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PROMOTION OF GROWTH AND SHIFT IN THE AUXIN DOSE/RESPONSE RELATIONSHIP IN MAIZE ROOTS TREATED WITH THE ETHYLENE BIOSYNTHESIS INHIBITORS AMINOETHOXYVINYLGLYCINE AND COBALT*

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SUMMARY

Indole-3-acetic acid (IAA) in concentrations from 10^{-12} M to 10^{-9} M does not stimulate elongation when applied to intact roots of maize (*Zea mays* L Bear Hybrid WF 9 × 38). Higher concentrations are inhibitory. In roots pretreated with the ethylene biosynthesis inhibitors, cobalt and aminoethoxyvinylglycine (AVG), IAA from 10^{-10} M to 10^{-8} M promotes growth. High concentrations of IAA (eg. 10^{-6} M) strongly inhibit growth in either pretreated or non-pretreated roots. The data indicate that low concentrations of auxin are capable of stimulating the growth of intact roots in which ethylene biosynthesis is suppressed. This suggests that auxin-induced ethylene biosynthesis may account, at least in part, for auxin inhibition of root growth at high concentrations and for the failure of auxin to stimulate intact root growth at low concentrations.

INTRODUCTION

Since the early reports of auxin promotion of root growth (Ref. cited by Thimann [1]) many workers have attempted to demonstrate auxin promotion of growth in intact roots. In 1957, Aberg [2] summarized these efforts by noting that '. . . direct stimulation of the longitudinal growth of intact roots by low auxin concentrations has never been convincingly demonstrated as a regular and reproducible phenomenon'. A factor which

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Abbreviations: AVG, aminoethoxyvinylglycine; Co/AVG, a solution containing 10^{-4} M $Co(NO_3)_2$ and 10^{-6} M aminoethoxyvinylglycine; IAA, indole-3-acetic acid.

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complicates the study of auxin action on root elongation is that auxin rapidly induces the biosynthesis of ethylene in roots and ethylene is an inhibitor of root elongation [3,4]. We have considered the possibility that auxin-induced ethylene production may mask a potential growth-promoting effect of auxin on roots and that this may contribute to the inconsistency in obtaining auxin promotion of root growth. With this in mind, we have examined the concentration dependence of auxin action on the elongation of maize roots which have been pretreated with cobalt and aminoethoxyvinylglycine (Co/AVG), both inhibitors of ethylene biosynthesis [5—8].

MATERIALS AND METHODS

Grains of maize (*Zea mays* L. Bear Hybrid 9 × 38, Customaize, Momence, IL, USA) were planted as described in Ref. 9. The seeds germinated at room temperature (20—24°C) under fluorescent room lighting (175 ± 25 $\mu E/m^2/s$) and the seedlings were used 3.5 days after planting. Growth was measured as the increase in the length of the root using intact seedlings mounted in a root auxanometer [10]. The root was immersed in approx. 75 ml of continuously oxygenated modified Hoagland's solution adjusted to pH 6.3 [11]. Experiments were carried out under fluorescent room lighting (175 ± 25 $\mu E/m^2/s$) at 20—24°C.

The ethylene level of roots was measured using the vacuum extraction method of Beyer and Morgan [12]. The collected gas sample was analysed by gas chromatography using a Hewlett Packard Model 5750 Gas Chromatograph, with a 6 ft × 1/8 in alumina column. The column was used in the isothermal mode at 100°C, with the injection port and flame ionization temperatures at 110°C. Nitrogen was used as the carrier gas.

IAA was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., AVG was obtained through the courtesy of Dr. G. Lee Benson, Maag Agrochemicals, HLR Sciences, Inc., Vero Beach, FL, U.S.A. and from samples provided by Dr. Richard Gladon, Department of Horticulture, Iowa State University.

