
MOVEMENT OF C14-TAGGED ALPHA-METHOXYPHENYLACETIC ACID OUT OF ROOTS

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Alpha-methoxyphenylacetic acid (MOPA) was reported in 1953 to have marked plant-growth-modifying properties and to be readily translocated by bean and other plants (6). When applied to leaves or stems, this compound was absorbed and translocated both upward and downward within the plant. It moved down into the roots and out of them and was absorbed by adjacent or nearby roots of an untreated plant; then it moved upward in the stem of this plant to partially developed leaves which subsequently became malformed (7). This phenomenon of plant regulators moving out of roots, however, is uncommon (7). MOPA moved from various kinds of bean plants to other bean plants and from other broad-leaved plants to bean, causing malformation and inhibition of new growth. There was no evidence at that time that the acid moved from corn to any other plant.

In studying the translocatability of a compound, it is, of course, necessary to identify the compound by either biological or chemical means after it has been moved through the plant. In a previous study (5) the downward transport and exudation of MOPA were proved by applying approximately 150 µg of C14-carboxyl-tagged MOPA to the stems of several bean plants. These plants were then grown with their roots immersed in aerated tap water. Three days later the water was found to contain radioactivity. A sample of the water was evaporated and the residue partitioned on paper. This residue was identified as MOPA. If any radioactive metabolites or degradation products were exuded by the roots, these were not detectable. These earlier results indicate that some of the methoxy acid was absorbed by the stems, translocated to the roots and exuded without detectable chemical change. The present investigation is concerned with the exudation of MOPA as affected by its absorption and translocation, the amount of MOPA applied and environmental conditions.

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Materials and Methods
Carboxyl-tagged alpha-methoxyphenylacetic acid was prepared on a micro scale from benzaldehyde and sodium cyanide-C14 by a modification of the standard procedure (1, 8) involving the successive preparation of mandelonitrile and mandelic acid and the conversion of the latter to MOPA by methylation with dimethyl sulfate.

Young plants of Pinto bean, approximately 5 inches (ca 12 cm) tall, were grown in aerated tap water or nutrient solution for these experiments. The plants, grown in pots containing soil, were removed, and after their roots were washed free of soil, they were placed in beakers containing the aerated tap water or nutrient medium.

Five µg of C14-tagged MOPA dissolved in water was then spread evenly on the upper surface of each primary leaf by means of a thin glass rod, making 10 µg per plant. Each beaker contained three plants.

After preliminary tests for periods up to 300 to 350 hours, it was arbitrarily decided that further experiments would be terminated after approximately 200 hours, as this was long enough to demonstrate the pattern of exudation. During this period, 20-ml portions of the solution were taken at successive intervals of 24 hours, except for the first 48 hours during which samples were taken more frequently, to determine the concentration of radioactive exudate. These aliquots were then evaporated in metal planchets and tested for radioactivity. Solutions in the beakers were always readjusted to their original volume and, whenever necessary, correction for self-absorption was made.

Results
The presence of MOPA in the tap water surrounding the roots was first detected about 5 hours after application of 5 µg of the acid to each leaf. The amount in the water surrounding the roots (the amount exuded less the amount reabsorbed by the plant through its roots and that adsorbed on the glass)
increased, reached a maximum, and then decreased (fig 1). This general pattern was observed in experiments carried out with plants subjected to a variety of environmental conditions. In experiments conducted during the following fall and winter, however, the maximum concentration of exudate in water surrounding the roots was more (50 %) than that in water surrounding the roots of comparable plants grown during the summer (fig 2).

Plants grown with their roots in nutrient solution exuded and reabsorbed MOPA in a pattern similar to those grown with their roots in tap water. There was, however, an apparent depression of the rate of exudation when the roots were maintained in the nutrient. This apparent depression was found to be due to the self-absorption of radioactivity by nutrient salts precipitated in evaporation of the aliquots. The self-absorption was found to be approximately 85 %. The correction for self-absorption showed the amount of MOPA exuded when the roots were in nutrient solution to be the same (within 10 %) as that exuded by roots in tap water.

In these tests, some of the plants were grown with their roots in tap water to which the required macro-nutrients, including calcium nitrate, potassium dihydrogen phosphate, and magnesium sulfate, had been added. Other plants were grown with their roots in tap water alone for comparison.

