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THE EXUDATION OF 14C-CACODYLIC ACID FROM THE ROOTS OF BEAN PLANTS AND ASH SEEDLINGS

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March 1973

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EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4738

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by

William A. Wells
Frank B. Anastasia

Chemical Laboratory

March 1973

DEPARTMENT OF THE ARMY
Headquarters, Edgewood Arsenal
Aberdeen Proving Ground, Maryland 21010

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EDGEWOOD ARSENAL TECHNICAL REPORT

EATR 4738

THE EXUDATION OF ^{14}C-CACODYLIC ACID FROM THE ROOTS OF BEAN PLANTS AND ASH SEEDLINGS

by

William A. Wells*
Frank B. Anastasia**

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March 1973

Task 1B562605AD2801

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FOREWORD

The work described in this report was conducted under Task 1B562605AD2801, Vegetation Control Technology (U). This work was started in January and completed in July 1971.

Material presented herein is the source material for Fort Detrick cleared manuscript 71-375, "Exudation of ^{14}C-Cacodylic Acid from the Roots of Bean Plants and Ash Seedlings," which was approved for oral presentation by Department of Army per AMC letter dated 7 December 1971. An oral presentation of the results was given to the Northeastern Weed Science Society, New York, New York, January 1972, and an abstract appeared in the proceedings of that society. The full manuscript has not appeared in published form.

Acknowledgment

The authors wish to acknowledge Dr. P. C. Kearney and Dr. J. R. Plimmer of the Pesticide Investigations Laboratories, US Department of Agriculture, for providing ^{14}C-cacodylic acid and Mr. Duane N. Sommerville for providing technical assistance.

DIGEST

Bean plants (*Phaseolus vulgaris* L. var. Black Valentine) and green ash seedlings (*Fraxinus pennsylvanica* Marsh.) were treated with sublethal concentrations of ^{14}C-cacodylic acid (hydroxydimethylarsine oxide) to determine if cacodylic acid is exuded from the roots of plants.

The bean plants were treated with three different concentrations of cacodylic acid (specific activity, 17.4 μCi/mg) in 0.5% (v/v) Tween 20 applied in five 10-μl droplets to each primary leaf. Similarly, 100 μl of cacodylic acid/adjuvant mixture (0.012 μCi) was applied to the ash seedlings on each leaf of the pair of leaves at the fourth node above the root collar. All plants were grown in 0.5-strength Hoagland's nutrient solution under controlled environmental conditions.

The presence of ^{14}C was detected in the nutrient solution of both bean plants and ash seedlings within 24 hours following treatment. The root exudation of radioactive material increased over the 9- and 16-day sampling periods for the bean plants and ash seedlings, respectively. The root exudation studies in bean plants showed 12.8% of the total applied radioactivity was exuded 3 days after application. For ash seedlings, the comparable rate after 3 days was 2.7%. The cumulative root loss of the total applied radioactivity for the bean plants was 19.1% after 9 days compared to 9.6% for the ash seedlings after 16 days.

In the bean plants, greater amounts of radioactivity were exuded with higher application rates unless the amount of herbicide applied was sufficient to cause tissue damage or growth reduction. When plant injury resulted, the exudation rate was inhibited even with an increase in the level of radioactivity applied to the plant.

At the termination of the experiment, the nutrient solutions were spotted on Whatman 3MM chromatography paper and developed in two solvent systems (2-propanol:H_2O, 7:3; 1-propanol:NH_4OH, 7:3). The radioactive areas on the chromatograms of the nutrient solution concentrate from both the treated bean plants and ash seedlings closely coincided with the chromatogram of the ^{14}C-cacodylic standard and the co-chromatogram of the nutrient solution-standard mix. Therefore, it would appear that ^{14}C-cacodylic acid is exuded as an unaltered molecule.