RESULTS AND DISCUSSION

When auxin from 10^{-10} M to 10^{-6} M is added to intact roots of maize, their rate of elongation is either unaffected or inhibited, depending on the concentration applied (Fig. 1). At 10^{-9} M, IAA is slightly inhibitory to elongation. With increasing concentration, the inhibition becomes more severe. Concentrations $<10^{-10}$ M neither promote nor inhibit elongation. Although in Fig. 1 the lowest concentration shown is 10^{-10} M, similar results were obtained with 10^{-11} M and 10^{-12} M.

In order to examine the concentration dependence of auxin action on root growth with minimal interference from auxin-induced ethylene production, the growth experiments were repeated using roots pretreated with

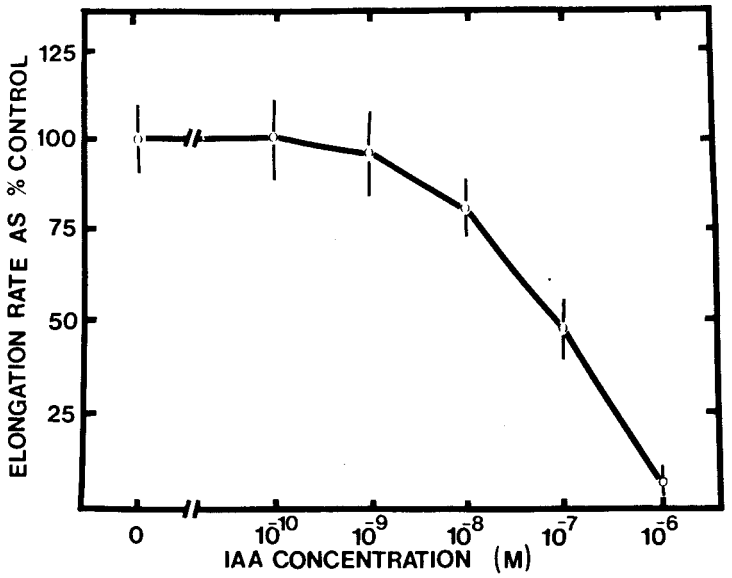

Fig. 1. Dependence of the elongation rate of intact roots of maize on the concentration of IAA. In each experiment, root growth was recorded for 1 h to obtain the control rate before adding auxin to the root. Typical control rates were about 1 mm · h^{-1}. For most inhibitory concentrations of auxin, there is a transient strong inhibition of growth followed by partial recovery to a steady inhibited rate (see Ref. 11). In this figure the inhibited rate is that rate measured 2 h after application of hormone, at which time the transient strong inhibitory period had passed. Means ± S.E. are shown.

