Technical Papers

Movement of Alpha-Methoxyphenylacetic Acid from One Plant to Another Through Their Root Systems

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Alpha-methoxyphenylacetic acid (*1*) (MOPA) was recently reported to have marked plant growth-modifying properties and to be readily translocated by bean and some other plants (*2*). In experiments reported here, MOPA (or a derivative of it) moved out of the roots of plants to which it was applied into nearby roots of untreated plants which then translocated it upward and developed modified leaves.

This movement of MOPA from one plant to another was not due to evaporation and movement of the compound through the air such as occur with volatile forms of 2,4-D (*3, 4*). Evaporation from three 1-g portions of MOPA stored at about 70 to 95°F for 4 mo averaged 16 μg/day, which was not sufficient to induce growth effects in young bean plants enclosed for 4 days in airtight bags (3-liter capacity) each with a 1-g portion of MOPA.

In another test, the stem of one plant was treated with 100 μg of MOPA mixed with 1 part of Tween 20 and 4 parts of lanolin applied as a narrow band around the stem. The untreated stem of another plant with roots in a separate pot was fastened 0.5 in. from the treated one by means of a piece of notched cardboard. For comparison, another treated and an untreated plant with their roots in the same pot were also fastened with their stems 0.5 in. apart. Both treated and untreated plants in the same pot showed effects of MOPA within 2 days. Untreated plants with their roots in pots separate from those of treated plants did not show effects of the chemical even after 3 wk.

Detectable amounts of MOPA moved from one plant to another within 9 hr after the chemical was applied. To determine the time required for transfer, 10 groups of 10 young bean plants (two plants in each pot) were selected. To the upper ¼ in. of the hypocotyl of one plant in each pot, 600 μg of MOPA, in the paste carrier previously described, was applied. Treated plants were severed at the soil surface from successive groups at 3-hr intervals after treatment. Subsequent observations showed that untreated plants grown for 9 hr or more in pots with intact treated ones developed malformed leaves, thus indicating transfer of a sufficient amount of MOPA to induce growth modification. The amount of MOPA transferred under the conditions described was equivalent to about 2 to 3 percent of that applied.

To indicate whether MOPA remained unchanged (aside from salt formation) when translocated upward through stems, about 2000 secondary galls induced by direct application of MOPA to stems of snap bean plants were collected (*2*). These were frozen, pulverized, boiled with 5 liters of water, and filtered. The filtrate was concentrated, acidified, and extracted repeatedly with ether, and the ether was evaporated. The resulting mixture of acids (about 1 g) was neutralized with 10 percent sodium hydroxide solution, 1 g of sodium chloride was added, and the *p*H of the solution was adjusted to 3.1. After standing for a few days at 0°C, the sodium acid salt of MOPA, together with small amounts of other acids, was separated. Washing with isopropyl alcohol removed alcohol-soluble impurities and left 25 mg of the sodium acid salt, with sodium chloride as an impurity. The material was treated with hydrochloric acid and extracted with chloroform; evaporation gave 11 mg of crude MOPA. The compound was identified by comparing its melting point (65°C) and mixed melting point (69°C) with that of an authentic sample (71°C), and by means of a methoxyl analysis (*5*) (theory, 18.7 percent; found, 14.8 percent, corresponding to a material 80-percent pure). The amount of MOPA recovered corresponded to 4.5 μg in the secondary gall of each plant. About 200 μg of the crude MOPA thus obtained was applied individually to the stems of young plants. Within 2 days, untreated bean plants in the same pots developed typical MOPA symptoms.

When plants treated with MOPA were grown with their roots in aerated water, the chemical was translocated to the roots and moved out into the water. In demonstrating this, three young bean plants approximately 5 in. tall were supported so that their roots were immersed in about 200 ml of tap water that was aerated by passing compressed air through it. About 400 μg of MOPA was applied to the hypocotyl and first internode of each plant. Three comparable untreated plants were similarly maintained in another container. As the experiment progressed, additional water was added to equal the amount lost from evaporation.

After 1 wk, the plants were removed. The water in which the treated plants had grown was evaporated; the residue was collected in ether, transferred to a vial, and dried. The residue was dispersed in 0.2 ml of water containing 0.01 percent of Tween 20. Four 0.01-ml aliquots of this mixture were then applied separately to the terminal buds of each of four young bean plants. Comparable plants treated with the residue from water in which untreated plants had grown served as controls. Residue from water in which the roots of MOPA-treated plants had grown induced typical MOPA effects. Plants treated with residue from water in which roots of untreated plants had grown were unaffected.

In a similar experiment, treated and untreated plants were grown separately with their roots in

aerated water. After 1 wk, the plants were removed and new plants were substituted. Four days later the trifoliate leaves of those growing in water that had previously supported growth of treated plants showed typical MOPA effects. Width of the modified leaves was 88 percent and their length 79 percent less than that of leaves of plants grown in water that had previously supported the growth of the untreated plants.