The amount of MOPA exuded from the roots was proportional to the amount applied to the leaves over the range of dosages used. Two and one-half \( \mu \text{g} \text{m} \) of MOPA was applied to each leaf in one group of plants, 5 \( \mu \text{g} \text{m} \) to each leaf in another group, and 10 \( \mu \text{g} \text{m} \) to each leaf in a third group. The average ratio of amounts of MOPA exuded by the roots in the different groups of plants was 1:2:4, the same as the ratio of the amounts applied to the leaves. These ratios remained alike throughout the experiment.

Exudation of MOPA was retarded by removal of the lower half of the root system. Pruned roots began to exude detectable amounts of the acid 4 to 24 hours later than did plants with comparable intact root systems. During the early part of the experiment, however, the concentration of MOPA in tap water surrounding pruned roots increased at a somewhat lower rate but eventually reached a higher level than it did in water surrounding the unpruned roots (fig 3). After the concentration of MOPA in water around the pruned roots reached a maximum, it decreased at a rate comparable to that in the tap water containing unpruned ones.

The amount of radioactive MOPA in water around the roots was not affected by continuously subjecting the roots to water containing stable MOPA (30 \( \mu \text{g} \text{m} \) in 150 ml of water), beginning 48 hours prior to application of radioactive MOPA to the leaves. In these tests, comparable plants with untreated roots were used for comparison. The results were adjusted to account for adsorption of the stable in place of the radioactive MOPA on the glass container.

Exudation of MOPA was greatly reduced by subjecting the roots to a lowered oxygen supply in the water surrounding them. Nitrogen instead of air was bubbled through tap water surrounding the roots to subject the roots to a reduced oxygen supply. Groups of comparable plants with their roots in aerated tap water were used as controls.

Plants subjected to the lowered oxygen supply became somewhat wilted within 1 day and subsequently
In considering the effect of the amount of the acid supplied to the leaves on the amount present in the water around the roots, one has to take into account the rate of absorption by the leaves, translocation through the stems into the roots and the rate of exudation from the roots. Since the amount of MOPA in the water was directly proportional to the amount applied to the leaves, it seems apparent that none of the processes mentioned were functioning at a maximum rate, even when 10 \( \mu \text{gm} \) was applied to each leaf.

Factors which influenced the amount of MOPA in water around the pruned roots were as follows: first, the effect of reduced root surface area due to removal of the immature portion of the root; secondly, the effect of initiation of new roots due to pruning along with mobilization of plant constituents associated with this production of new roots; and finally, a decrease in the amount of MOPA absorbed and translocated to the pruned roots with the resultant depression in the amount of MOPA in the water surrounding them.

Removal of the lower part of the roots involved loss of the less mature portions including many root tips. This, along with the fact that the total surface area of the roots was greatly reduced, temporarily resulted in a marked reduction in the amount of acid in the water. Thus, the water surrounding the pruned roots contained only 13% as much MOPA after 35 hours as did that surrounding unpruned ones as shown in segment 1 (fig 3). The lack of a sudden increase in the amount of MOPA in the water immediately after pruning (up to 30 to 40 hours) indicates that a detectable amount of MOPA was not exuded from the injured surfaces.

Considering now the second segment of the curve (fig 3), the marked increase in the amount of MOPA present in the water around the pruned roots may have been partly due to the rapid increase in lateral root development with its resultant increase in root surface. Utilization of carbohydrates, nitrogenous and other plant constituents required in the production of new roots was notably accelerated as the result of the removal of the lower half of the roots. When exogenous compounds are applied to plants, they, together with carbohydrates, are translocated downward (2, 3, 4, 9, 10). This accelerated mobilization of carbohydrates required for root production probably increased the rate at which MOPA was translocated downward and thus resulted in a somewhat greater rate of MOPA exudation than occurred in the unpruned roots.

In segment 3 of the curve (fig 3), the reduced amount of MOPA in water around the roots may have been due to decreased absorption by the leaves and decreased transport of the acid to the roots, thereby allowing reabsorption by the roots to be the controlling factor.

Turning now to a consideration of plants with roots exposed to stable MOPA and leaves treated with radioactive MOPA, the roots absorbed the non-radioactive acid from the solution around the roots. This absorption was obvious from the fact that the stable

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**Fig. 3.** Average amounts of detectable MOPA C\(^{14}\) exudate in tap water surrounding pruned bean roots compared with the average amounts in tap water surrounding roots that were not pruned.

