roots to shoots. It is known, however, that movement of 2,4-D from roots to shoots is very slow (Crafts 1961), and it is more likely that *Z. mays* shoots accumulate the auxin slowly. This is substantiated by measurements of 2,4-D accumulation in the shoots (Fig. 7). The extra shoot growth found could reflect the attainment of concentrations of 2,4-D that promote growth.

Inhibition of growth of the primary seminal roots was found to take place very rapidly on addition of concentrations of 2,4-D inhibiting growth (Fig. 5). The concentration of the 2,4-D in the tissue after treatment with 100 mg/l 2,4-D for 5 min was less than one-quarter of the concentration in the external solution. The concentration of 2,4-D in the tissue, however, was an average concentration and it is evident that the more densely cytoplasmic section (0—1 cm) had the greatest concentration of 2,4-D (Fig. 7). This suggests that 2,4-D is located primarily in the cytoplasm.

Inhibition of growth of primary seminal roots by 2,4-D after a short treatment was reversible and the tissue was able to recover. If, however, the treatment were continuous there was no recovery (Figs. 3 and 4). In these respects 2,4-D is similar to IAA and this is further evidence that the biochemical site of action of these two compounds is similar.

2,4,6-T also caused a growth inhibition at concentrations in excess of $4{\cdot}5\times10^{-5}$M (Fig. 4) but, unlike the inhibition produced by 2,4-D, it was not produced by treatment for 5 min and furthermore the plant was able to recover from the inhibition despite prolonged treatment (Fig. 6). It could, therefore, be postulated that the biochemical site of this growth inhibition by 2,4,6-T is different from that of 2,4-D.

The initial rapid uptake of 2,4-D shown in Figure 7 has been reported previously by Johnson and Bonner (1956). They were able to show that the radioactivity in the initial rapid uptake was freely exchangeable with non-radioactive material, and attributed the rapid rate to an adsorption phenomenon. The larger uptake in the cell-tip region was possibly due to the higher concentration of cytoplasm in which the substance is adsorbed. If the reversal of the inhibition observed following a short-term treatment (Fig. 5) was due to the leaching of the adsorbed 2,4-D from the tissue there must be only a weak adsorption. If this is true, even weakly adsorbed 2,4-D must have been capable of causing growth inhibition.

It is not possible to explain the loss of 2,4-D after the rapid adsorption, unless it reflects chemical changes taking place in the adsorption layer. A similar loss was observed by Blackman (1961) in plants resistant to 2,4-D. Growth response measured in these studies was primarily due to cell elongation, particularly in the short-term studies. The changes in root length therefore reflect the ability of the cells to take up water, which is the major component of cell elongation, or an inability of the cells to mature in the presence of 2,4-D. Since shoot growth was initially stimulated by high concentrations of 2,4-D despite an inhibition of root growth, it is evident that the cells of the roots were still able to take up water, which is then translocated to the shoots. It was observed that xylem elements 2·5 mm from the root tip retained their cytoplasm after treatment with 1000 mg/l 2,4-D for 48 hr. The control roots grew at the rate of 2 mm/hr and hence these cells would normally be in the region of 10 cm from the root tip and have lost all cytoplasmic elements. It would appear, therefore, that 2,4-D inhibited cell maturation. Conversely, low concentrations of 2,4-D stimulated growth and also stimulated plant development as shown by decreased time for plumule emergence (Fig. 2). This indicates that 2,4-D exerts some control on the ontogony of the plant.

The site of cell division was not affected by 2,4-D since cell division continued to take place only in the specialized regions of the pericycle. The secondary lateral seminal roots formed from the pericycle in the normal manner as reported by Kiesselbach (1949), except that they were much closer together than usual. Nor does 2,4-D have any effect on the fate of previously differentiated tissue. The lateral seminal roots have their initials present in the embryo (Kiesselbach 1949) and 2,4-D has no effect on their later appearance.

The anatomical studies gave no indication of cell "poisoning" by 2,4-D, such as disintegrating cytoplasm, or deteriorating structures. Rather, the response was that described for 2,4-D in other plants (Beal 1945; Eames 1949; Wilde 1951), namely, increased proliferation of the primordia and decreased root development, as shown by the retention of the xylem cytoplasm and lack of root elongation. The action of IAA was similar to 2,4-D while that of 2,4,6-T was dissimilar, and caused no antagonism, to the 2,4-D inhibition.

Eliasson (1961) observed that root growth stopped in less than 4 hr after auxin treatment, and with biochemical analyses it has been found that similar times must elapse for changes to be observed after auxin treatment (Cleland 1963; Key 1963). Truelsen and Galston (1966) have shown biochemical changes 24 hr after a pulse treatment with auxin but in most cases much longer times of treatment are used in order to observe changes in enzyme activities, or changes in substrate or product levels. In such work it is difficult to have adequate controls and Hilton and Jansen (1963) have shown that many of the results obtained by continuous 2,4-D treatment may be obtained by uprooting the plants and letting them die. The necessity for precise information on the time course of auxin inhibition is clear because any biochemical changes which cause growth inhibition must take place before the expression of the inhibition. This study shows that growth inhibition is an immediate response to the application of growth-inhibitory concentrations of auxin.

V. References

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