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THE EXUDATION OF ^{14}C-CACODYLIC ACID FROM THE ROOTS OF BEAN PLANTS AND ASH SEEDLINGS

I. INTRODUCTION.

The last decade has seen the appearance of numerous reports on the root exudation of exogenously applied compounds, including herbicides and other substances that regulate plant growth. The papers of Mitchell and Linder,[1] Rovira,[2] and Foy *et al.*[3] summarize the main areas of experimental emphasis with relation to studies employing foliar applications of labeled compounds. Although these studies have covered a number of families of chemical compounds, no reports have appeared concerning arsenic-containing pesticides. The organic arsenicals form a group of special interest within this family of compounds both because of their increasingly widespread use as cotton defoliants and general herbicides and the controversy surrounding their utilization. One of these organic arsenicals, cacodylic acid (hydroxydimethylarsine oxide), has recently received much attention, largely because of its military employment as an anticrop agent in Southeast Asia. However, the nonavailability of labeled cacodylic acid has posed a problem in studies concerning its fate when applied to plants. Therefore, the recent synthesis of ^{14}C-cacodylic acid and its availability to this laboratory initiated a series of physiological and biochemical studies of this compound in plant applications. This report represents one segment of a series of studies concerned with the absorption, uptake, distribution, and exudation of cacodylic acid and gives evidence for inclusion of the arsenicals as members of that group of exogenous compounds which are exuded from plant roots.

II. MATERIALS AND METHODS.

Bean plants (*Phaseolus vulgaris* L. var. Black Valentine) were germinated in sand and transferred to pots containing 800 ml of aerated 0.5-strength Hoagland's nutrient solution which was replenished daily. The plants, two per pot, were grown in a controlled environmental chamber. The relative humidity of the growth chamber was 50% ± 5%; and the temperature, 25° ± 1°C. A 16-hour photoperiod of 1450 ± 50 ft-c illumination at plant-top level was provided by a mixture of fluorescent and incandescent lamps.

Uniform plants in which the first trifoliolate leaf had just opened (6 days old) were selected for use. Using a microliter syringe, sublethal dosages of ^{14}C-labeled cacodylic acid (specific activity, 17.4 µCi/mg) were applied in five 10-µl droplets to each of the five main leaf veins on each primary leaf. Two series of plants were treated, each with a different concentration of cacodylic

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[1] Mitchell, J. W., and Linder, P. J. Absorption, Translocation, Exudation, and Metabolism of Plant Growth-Regulating Substances in Relation to Residues. pp 51-76. 1963. In Residue Reviews, Volume 2 (Francis A. Gunther, ed.).

[2] Rovira, A. D. Plant Root Exudates. Bot. Rev. 35, 35-58 (1959).

[3] Foy, C. L., Hurtt, W., and Hale, M. G. Root Exudation of Plant Growth Regulators. pp 75-83. 1971. In Biochemical Interactions Among Plants, National Academy of Sciences, Washington, DC.

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acid. The first series received 100 μl (0.006 μCi) of labeled cacodylic acid per plant or 200 μl (0.012 μCi) per container of two plants, and the second series received 100 μl (0.012 μCi) per plant or 200 μl (0.24 μCi) per container.

In order to make a further comparison of the effect of application concentration on exudation, a third series of plants was treated in a similar manner with a lethal concentration of cold cacodylic acid (100 μg arsenic) in combination with labeled cacodylic acid at a higher radioactivity level (0.56 μCi) per plant or 1.12 μCi per container of two plants.

In order to compare the exudation rates of a woody species with those of the herbaceous bean plants, a fourth series of plants was prepared utilizing ash seedlings as the test species. The inclusion of the slower growing woody species also enabled an experiment of longer duration than was possible with the faster growing bean plants.

Green ash seedlings (*Fraxinus pennsylvanica* Marsh.) obtained from commercial nursery stock were selected for use on the basis of uniformity of size (stem and root lengths of approximately 40 and 25 cm, respectively, and 6 to 7 whorls of leaves). The ash seedlings were approximately 6 weeks old.

These seedlings were transferred to nutrient solution culture in the same manner and under the same conditions as previously described for the bean plants. The pair of leaves at the fourth whorl above the root collar on each plant was treated in the same fashion as the bean plants with a dosage of 100 μl (0.012 μCi) of labeled cacodylic acid.