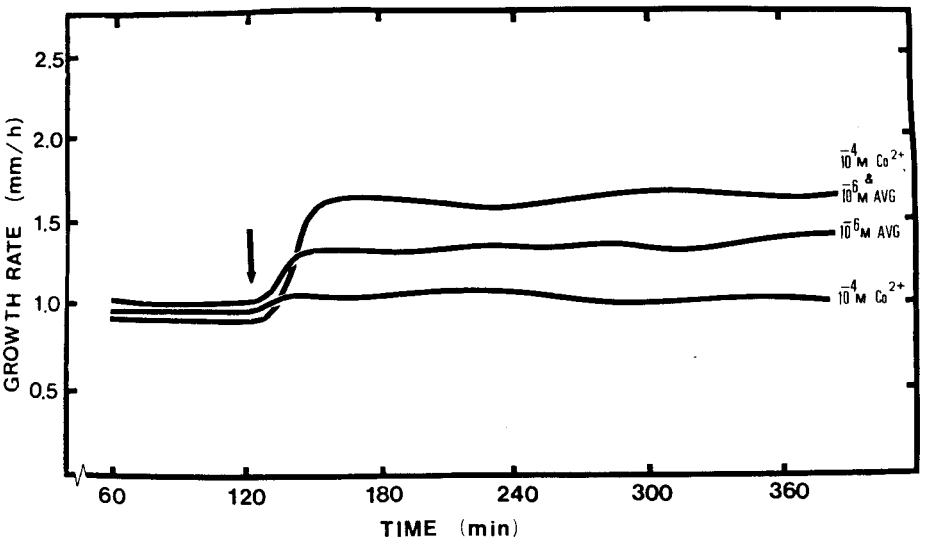

Fig. 2. Effect of AVG and cobalt on the elongation rate of intact roots of maize. At the arrow the root was treated with 10^{-4} M cobalt nitrate (lower curve) or 10^{-6} M AVG (middle curve) or both (upper curve).

Co/AVG. The effect of cobalt, AVG or CO/AVG, was first examined in the absence of applied auxin (Fig. 2). Application of 10^{-4} M cobalt nitrate caused a slight (approx. 10%) promotion of the elongation rate. Application of 10^{-6} M AVG caused a somewhat greater (approx. 25%) enhancement of the elongation rate, while the combination of cobalt plus AVG resulted in a substantial (approx. 50%) promotion of elongation after a lag of 9—14 min. Although the cause of the Co/AVG effect on root growth is unknown, we interpret their action as a suppression of ethylene production by the roots. We find that the apical portion of pretreated roots contains only about 25% as much ethylene as in control roots (Fig. 3).

Figure 4 shows the concentration dependence of auxin action on elongation in roots pretreated with Co/AVG for 1 h. In pretreated roots, IAA from 10^{-10} M to 10^{-8} M promotes elongation. This contrasts with control roots in which no promotion of growth by these concentrations of auxin is observed. In pretreated roots, high concentrations of auxin (eg. 10^{-6} M) are

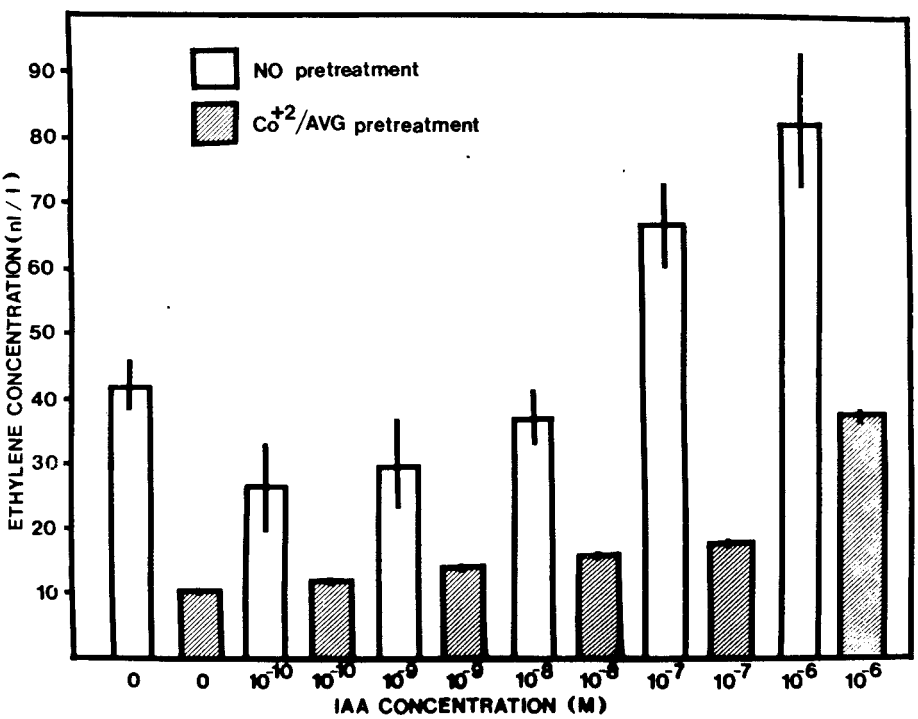

Fig. 3. Effect of pretreatment with Co/AVG on the ethylene content of roots in the presence or absence of various concentrations of IAA. Roots of intact seedlings were treated with 10^{-4} M cobalt nitrate plus 10^{-6} M AVG for 1 h before transferring them to solutions containing IAA concentrations from zero to 10^{-6} M (plus Co/AVG). Two hours after the transfer, the apical 2 cm of the roots was excised and vacuum extracted for collection and determination of ethylene. The experiment was repeated 4 times using 400 roots in each sample. Means ± S.E. are shown.