Grew very little. Although plants in water of lowered oxygen content exuded a detectable amount of MOPA at about the same time as did plants in aerated water, the radioactive exudate was greatly reduced by the lack of oxygen. During the latter part of the experiment roots in water with reduced oxygen exuded only about 20% as much acid as did plants with their roots in aerated water.

**Discussion**

The amount of MOPA absorbed by the leaves, the amount translocated and exuded by the roots, the amount adsorbed on the glass container, and also the amount of the exuded MOPA reabsorbed by the plant through its roots are factors which account in part, at least, for variation observed in the amount of the acid in the water.

It is obvious that during the period in which the amount of MOPA in the water increased, exudation exceeded the rate of reabsorption. As absorption of MOPA by the leaves and its translocation to the roots decreased with time, reabsorption of the acid by the roots exceeded exudation. This apparently accounts for the marked decrease in the amount of MOPA detected in the water during the latter part of the experiments.

A possible explanation for parts of the decrease that occurred in the amount of MOPA in the water during the latter part of the experiment is that bacteria or other microorganisms metabolized some of the MOPA in the water and loss of radioactive carbon dioxide from the water resulted. Both the aerated tap water and the nutrient solution remained clear. Notwithstanding this, some of the MOPA may have been metabolized by the organisms and lost as \( \text{C}^{14}\text{O}_2 \).
form of the acid caused the plant to develop detectable morphological responses in the young leaves even before the radioactive MOPA was applied. The absorption and upward transport of stable MOPA did not interfere detectably with absorption and downward transport of radioactive MOPA from the leaves. Furthermore, the movement of the radioactive MOPA out of the roots and its partial reabsorption by the roots were not detectably affected by the presence of the stable acid.

It should be mentioned, however, that radioactive MOPA in the water surrounding the roots reached a greater level when the roots were placed in water containing stable MOPA before the radioactive form of the acid was applied to the leaves. This increase was apparently due to adsorption of the stable MOPA on the surface of the glass container before the radioactive MOPA was exuded by the roots. This reduced the amount of radioactive MOPA adsorbed on the glass surface and thus caused an increase in the amount detected in the water.

An amount of radioactive MOPA approximately equal to that in water around roots of plants in previous experiments (1.5 μgm per 150 ml) was added to water in a beaker. The amount adsorbed to the glass was about 28% of the amount added. This percentage of the amount of MOPA detected in the water surrounding the roots was, therefore, subtracted to make the results comparable.

Assay of ground parts from plants subjected to a reduced oxygen supply revealed that relatively little radioactive MOPA was translocated from the leaves to the roots of these plants (50% less radioactivity in roots exposed to a lowered oxygen supply than in roots with an adequate oxygen supply). This probably accounted for the fact that relatively little MOPA exudate was detected in the water surrounding the oxygen-deficient roots.

SUMMARY

1. C₁⁴-labeled MOPA was applied to primary leaves of young Pinto bean plants growing in aerated tap water or nutrient solution so that exudation and reabsorption of the acid by the root could be studied.
2. The amount of MOPA exudate present in the water surrounding the roots (the amount exuded from the roots less the amount reabsorbed by the plant through its roots and that adsorbed on the glass) increased, reached a maximum, and then decreased within a period of approximately 200 hours. This general pattern was observed in experiments carried out with plants subjected to a variety of environmental conditions.
3. Plants grown with their roots in nutrient solution exuded and reabsorbed MOPA in a pattern similar to those grown with their roots in tap water.
4. The amount of MOPA exuded from the roots was proportional to the amount applied to the leaves over the range of dosages used.
5. Exudation of MOPA was retarded by the removal of the lower half of the root system. Pruned roots began to exude detectable amounts of the acid 4 to 24 hours later than did plants with comparable intact root systems. The concentration of MOPA in tap water surrounding pruned roots increased at a somewhat lower rate and eventually reached a higher level than in water surrounding the unpruned roots.
6. The amount of radioactive MOPA exuded into water around the roots was not affected by continuously subjecting the roots to water containing stable MOPA beginning 48 hours prior to application of the radioactive MOPA to the leaves.
7. Exudation of MOPA was greatly reduced by subjecting the roots to a lowered oxygen supply in the water surrounding them.

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LITERATURE CITED