At 24-hour intervals, the nutrient solution was brought up to 800 ml and 10-ml samples were taken. The experiment covered a 6-day or 9-day sampling period in the case of the bean plants and a 16-day sampling period in the case of the ash seedlings. The nutrient solution samples were evaporated to dryness, brought up in 15 ml of scintillation fluid, and counted in a Nuclear-Chicago Mark I liquid scintillation counter to determine the amount of ^{14}C present. The background count was determined from nutrient solution samples of untreated plants.

At the termination of the experiment the nutrient solutions of the treated plants were combined, filtered, and evaporated to a 320-fold decrease in volume. The solution was then spotted on Whatman 3MM chromatography paper and run in one of two solvent systems (2-propanol:H_2O, 7:3; 1-propanol:NH_4OH, 7:3). The nutrient solution from control plants was also co-chromatographed with labeled cacodylic acid standard. Following development, the chromatograms were subdivided by R_f values and counted in liquid scintillation.

III. RESULTS.

A. Bean Plant Exudation.

The radioactivity of the mean cumulative total ^{14}C exuded at each sampling period from the second series of bean plants is shown in figure 1. Loss of ^{14}C was observed within 24 hours from all replicates and increased over the 9-day period with the exception of a slight decrease on the fifth and seventh days of sampling. These decreases may have been due to recycling of the nutrient solutions into the plants themselves.

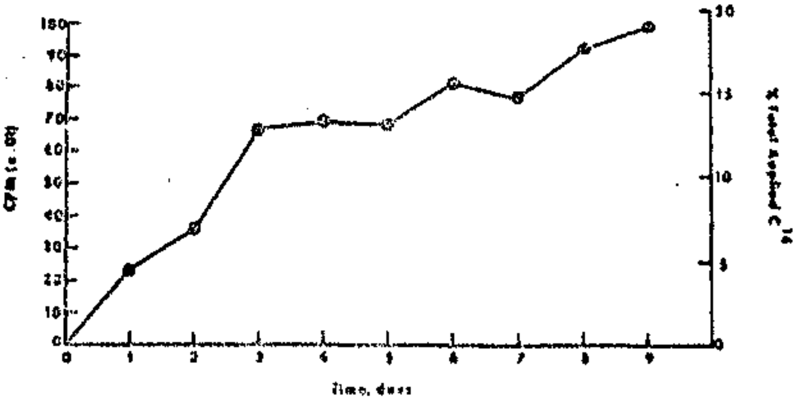

Figure 1. Cumulative ^{14}C Loss from Roots of Bean Plants after Foliar Treatment with 100 µl (0.012 µCi) ^{14}C-Cacodylic Acid per Plant or 200 µl (0.024 µCi) per Container of Two Plants

Each point represents the mean of six replications.

The amount of radioactivity lost by the roots increased from 4.5% of the total amount applied at the end of 24 hours to 19.1% at the end of 9 days. Comparative values for 6-day bean plant experiments utilizing a lower sublethal application rate and a tenfold higher rate of application with incorporation of cold cacodylic acid at a lethal dosage can be seen in the table. It is of interest to note that greater amounts of radioactivity are exuded with greater rates of application unless sufficient amounts of herbicide are present to cause tissue damage or growth reduction in the treated plants. In the latter case, the exudation rate is greatly inhibited in spite of the large increase in applied radioactivity.

Table. Comparison of the Effects of Different Application Rates of Cacodylic Acid on the Rate of Exudation from Bean Roots at Days 1, 3, and 6 after Application

Application rate		Exudation rate					
		Day 1		Day 3		Day 6	
^{14}C	Arsenic	CPM*	Percent of total applied	CPM*	Percent of total applied	CPM*	Percent of total applied
µCi	µg						
0.012	1.0	400	1.38	1445	4.98	3054	10.60
0.024	1.4	2355	4.50	6657	12.80	8068	15.50
1.12	100.0	3692	0.62	8162	1.77	9896	1.66

* CPM – counts per minute.

