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Short Communications

(Aminooxy)acetic Acid Inhibits Petunia Growth and Gibberellin- and Cytokinin-Stimulated Growth in Bioassays

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Abstract. (Aminooxy)acetic acid (AOA) was applied to greenhouse-grown petunias and was used in bioassays for three plant growth hormones so that its growth regulator properties could be studied. In greenhouse studies foliar sprays of 4.8–12 mM AOA inhibited vegetative growth of petunia seedlings (*Petunia xhybrida* Vilm. 'White Flash'). When gibberellin A_3 (GA_3) was applied to shoot tips previously treated with AOA, plant growth was stimulated, but there was no AOA × GA_3 interaction. Some changes in petunia leaf morphology induced by AOA were reversed by GA_3. AOA inhibited elongation of corn coleoptile segments (*Zea mays* L. B73 × Mol7) whether or not 10 μM indole-3-acetic acid (IAA) was present, but there was no AOA × IAA interaction. AOA reduced lettuce hypocotyl (*Lactuca sativa* L. 'Grand Rapids') elongation induced by GA_3 and radish cotyledon (*Raphanus sativus* L. 'Champion') expansion induced by benzyladenine (BA). We propose that AOA interferes with postsynthetic metabolism of plant hormones during cell elongation induced by GA_3 and cell expansion induced by BA.

(Aminooxy)acetic acid (AOA) inhibits ethylene (C_2H_4) biosynthesis (Yang 1980) and retards vegetative growth of petunias (Laffe 1986). C_2H_4 facilitates normal vegetative growth of whole plants or plant parts (Abeles et al. 1992, Sinska and Gladon 1984, Zobel 1974, Zobel and Roberts 1978), but it is known more as a modulator than as a promoter of vegetative growth (Abeles et al. 1992, Lieberman 1979). When AOA was applied to petunias, some of its manifestations resembled those of ethephon (Laffe 1986), a chemical that releases C_2H_4 upon degradation (Tija and Buxton 1977, Yang 1969). These observations suggested that inhibition of C_2H_4 biosynthesis was not the primary mechanism by which AOA regulated vegetative plant growth.

AOA inhibits enzymes mediated by pyridoxal phosphate (e.g., transaminases) (John et al. 1978) and affects amino acid metabolism (Halevy 1963, Havir 1981, Hoagland and Duke 1982, Lieberman 1979). Leopold (1971) has suggested that growth retardants interfere with growth-actuating systems in plants, and growth retardants are known to interfere with synthesis (Coolbaugh et al. 1978, Dennis et al. 1965) or action (Cleland 1965, Halevy 1963) of plant hormones. However, the mechanism by which AOA regulates vegetative plant growth has not been established.

The overall objective of this research was to determine the effect of AOA on changes in vegetative plant growth caused by combinations of AOA with several plant growth hormones. The specific objectives of this research were to determine interactive effects of applied AOA and GA_3 on vegetative growth and development of petunias and to determine the effect of AOA on plant growth stimulated by auxin, gibberellin, and cytokinin in bioassays.

Materials and Methods

Petunia Growth

Petunia (*Petunia xhybrida* Vilm. 'White Flash') seeds germinated in plug trays (Blackmore Transplanter Co., Ypsilanti, MI) that contained a soil-free medium of 35% *Sphagnum* moss peat, 35%

Abbreviations: AOA, (aminooxy)acetic acid; GA_3, gibberellin A_3; IAA, indole-3-acetic acid; BA, benzyladenine.