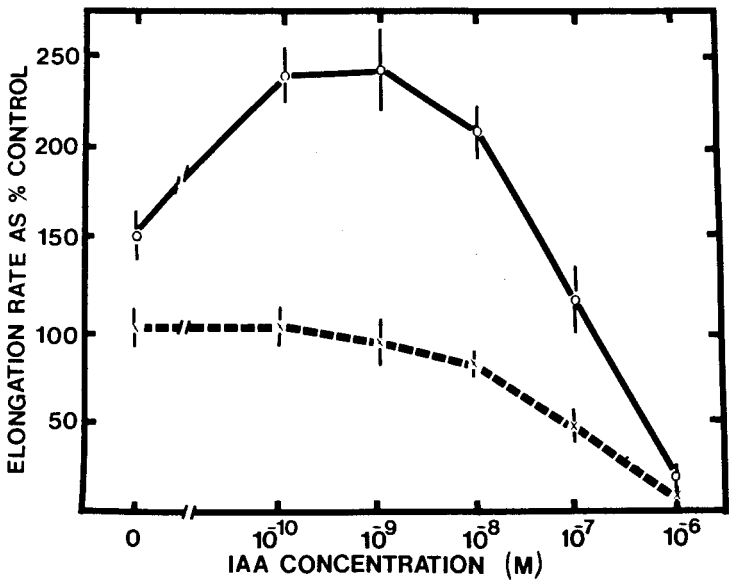

Fig. 4. Dependence of the elongation rate of intact roots of maize pretreated with cobalt plus AVG on the concentration of IAA. Solid line: roots pretreated with 10^{-4} M cobalt nitrate plus 10^{-6} M AVG for 1 h and then transferred to the indicated concentration of IAA (plus Co/AVG). The growth rate was measured 2 h after transfer to the IAA-containing solutions. Dashed line: control roots held in buffer for 1 h and then treated with IAA. The growth rate was measured 2 h after transfer to IAA. Means ± S.E. are shown.

nearly as inhibitory to growth as they are in control roots. Since roots pretreated with Co/AVG grow faster than control roots and their growth can be promoted by IAA, it may be that auxin-induced ethylene production interferes with the promotive action of low concentrations of auxin on the growth of intact roots. While this interpretation is straightforward, there are certain aspects of our data on auxin-induced ethylene production which remain puzzling. For example, in Fig. 3, although the level of apparent ethylene biosynthesis is less in pretreated roots than in non-pretreated roots, the level of ethylene biosynthesis in non-pretreated roots exposed to 10^{-10} M or 10^{-9} M IAA is also less than in the control. This is surprising since it indicates that very low levels of auxin suppress ethylene production relative to controls receiving no added auxin. Nevertheless, we do not see promotion of growth by low concentrations of auxin except in the presence of ethylene biosynthesis inhibitors which suppress the ethylene level substantially below that seen in the presence of low concentrations of IAA alone. This suggests that a very low level of ethylene, such as that seen in roots receiving Co/AVG plus 10^{-10} M or 10^{-9} M IAA, is required to allow a strong expression of the growth promoting activity of IAA. The fact that 10^{-6} M IAA inhibits growth even in the presence of inhibitors of ethylene biosynthesis, indicates that the inhibitory action of high concentrations of

auxin on root growth is independent of ethylene formation as suggested by Rauser and Horton [13].

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