Persistence and Translocation of Exogenous Regulating Compounds That Exude from Roots

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The persistence and translocation of six regulating compounds were compared to learn why plants exude some regulating substances from their roots but not others. Three of these growth regulators, 2,3,6-trichlorobenzoic, α-methoxyphenylacetic, and 2-methoxy-3,6-dichlorobenzoic acids, were translocated to the roots and exuded in detectable amounts as the compound applied. Exudation from the roots of one bean plant during the first 24-hour period varied from less than 1% of the amount applied for 2-methoxy-3,6-dichlorobenzoic acid to as much as 10% for α-methoxyphenylacetic acid. 2,5-Dichlorobenzoic, 3,4-dichloro-α-methoxyphenylacetic, and naphthaleneacetic acids were apparently degraded or bound by the plant since they failed to reach the roots after application to leaves and, therefore, were not exuded. Relatively rapid degradation or other type of inactivation of naphthaleneacetic acid in detached leaf blades of bean plants was demonstrated. There was no relationship between the potency of a compound as a regulator and the ability of the plant to exude it.

Plants are capable of exuding many kinds of endogenous compounds from their roots into surrounding soil (*1–3, 10*). They are also capable of exuding some exogenous regulating substances into soil after application of the compounds to leaves and stems. Six regulators are known to be exuded in readily detectable amounts from roots. These compounds are α-methoxyphenylacetic; *m*-chloro-α-methoxy-phenylacetic; *m*-fluoro-α-methoxy-phenylacetic; *p*-fluoro-α-methoxy-phenylacetic; 2,3,6-trichlorobenzoic; and 2,3,5,6-tetrachlorobenzoic acids (*4, 5, 7, 8*).

An additional compound, 2-methoxy-3,6-dichlorobenzoic acid, is reported here as also being exuded from roots. All these regulators were exuded from roots of treated plants into soil in amounts sufficient to affect untreated plants growing with their roots next to those of the treated plants. On the other hand, there are many regulators which apparently are not exuded from roots into soil in detectable amounts.

The objective of this research was to gain an understanding of the persistence and translocation of several growth-regulating chemicals in relation to their exudation from roots. Chromatographic and bioassay techniques were used. Three regulating compounds which are exuded from roots in detectable amounts were compared with three regulators not known to be exuded. Four of these compounds were paired on the basis of similarity in chemical structure and the other two—namely, 2-methoxy-3,6-dichlorobenzoic and naphthaleneacetic acids—on the basis of their marked regulating activity. The regulators exuded from roots were 2,3,6-trichlorobenzoic, α-methoxyphenylacetic, and 2-methoxy-3,6-dichlorobenzoic acids. These were paired, respectively, with 2,5-dichlorobenzoic, 3,4-dichloro-α-methoxyphenylacetic, and naphthaleneacetic acids, which are not exuded from roots in detectable amounts.

Experimental

Bean plants of the Pinto variety about 13 cm. tall were selected for this study.

To collect exudates, roots of intact plants were washed free of soil and immersed in aerated tap water. Approximately 63 μg. of each chemical in a Tween 20-lanolin mixture was spread over 1 sq. cm. of the upper surface of each primary leaf near the petiole. The water containing the exuded material from roots was collected 24 hours after application of the chemical. The exudate was concentrated and chromatographed by using several solvent systems. Similar plants growing in soil were also treated to provide necessary plant tissue, such as stems and roots, for extraction and subsequent chromatography. After chromatography, identification was made by bioassay of the regulating compounds or their growth-regulating metabolites which were exuded or which remained in the tissues of stems or roots. Eluate from each segment of the chromatogram was placed in lanolin, and each mixture was applied separately to the second internode of a young bean plant (*9*). Observations were made 1 to 4 days after application of the eluate-lanolin mixture to detect growth responses in terminal buds. The R_f of the regulator in the eluate was chromatographically compared with the R_f of the pure compound by using the standard and the unknown separately and with cochromatography.

Compounds used in these tests, except for C^{14}-labeled α-methoxyphenylacetic acid, were nonradioactive. Identity of this compound in the plant and in the exudate using radioactivity was reported earlier (*6*). Quantitative estimations of the regulator exuded from roots or extracted from the plants were made by comparing the magnitude of the growth responses induced by the exudates or extracts with those induced by known amounts of the compounds involved. Comparable results were obtained in three replicated experiments in the case of each determination.

The persistence of naphthaleneacetic acid in the blades of bean leaves and the part played by enzymes in the fate of the acid were studied. Approximately 10 μg. of the acid was spread evenly over the upper surface of an attached primary leaf using the carrier previously described. The opposite primary leaf of the plant was treated in the same manner. These two leaf blades were immediately detached from the petioles and placed together between two pieces of wire mesh. The wire mesh was then wrapped in moist filter paper. The leaves, mesh, and filter paper were sealed in an airtight cellophane envelope. This procedure was repeated resulting in four packets, each containing a pair of primary leaves. Leaves in two of the packets enclosed in this manner were frozen immediately by surrounding the packets with dry ice. The remaining packets were placed in diffused light at room temperature for 24 hours and then frozen. Each pair of leaves was homogenized separately and extracted with ethanol. The alcohol was evaporated, water added, and the mixture extracted with ether at a pH of 2.5 to remove and partially purify any naphthaleneacetic acid present. The pH of the mixture was

Table I. Identity of 2-Methoxy-3,6-dichlorobenzoic Acid (Dicamba) in Root Exudate