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Hypnum moss peat, and 30% vermiculite (grade 3) (v/v/v). The temperature of the germination chamber was 26 ± 1°C, and continuous irradiance from cool white fluorescent lamps was 60–70 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Seedlings with two pairs of true leaves were transplanted into plastic cell packs that contained a soil-free medium of 70% coarse *Sphagnum* moss peat and 30% vermiculite (grade 3) (v/v) amended with 3 kg of $CaSO_4 \cdot 2H_2O$, 3 kg of superphosphate (0:20:0, 4.9% P), and 3 kg of ground dolomitic limestone/m^3 of soil-free mix. Plants grew in a greenhouse under natural photoperiods during spring; the greenhouse temperature was 24 ± 1°C during the day and 18 ± 1°C at night. Plants were fertilized weekly with 150 mg · $liter^{-1}$ N from 15:3.9:13.9 (N/P/K) fertilizer (Peter's 15-17-17 Peat Lite Special®) for the first 4 weeks and thereafter weekly with 250 mg · $liter^{-1}$ N from the same source. Each fertilization included 22 mg · $liter^{-1}$ Fe from Sequestrene 330®. Six days after being transplanted, cell packs of uniform seedlings with a rosette diameter of 1.5–2.5 cm were sprayed to runoff (mean of 0.44 ml/plant) with 0, 4.8, 7.2, 9.6, or 12 mM AOA that included 1 ml · $liter^{-1}$ of Triton X-100® as an adjuvant. The next day, 0, 0.1, 1.0, or 10 nmol of GA_3 was applied to the shoot tips as a 10-µl droplet. The AOA and GA_3 treatments were reapplied in 7 days. This experiment was a complete factorial of five AOA concentrations and four GA_3 doses in a completely randomized design. Each treatment was replicated three times with six observations (plants)/replicate. Plant height was recorded periodically. A multiple regression model was fit to the data by using partial F tests to determine the main effect and interaction significance.

Laboratory Bioassays

Three sensitive, specific bioassays were chosen so that AOA × hormone interactions could be studied selectively (Frankland and Wareing 1960, Jones and Moll 1983, Letham 1971). The hormone concentration chosen for each bioassay was less than supraoptimal but elicited a significant growth response. When AOA interacted with a given hormone, the range of AOA concentrations was narrowed to define response curves more precisely. All bioassays were conducted on the laboratory bench under fluorescent lighting. The bioassays were as follows.

Corn Coleoptile Elongation Bioassay for Auxin. Corn coleoptiles (*Zea mays* L. B73 × Mol7) were produced by the method of Hatfield and LaMotte (1984) with these modifications after the 12-h soak: (1) seeds were planted directly into moist vermiculite, and (2) seedlings grew at 25 ± 1°C in continuous, dim red light. IAA and AOA solutions were prepared in 10 mM citrate KOH buffer (pH 5.0). Uniform seedlings 3–5 cm long were harvested 60 h after planting. Each seedling was decapitated 3 mm from the shoot tip, and a 5-mm segment immediately below the point of decapitation was used for the bioassay. Segments were preincubated in buffer for 2 h at 20 ± 1°C in darkness and then treated as follows under dim green light. Segments were distributed to Petri dishes that contained 20 ml of test solution and then incubated in darkness for 6 h at 20 ± 1°C. Segment length was measured to the nearest 0.1 mm with a 10× ocular micrometer. This experiment was a complete factorial with two replications (Petri dishes) of each treatment and 10 observations (segments)/replicate.

Lettuce Hypocotyl Elongation Bioassay for Gibberellins. This bioassay was based on the methods of Frankland and Wareing (1960) and Biran et al. (1964). Lettuce seeds (*Lactuca sativa* L. 'Grand Rapids') germinated on moist Whatman no. 1 filter paper in darkness at 23 ± 1°C for 30 h. Uniform seedlings with unemerged cotyledons and radicles 2–8 mm long were placed in beakers that contained filter paper wetted with 2 ml of test solution. Beakers were covered and placed under continuous irradiance (mean 54 $\mu mol \cdot s^{-1} \cdot m^{-2}$) from cool white fluorescent lamps. After 30 h, hypocotyl length was measured to the nearest 0.1 mm with a 10× ocular micrometer. Test solutions were prepared in one-quarter-strength Hoagland's solution and adjusted to pH 6.0 (Berry 1978). This experiment was a complete factorial with two replicates (beakers) of each treatment and 10 observations (seedlings)/replicate.

Radish Cotyledon Expansion Bioassay for Cytokinins. This test was adapted from Letham (1971). Radish seeds (*Raphanus sativus* L. 'Champion') germinated on moist Whatman no. 1 filter paper at 23 ± 1°C in darkness except for occasional exposure to dim green light. After 40 h the smaller (inner) cotyledon was excised, residual hypocotyl tissue was removed with a scalpel, and original fresh mass was recorded. Excised cotyledons were held temporarily in 2.0 mM KH_2PO_4 (pH 6.0) and then distributed to Petri dishes that contained filter paper wetted with 3 ml of test solution. Petri dishes were irradiated at a mean of 7.2 $\mu mol \cdot s^{-1} \cdot m^{-2}$ irradiance from cool white fluorescent lamps. After 48 h, cotyledons were blotted dry and weighed. AOA and BA solutions were prepared in 2 mM KH_2PO_4 adjusted to pH 6.0. Two to 3 ml of 0.1 N HCl was used to dissolve the BA. This experiment was a complete factorial with two replicates (Petri dishes) of each treatment and seven observations (cotyledons)/replicate.