B. Ash Seedling Exudation.

The radioactivity of the mean cumulative total ^{14}C exuded at each sampling period of the 16-day ash seedling experiment is shown in figure 2.

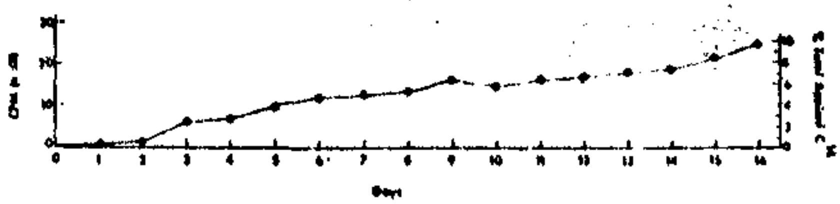

Figure 2. Cumulative ^{14}C Loss from the Roots of Green Ash Seedlings after Foliar Treatment with 100 μl (0.012 μCi) of ^{14}C-Cacodylic Acid per Plant
Each point represents the mean of six replications.

Loss of ^{14}C was observed within 24 hours from all seedlings and increased over the 16-day period with the exception of a slight decrease on the tenth day. This change in cumulative radioactivity at 10 days is not easily explained. A possible explanation is a sudden change in recycling of ^{14}C from the nutrient solution to the seedlings.

The amount of radioactivity lost by the roots, expressed as a percentage of the total amount of radioactivity applied, increased from 0.1% at the end of 24 hours to 9.6% after 16 days.

C. Chromatography of Nutrient Solutions.

The areas of radioactivity on the chromatograms of the nutrient solution concentrate from the treated plants closely coincided with the chromatograms of ^{14}C-cacodylic acid standard and the co-chromatograms of the nutrient solution-standard.

Figure 3 shows the areas of ^{14}C activity on paper chromatograms of ^{14}C-cacodylic acid standard, nutrient solution from the second series of bean plants and a co-chromatogram of bean nutrient solution and ^{14}C-cacodylic acid standard, developed in 2-propanol:H_2O (7:3). Chromatograms of the nutrient solutions from the ash seedlings run in this solvent system yielded very similar results. Figure 4 shows the resulting areas of radioactivity when the ^{14}C-cacodylic acid standard and ash nutrient solution were run in the 1-propanol:NH_4OH (7:3) system. Similar results were also obtained from chromatograms of the bean nutrient solution developed in this solvent system.

In the case of the ash nutrient solution chromatograms (figure 4), some retardation of the radioactive areas was evident when compared to the chromatogram of the ^{14}C-cacodylic acid standard. Due to the relatively long duration of the ash seedling experiment, the nutrient solution may have contained large amounts of salts and proteins exuded by the plants in addition to the salts in the nutrient solution itself. Because of the close agreement of the chromatograms developed in the 2-propanol:H_2O (7:3) system in the case of both the bean and ash nutrient solutions and the similarity of the nutrient solution chromatograms and the co-chromatograms run in the 1-propanol:H_2O (7:3) system, this retardation is most likely due to the heavy concentration of salts in the nutrient solution rather than the presence of a metabolite of the cacodylic acid.