Solvent System	R_f of Sample Chromatographed: Dicamba	Exudate	Exudate + dicamba
Butanol–benzene–phosphate buffer (16:1:3 v./v.)	0.92	0.91	0.90
Benzene–acetic acid–water (1:1:2 v./v.)	0.90	0.90	0.90
Ammonium hydroxide–butanol (1:2 v./v.)	0.93	0.90	0.85
Ammonium hydroxide–ammonium carbonate–butanol (1:1:2 v./v.)	0.68	0.70	0.68
Butanol–ethanol–ammonium hydroxide/ammonium carbonate (40:11:19 v./v.)	0.70	0.70	0.72

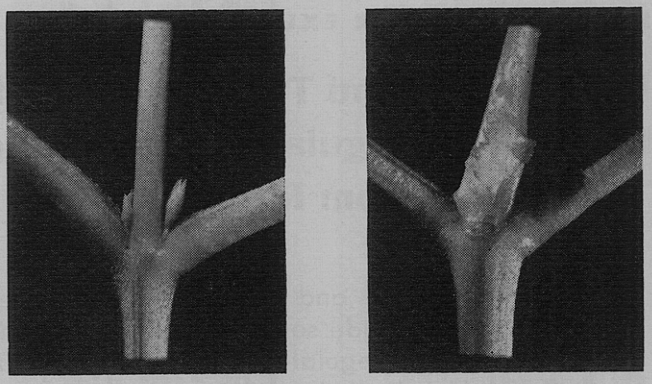

Figure 1. Stems of intact assay plants used to detect residual naphthaleneacetic acid extracted from bean leaf blades treated previously with this acid

The extract that induced a gall, *right*, was obtained from treated leaves immediately frozen and stored for 24 hours, then assayed for naphthaleneacetic acid. Extract applied to stem, *left*, was obtained from treated leaves stored in diffused light at room temperature for 24 hours before being frozen, extracted, and assayed for naphthaleneacetic acid. Lack of gall development indicates that naphthaleneacetic acid was apparently metabolized by the unfrozen stored leaves

then adjusted to 8 and the naphthalene salt extracted into water. The pH was again adjusted to 2.5 and the naphthaleneacetic acid once more dissolved in ether. Finally, the ether was evaporated and the naphthaleneacetic acid was dispersed in lanolin and applied to test plants as described above. Response of the test plants to naphthaleneacetic acid was evidenced as cellular proliferation which occurred in the region of the stem to which the mixture was applied.

Results

2,3,6-Trichlorobenzoic acid was translocated from the leaves into the stems, then into the roots, and finally was exuded into aerated water surrounding the roots. No chemical change could be detected chromatographically in the exuded compound as compared with the substance applied.

When 100 μg. of this compound was applied to the leaves of a bean plant, about 1 μg. of the acid was recovered from the stem of each plant 4 days after treatment. Less than 1 μg. was detected in the root. The roots of each plant exuded about 1.5 μg. of 2,3,6-trichlorobenzoic acid per day during the 2 days immediately after application of the chemical to the leaves.

Another regulator, 2,5-dichlorobenzoic acid, closely related to the trichloro acid but slightly less active, was also translocated in an unchanged form from the leaves to the stems. However, unlike the trichloro acid, 2,5-dichlorobenzoic acid was not detected in the roots nor was it exuded in detectable amounts.

Two active regulators in a different family, α-methoxyphenylacetic acid and 3,4 - dichloro - α - methoxyphenylacetic acid, were also compared. Like the 2,3,6-trichlorobenzoic acid, the unsubstituted methoxy acid was detected in the roots and was also exuded. Approximately 2 to 3 μg. of the α-methoxyphenylacetic acid was found in the root, while a little less than 10 μg. of the same acid was exuded from each root during the first 24-hour period after treatment.

The other regulator in this methoxy group, 3,4 - dichloro - α - methoxyphenylacetic acid, was not found in a detectable amount within the roots of plants after its application to the leaves. Neither was a detectable amount exuded from roots of treated plants. The bioassay methods were sensitive to 0.04 μg. of extractable 3,4-dichloro-α-methoxyphenylacetic acid per root.

Another pair of compounds, related as markedly active growth regulators rather than as to similarity in chemical structure, was studied. They were 2 - methoxy - 3,6 - dichlorobenzoic acid, which was exuded from roots of treated plants, and naphthaleneacetic acid, which was not exuded from roots in detectable amounts.

The methoxy-substituted benzoic acid was detected in the roots, exuded, and identified as the compound applied. On the basis of bioassays, it was estimated that less than 1% of this methoxy acid was exuded during the first 24-hour period after application of 100 μg. per plant. In identifying the exuded regulator chromatographically, five different solvent systems were used in separate comparisons and in cochromatography (Table I).

Naphthaleneacetic acid was not found in the roots, nor was it exuded in detectable amounts. With the assay method used, it is possible to detect 1 μg. of extractable naphthaleneacetic acid per root.

Experiments designed to measure the persistence of naphthaleneacetic acid in bean leaves showed that this acid was apparently degraded or bound by the leaf blades. After application of approximately 10 μg. of this acid to each leaf blade, so much of the compound was changed or bound that a detectable amount could not be extracted from the treated leaf blades 24 hours after application. Persistence of the compound in leaves that were immediately killed after treatment indicated that the compound may have been enzymatically degraded or that binding of the compound involved vital processes within the leaves (Figure 1).

Acknowledgment

Wilkins Reeve, Chemistry Department, University of Maryland, synthesized α-methoxyphenylacetic acid and 3,4-dichloro-α-methoxyphenylacetic acid. 2,3,6-Trichlorobenzoic acid and 2,5-dichlorobenzoic acid were obtained from Amchem Products, Inc., Ambler, Pa. 2-Methoxy-3,6-dichlorobenzoic acid was obtained from Velsicol Chemical Corp., Chicago, Ill.

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