Statistical Analysis

Entire bioassay experiments were repeated as described in the figure legends. Data were pooled by using the replications as blocks in randomized complete block designs. Multiple regression models were fit, and partial F tests were used to compare slopes and intercepts of estimated regression functions in the presence or absence of exogenous hormone.

Results

Petunia Growth

Phytotoxicity, in the form of leaf necrosis and subsequent death, was observed on some plants 1 week after they were treated with 12 mM AOA (data not presented). Plants treated with nonphytotoxic concentrations of AOA (≤9.6 mM) were shorter than the controls after 5 weeks, and growth decreased as the AOA concentration increased (Fig. 1). Within each AOA concentration treatment, plant height increased with each increase of GA_3 dosage. The AOA × GA_3 interaction was not significant ($P > 0.13$), but each increase in GA_3 dose shifted the curve farther away from the abscissa. The same

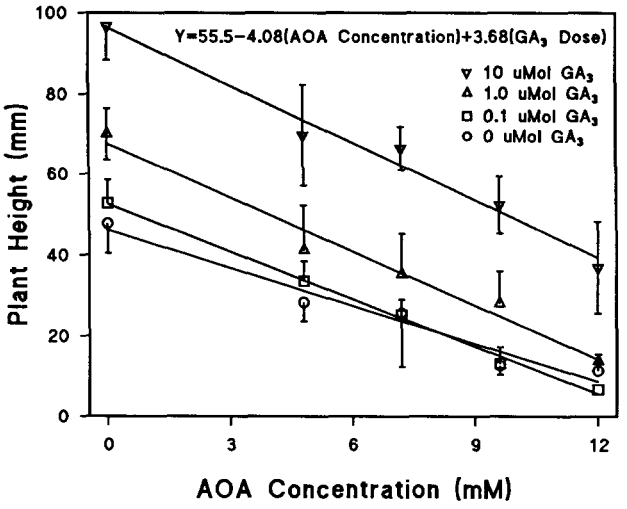

Fig. 1. Effects of AOA and GA_3 on 'White Flash' petunia height. Points are the means of three replicates ± S.D. Main effects of both AOA and GA_3 were significant ($P < 0.001$), but the AOA × GA_3 interaction was not ($P > 0.13$). For the entire model, $R^2 = 0.86$ and $P < 0.001$.

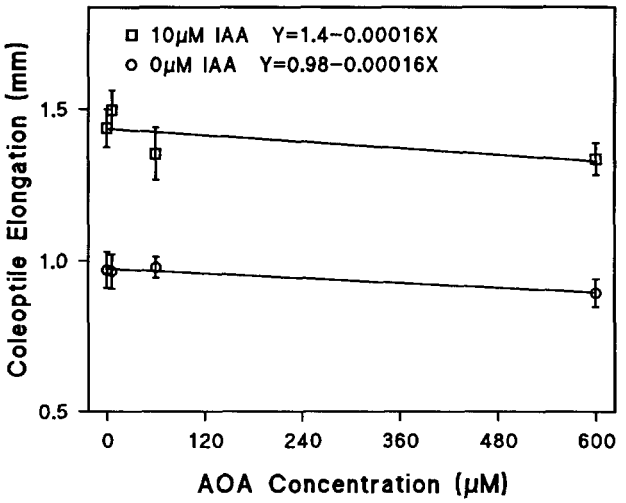

Fig. 2. Effects of AOA concentration on corn coleoptile elongation stimulated by IAA. Points are means ± S.D. of six replicates from three separate runs of the experiment. AOA inhibited coleoptile elongation ($P = 0.027$). Treatment with 10 μM IAA increased coleoptile elongation ($P < 0.001$) but did not affect the slope of the AOA elongation regression line ($P > 0.58$). For the entire model, $R^2 = 0.92$ and $P < 0.001$.

trend was observed each time plant height was measured (data not presented). Plants treated with 9.6 mM AOA and 1.0 nmol of GA_3 were morphologically similar to untreated control plants (data not presented). By visual examination we found that neither AOA nor GA_3 affected lateral shoot development.