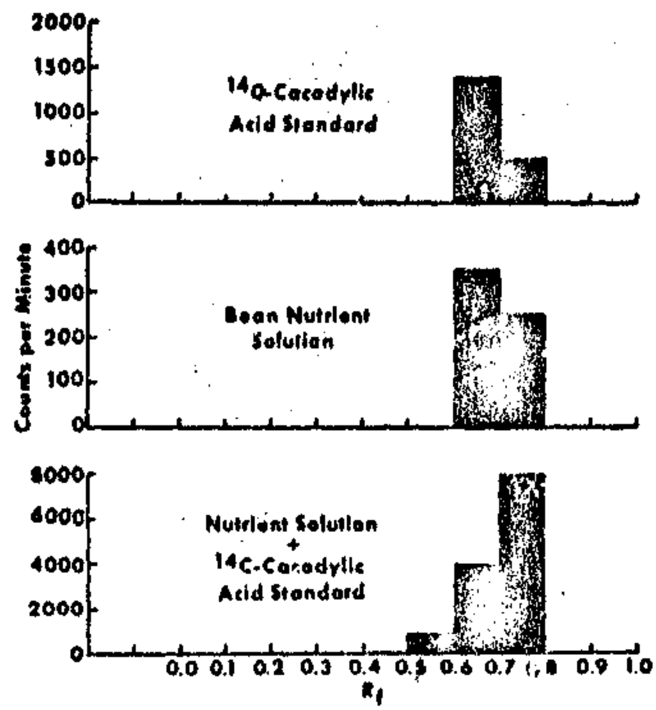

Figure 3. Areas of ^{14}C Activity on Paper Chromatograms Developed in 2-Propanol:H_2O (7:3) of (1) ^{14}C-Cacodylic Acid Standard, (2) Nutrient Solution from Bean Plants Treated with ^{14}C-Cacodylic Acid, and (3) (Co-Chromatogram) Bean Nutrient Solution and ^{14}C-Cacodylic Acid Standard

Values in CPM, corrected for background and counting efficiency, are significantly above background at the 95% confidence level.

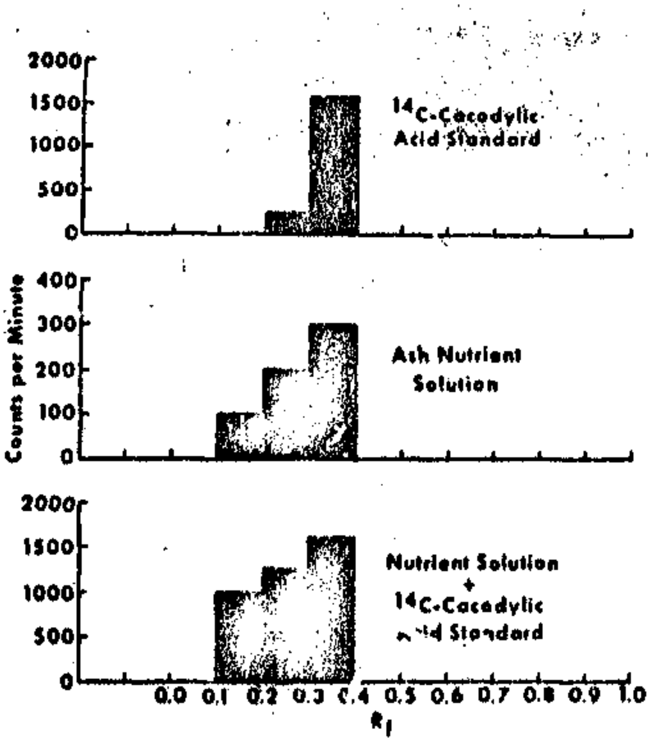

Figure 4. Areas of ^{14}C Activity on Paper Chromatograms Developed in 1-Propanol:NH_4OH (7:3) of (1) ^{14}C-Cacodylic Acid Standard, (2) Nutrient Solution from Green Ash Seedlings Treated with ^{14}C-Cacodylic Acid, and (3) (Co-Chromatogram) Ash Nutrient Solution and ^{14}C-Cacodylic Acid Standard

Values in CPM, corrected for background and counting efficiency, are significantly above background at the 95% confidence level.

IV. DISCUSSION.

These studies revealed that 19.1% of the total amount of ^{14}C-cacodylic acid applied to bean plants grown in nutrient solution is exuded after 9 days in culture and that, after 16 days, 9.6% of the total applied ^{14}C-cacodylic acid is exuded from the roots of green ash seedlings. This would indicate that the rate of exudation of cacodylic acid from woody species may be considerably less than its rate of exudation from herbaceous species.