Snap bean, sunflower, cucumber, buckwheat, cotton, and corn seeds were germinated together in composted soil in 6-in. clay pots to determine whether MOPA would move from one kind of plant to another through their roots systems. After the pots had been divided into six groups with equal numbers of plants, MOPA was applied in the carrier as a band around the stems of all plants of one species in the first group. Plants of another species were treated in the second group, and so on, until each species (except corn) had been treated and was growing with untreated plants of the remaining species. Corn plants in one group were treated by applying a band of the paste, about 1 cm wide, across the base of the first true leaf. On the basis of the formative effects induced, MOPA moved from bean plants to sunflower, cucumber, buckwheat, and cotton; from cucumber to bean, sunflower, buckwheat, and cotton; from sunflower to bean; from buckwheat to bean; and from cotton to bean. There was no evidence that MOPA moved from corn to any other plants.

A wide variety of growth-modifying compounds known to induce formative effects when applied to bean plants were tested to determine whether they moved from one plant to another through the root systems. In these tests, about 150 to 200 μg of each compound tested was applied in the Tween-20-lanolin carrier as a band around the stem of one of two bean plants growing in the same pot. The following compounds (*6*) were tested: 2-naphthoxyacetic acid; 3,4-xylyloxyacetic acid; 2,4-dibromophenoxyacetic acid, 2,3-dichloropropyl ester; para-chlorophenoxyacetic acid, 2-butenyl ester; para-chlorophenoxyacetic acid, 2-methylallyl ester; 2,4-dichlorophenoxyacetamide; 2,3,5-triiodobenzoic acid; and 2,4-dichlorophenoxyacetic acid and 6 of its salts and esters. In addition, 14 other chlorine-substituted phenoxyacetic acids (*7*) were tested, all of which were known to induce growth modifications when applied directly to bean plants. None of the compounds was moved from the treated plants to the untreated ones in sufficient amounts to induce visible growth effects.

MOPA apparently did not accumulate and remain in detectable amounts in soil in which treated plants were grown. Bean plants treated with about 150 μg of MOPA on the first internodes were removed from soil in which they had grown for 2 wk. Bean seeds were immediately planted in the soil, but the resulting plants did not develop growth modifications.

When a treated and an untreated plant were grown in soil contained in a pot, MOPA may have moved from roots of the treated one through the water films into roots of the untreated one and apparently through those roots that were in contact with each other, or at least through those that grew very near to each other. Close proximity of bean roots was repeatedly observed, sometimes as many as five roots growing in contact with each other for a distance of 1 in. or more.

References and Notes

1. Available from the Polynitro Chemical Co., Box 374, College Park, Md.
2. J. W. Mitchell and W. H. Preston, Jr. *Science* **118**, 518 (1953).
3. P. C. Marth and J. W. Mitchell. *Botan. Gaz.* **110**, 632 (1949).
4. W. R. Mullison and R. W. Hummer. *Botan. Gaz.* **111**, 77 (1949).
5. Carried out by Kathryn Gerdman.
6. Supplied by Chemical-Biological Coordination Center.
7. Supplied by American Chemical Paint Co., Ambler, Pa.

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Central Nervous System Gliogenesis in Piromen-Treated Rat Embryos

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In addition to its pyrogenic properties, the bacterial polysaccharide Piromen has been shown by Windle, *et al.* (*1*) to possess the ability to inhibit glial proliferation in traumatic lesions of the spinal cord and brain of mammals. These investigators employed a dose of 0.5 micrograms per kilogram (μg/kg) of body weight, administered intravenously daily for 2 wk.

Because of these results, we have attempted to inhibit the normal maturation of central nervous system glial tissue in the rat embryo and in the developing rat with Piromen. Since intravenous administration was impractical, relatively large doses were given subcutaneously and intraperitoneally. Fischer females were bred with Agouti males (both are Curtis-Dunning lines). The Piromen treatment schedule was varied in three ways.

A-1. Injections were begun on the 10th to 12th days of pregnancy via the intracardiac or intraperitoneal route. Four animals were treated for 1 to 5 days and received total doses of 4 to 10 μg/kg. Embryos were obtained at the 12th and 15th days of pregnancy and immediately after birth.

A-2. Injections were begun on the 16th to 19th days of pregnancy via the intraperitoneal route. Five animals were treated for 1 to 4 days and received total doses of 0.4 to 1.8 μg/kg. The animals were allowed to come to term, and 25 young rats were sacrificed at postpartum days, 0, 1, 2, 3, 7, 12, and 40.

B. Siblings from animals treated under schedule A-2 were given daily injections after birth. The injections were first made subcutaneously and later intraperitoneally as the animals grew. Three animals sacrificed at the age of 2 or 3 days had received total doses of 0.5 to 2 μg/kg. Nine other animals received