Bioassays

Corn Coleoptile Elongation. AOA inhibited elongation of corn coleoptile segments ($P = 0.027$), but there was no AOA × auxin interaction ($P > 0.13$) (Fig. 2). Thus, AOA did not affect growth stimulated by auxin.

Lettuce Hypocotyl Elongation. AOA inhibited lettuce hypocotyl elongation induced by exogenous gibberellin, but it did not affect elongation in the absence of applied gibberellin (Fig. 3, *A* and *B*). Hypocotyl elongation induced by GA decreased as the AOA concentration increased, and at 360 μM 70% of the growth stimulated by GA was inhibited (Fig. 3*A*). Elongation was inhibited only slightly by greater AOA concentrations (Fig. 3*B*).

Radish Cotyledon Expansion. Without regard to the presence of BA, AOA at concentrations <60 μM did not inhibit radish cotyledon expansion (data not presented). AOA concentrations >60 μM inhibited expansion of radish cotyledons induced by BA, and AOA at 450–600 μM inhibited expansion completely (Fig. 4). AOA at concentrations >60 μM also inhibited radish cotyledon expansion when BA was not applied (Fig. 4).

Discussion

Growth regulators with antiauxin activity can increase lateral shoot development because they overcome the apical dominance controlled by auxin. AOA did not stimulate lateral shoot development of petunia in our experiments or those of Laffe (1986). We also observed that AOA did not reduce the response of corn coleoptiles to applied IAA. Thus, AOA does not affect the stimulation of growth by auxin, and we conclude that AOA retards plant growth by a mechanism that does not involve inhibition of auxin action.

In petunia, retardation of growth and morphologic changes induced by AOA were altered by subsequent application of GA_3; therefore, AOA seems to have antigibberellin properties. We cannot conclude directly, however, that AOA inhibits GA_3 action because interaction effects between AOA and GA_3 were not observed in the petunia experiment; that is, the response of petunia to GA_3 was not