In the only other reported studies on the exudation of labeled herbicides from woody species, those by Reid and Hurtt[4] on the exudation of ^{14}C-picloram and ^{14}C-2,4,5-T from ash and

[4] Reid, C. P. P., and Hurtt, W. Root Exudation of Herbicides by Woody Plants: Allelopathic Implications. Nature 225: 291 (1970).

maple seedlings, the percentages of herbicide exuded are less than those reported in these studies. Their reported 8.4% of ^{14}C-2,4,5-T exuded from maple roots after 22 days is the only amount comparable in magnitude to the amount of ^{14}C-cacodylic acid exuded after 16 days from ash roots. Moreover, the 8.4% exudation rate reported by Reid and Hurtt is the percentage of labeled herbicide actually entering the plant leaf and therefore represents an even smaller percentage when expressed as percent of total applied radioactivity.

The existence of a greater number of published studies on the exudation of exogenous compounds from herbaceous plants offers a somewhat broader base for comparison. A number of these studies were also conducted with bean plants.

Mitchell *et al.*[5] reported that the loss of ^{14}C-α-methoxyphenylacetic acid from pinto bean roots was 16% after 3 days. This percentage is in excess of the 12.7% of cacodylic acid exuded after 3 days as reported in this paper (figure 1). Even greater losses of labeled Diazinon have been demonstrated by Kansouh and Hopkins[6] who report 68% loss after 2 days' duration in studies with bean plants.

The contamination of soil by residues of substances exuded from plants represents a potential problem with regard to influencing the growth of nearby plants or crops subsequently grown in the same soil. Foy *et al.*[3] report three substances, picloram, dicamba, and 2,3,6-TBA, as being the most potent of 31 herbicides tested with regard to causing injury to untreated plants growing in the proximity of treated plants. They report 21 benzoic acid derivatives, 5 phenylacetic acids, and 5 picolinic acids or substituted pyridines which were excreted by bean roots.

The report of Foy *et al.* represents an expansion of the two family classification of exuded compounds reported by Mitchell and Linder.[1] Likewise, the exudation of cacodylic acid, a methylated arsenical, adds a new class of compounds which are lost from the roots of treated plants.

Because cacodylic acid is an arsenic-containing compound, the finding that almost 20% of the total application is lost from roots may initially seem to pose a problem of serious consequence. However, these findings are based on experimentation with nutrient solution cultures, a situation which in all likelihood does not reflect that encountered in crops grown in soil. Similarly, root exudation into the soil opens the possibilities of degradation by microorganisms, complexing with soil ions, and the influence of climatic conditions. All of these possibilities could render a phytotoxic exudate relatively harmless to other plants. In the case of cacodylic acid,

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[5] Mitchell, J. W., Linder, P. J., and Robinson, M. B. Mechanism of Root Exudation of α-Methoxyphenylacetic Acid in the Bean Plant. Bot. Gaz. *123*, 134-137 (1961)

[6] Kansouh, A. S. H., and Hopkins, T. L. Diazinon Absorption, Translocation, and Metabolism in Bean Plants. J. Agr. Food Chem. *16*, 446-450 (1968).

evidence exists both for microbial action,[7] which may produce a more toxic residue, and complexing within the soil,[8]* which would make the arsenic unavailable and thus remove its phytotoxic capabilities.

In these studies, the amount of arsenic applied in the amount of labeled material used is extremely low. The low application rate with 0.006 μCi per 100 μl contained only 1 μg arsenic per 200 μl. The 0.012 μCi per 100 μl treatment, which resulted in 19.1% of the total application being exuded after 9 days, contained only 1.4 μg arsenic per 200 μl. When arsenic levels are present that more closely approximate levels used in agriculture practices, the physiological and morphological changes in the plant greatly inhibit exudation. Even with applications of extremely high radioactivity (0.56 μCi per 100 μl), the inclusion of 100 μg arsenic per 200 μl resulted in only 1.6% of the total application being exuded after 6 days. Additionally, we find no evidence to suggest that the exudate from the cacodylic acid plants contains any metabolite resulting from the application of the methylated organic arsenical. Our results indicate that cacodylic acid is exuded from plant roots as an unaltered molecule. This is of considerable importance since the demethylated and inorganic forms of arsenic exhibit more toxic characteristics than cacodylic acid which has an acute oral toxicity approximating that of common aspirin.