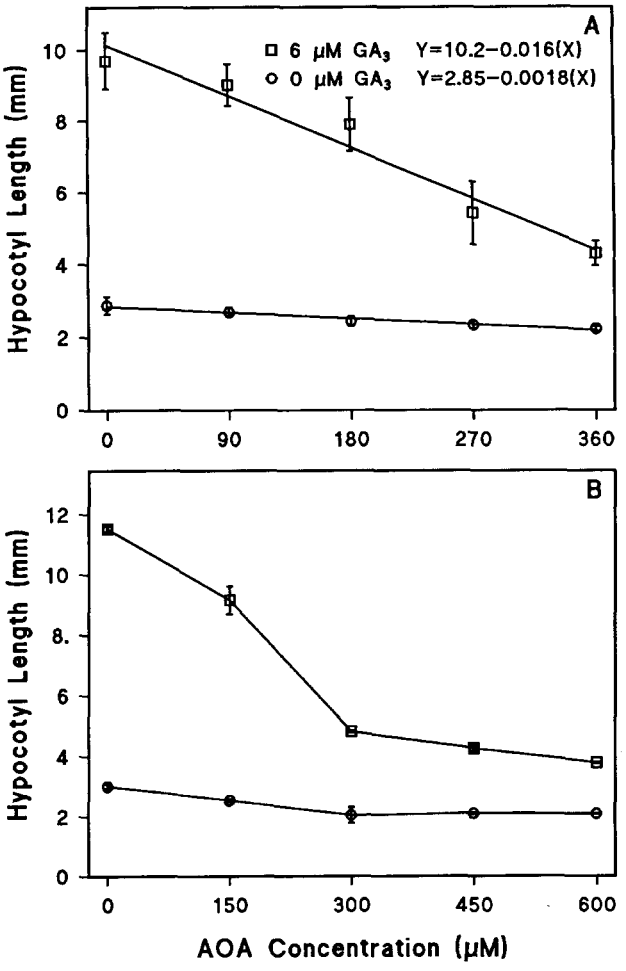

Fig. 3. Effect of AOA on lettuce hypocotyl elongation stimulated by GA_3. *A*, points are means ± S.D. of six replicates from three separate runs of the experiment. AOA inhibited hypocotyl growth at both GA_3 concentrations ($P < 0.001$). For seedlings treated with 6 μM GA_3, the intercept of the AOA elongation regression line was greater ($P < 0.001$), and the slope was steeper ($P < 0.001$) than for seedlings treated with 0 μM GA_3. For the entire model, $R^2 = 0.96$ and $P < 0.001$. *B*, points are means ± S.D. of two replicates. The main effects of AOA and GA_3 and the AOA × GA_3 interaction were significant ($P < 0.001$).

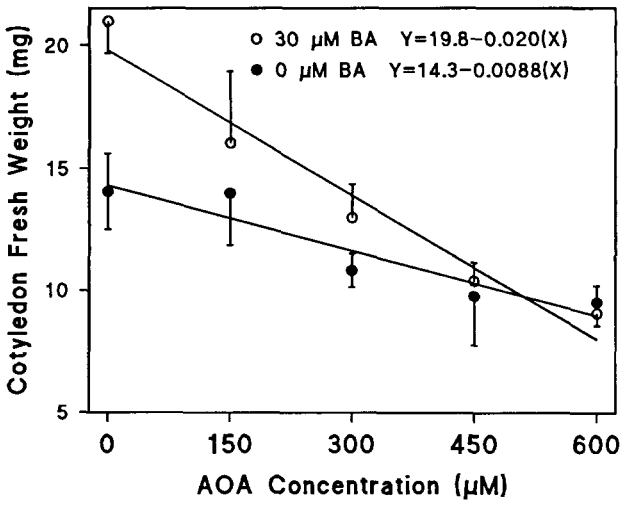

Fig. 4. Effect of AOA on radish cotyledon expansion stimulated by BA. Mean original fresh mass was 6.1 mg/cotyledon over all experiments. Points are means ± S.D. of six replicates from three separate runs of the experiment. For cotyledons treated with 30 μM BA, the intercept of the AOA expansion regression line was greater ($P < 0.001$), and the slope was steeper ($P < 0.001$) than for cotyledons treated with 0 μM BA. For the entire model, $R^2 = 0.78$ and $P < 0.001$.

changed entirely by AOA. In contrast, an AOA × GA_3 interaction was observed in the lettuce hypocotyl test, and this result indicates that AOA changes the mode of action of GA_3. When these results are considered together, they lead to the conclusion that part of the growth control caused by AOA was exerted through inhibition of GA_3 action.

Letham (1971) showed that radish cotyledon tissue is sensitive to applied cytokinin and insensitive to applied auxins and gibberellins. AOA reduced radish cotyledon growth in the absence of applied BA, and the significant AOA × BA interaction in the presence of applied BA indicates that AOA interfered with cytokinin action. Thus, we infer that the observed inhibition of radish cotyledon growth is due to the effect of AOA on the action of endogenous and exogenously applied cytokinins.

Paleg et al. (1964) suggested five ways that growth retardants inhibit responses induced by GA, and their model may be applied to other plant hormones as well. This model suggests that growth retardants may: (1) inhibit hormone synthesis; (2) decrease the concentration of a substance (e.g., receptor(s)) with which it reacts; (3) inactivate or destroy the hormone directly; (4) inhibit hormone binding; or (5) depress reactions that occur after the hormone binds to its receptor. We used exogenously applied hormones, so we did not test the first hypothesis directly. Competition for hormone binding sites (hypothesis 4) is unlikely because AOA is not structurally similar to gibberellins, cytokinins, or known inhibitors of cytokinin action (Skoog et al. 1973). Furthermore, AOA was effective only at concentrations about tenfold greater than of the applied chemicals.

Of the remaining hypotheses, the fifth best explains our results. Lettuce hypocotyls and radish cotyledons grow primarily by cell expansion (Jones and Moll 1983, Letham 1971). Our results suggest that after the hormone binds to its receptor, AOA interferes with a cell expansion pathway common to cytokinins and gibberellins. Subsequent experi-

ments that examine the effect(s) of AOA on other gibberellin and cytokinin responses could test this hypothesis and elucidate more precisely the mechanism by which AOA regulates vegetative plant growth.

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