Mitchell and Linder[1] in their 1963 review paper state: "There is no evidence that root exudation is a problem in soil contamination as far as the present use of growth regulating substances is concerned." This statement may still be valid. However, the consideration of more recent reports such as this paper and those of Rovira[2] and Foy *et al.*[3] clearly show the need for serious study in the allelopathic effects of foliar applied exogenous compounds.

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[7] Challenger, F., and Higginbottom, C. The Production of Trimethylarsine by *Penicillium brevicaule (Scopulariopsis brevicaulis)*. Biochem. J. 29, 1757-1778 (1935).

[8] Dickens, R., and Hiltbold, A. E. Movement and Persistence of Methanearsonates in Soil. Weeds *15*, 299-304 (1967).

* Woolson, E. A. The Chemistry and Toxicity of Arsenic in Soil. Unpublished Ph.D. Dissertation, University of Maryland, College Park, Maryland. 1969.

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4. Reid, C. P. P., and Hurtt, W. Root Exudation of Herbicides by Woody Plants: Allelopathic Implications. Nature *255*, 291 (1970).

5. Mitchell, J. W., Linder, P. J., and Robinson, M. B. Mechanism of Root Exudation of α-Methoxyphenylacetic Acid in the Bean Plant. Bot. Gaz. *123*, 134-137 (1961).

6. Kansouh, A. S. H., and Hopkins, T. L. Diazinon Absorption, Translocation, and Metabolism in Bean Plants. J. Agr. Food Chem. *16*, 446-450 (1968).

7. Challenger, F., and Higginbottom, C. The Production of Trimethylarsine by *Penicillium brevicaule* (*Scopulariopsis brevicaulis*). Biochem. J. *29*, 1757-1778 (1935).

8. Dickens, R., and Hilbold, A. E. Movement and Persistence of Methanearsonates in Soil. Weeds *15*, 299-304 (1967).

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13. ABSTRACT

Bean plants (*Phaseolus vulgaris* L. var. Black Valentine) and green ash seedlings (*Fraxinus pennsylvanica* Marsh.) were treated with sublethal concentrations of ^{14}C-cacodylic acid (hydroxydimethylarsine oxide) to determine if cacodylic acid is exuded from the roots of plants. The bean plants were treated with three different concentrations of cacodylic acid (specific activity, 17.4 μCi/mg) in 0.5% (v/v) Tween 20 applied in five 10-μl droplets to each primary leaf. Similarly, 100 μl of cacodylic acid/adjuvant mixture (0.012 μCi) was applied to the ash seedlings on each leaf of the pair of leaves at the fourth node above the root cellar. All plants were grown in 0.5-strength Hoagland's nutrient solution under controlled environmental conditions. The presence of ^{14}C was detected in the nutrient solution of both bean plants and ash seedlings within 24 hours following treatment. The root exudation of radioactive material increased over the 9- and 16-day sampling periods for the bean plants and ash seedlings, respectively. The root exudation studies in bean plants showed 12.8% of the total applied radioactivity was exuded 3 days after application. For ash seedlings, the comparable rate after 3 days was 2.7%. The cumulative root loss of the total applied radioactivity for the bean plants was 19.1% after 9 days compared to 9.6% for the ash seedlings after 16 days. In the bean plants, greater amounts of radioactivity were exuded with higher application rates unless the amount of herbicide applied was sufficient to cause tissue damage or growth reduction. When plant injury resulted, the exudation rate was inhibited even with an increase in the level of radioactivity applied to the plant. At the termination of the experiment, the nutrient solutions were spotted on Whatman 3MM chromatography paper and developed in two solvent systems (2-propanol:H_2O, 7:3; 1-propanol:NH_4OH, 7:3). The radioactive areas on the chromatograms of the nutrient solution concentrate from both the treated bean plants and ash seedlings closely coincided with the chromatogram of the ^{14}C-cacodylic standard and the co-chromatogram of the nutrient solution-standard mix. Therefore, it would appear that ^{14}C-cacodylic acid is exuded as an unaltered molecule.

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Exudation						
Bean plants						
Ash seedlings						
Roots						
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