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Phytotoxicity, Absorption, and Translocation of 4-Aminopyridine in Corn and Sorghum Growing in Treated Nutrient Cultures and Soils

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There were no visible phytotoxic effects when 1-month-old corn (*Zea mays*) or sorghum (*Sorghum vulgare*) seedlings were grown 1 week in nutrient cultures containing 0.1–100 ppm of 4-aminopyridine. Autoradiograms of 1-month-old plants grown 1 week in treated nutrient solutions containing 5 or 10 ppm of 4-[^{14}C]aminopyridine revealed a general distribution of radioactivity throughout the plants, with higher concentrations in the roots, lower stem, and leaf sections nearest the stalks. The quantity of 4-[^{14}C]aminopyridine translocated into shoot tissues of 2- and 3-month-old plants was inversely proportional to age, with most of the radioactivity in these older plants contained in the lower sections. Carbon-14 was not detected in corn seeds, but trace amounts were detected in seeds of sorghum plants. Shoots of 1-month-old corn plants grown in treated soils contained only small quantities of radioactivity, indicating that the compound was highly adsorbed onto soil colloids and thus relatively unavailable for root absorption.

Damage to ripening cereal grains by flocks of feeding blackbirds is a problem of economic concern in the United States (Neff, 1949; Neff and Meanley, 1957). Recent surveys in major corn-producing states revealed that direct losses attributable to birds were about 6 million bushels during both 1970 and 1971 (Stone *et al.*, 1972; Stone, 1972).

One of the most effective means found to date for reducing blackbird damage to corn involves the use of 4-aminopyridine, a chemical frightening agent (De Grazio *et al.*, 1971), whose utility in reducing problems caused by birds was first demonstrated by Goodhue and Baumgartner (1965). Cracked corn treated with this compound and broadcast in fields (De Grazio *et al.*, 1972) causes birds that ingest treated baits to fly erratically and emit distress calls, thereby inducing other members of the flock to abandon the area.

As with other toxic chemicals intended for soil application in the vicinity of food or feed crops, it is necessary to understand the fate of 4-aminopyridine in soils and its phytotoxicity, translocation, and metabolism in plants, in order to evaluate potential hazards from its use. With a

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view to the registration of 4-aminopyridine-treated baits for ground application in field corn and grain sorghum, we undertook phytotoxicity and translocation studies with these plants. The degradation of translocated 4-[^{14}C]aminopyridine in corn and grain sorghum will be described in a paper to be published (Starr and Cunningham, 1974).

MATERIALS AND METHODS

Chemicals and Reagents. Analytical grade 4-aminopyridine was either furnished by the Phillips Petroleum Co. or purchased from the J. T. Baker Laboratories and was usually recrystallized from an acetonitrile solution before use. α-Labeled 4-[^{14}C]aminopyridine, purchased from the International Chemical and Nuclear Corporation, was recrystallized with unlabeled 4-aminopyridine before use and was estimated to be 98% radiochemically homogeneous by thin-layer chromatography-autoradiography, with a specific activity of 0.15 mCi/mmol.

All reagents used were of analytical grade quality unless otherwise specified.

Radioactivity Measurements. *Liquid Scintillation Analysis.* Plant tissues to be analyzed by liquid scintillation spectrometry were air-dried and macerated with a Virtis "45" blender and then oxidized by Schöniger oxygen flask combustion. Between 50 and 100 mg of each plant sample was combusted in duplicate, unless otherwise stated, and the combustion products were absorbed in 10 or 15 ml of a solution containing 12% (v/v) of scintillation grade ethanolamine in methanol (Nuclear-Chicago Corp., 1966). A 5-ml aliquot of this solution was withdrawn and was added to a vial containing 15 ml of a scintillation solution (4-6 g of 2,5-diphenyloxazole plus 50-75 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene per liter of toluene). The mean recovery and standard deviation of ^{14}C from triplicate 150-mg portions of filter paper treated with 4-[^{14}C]aminopyridine and combusted was 94.7 ± 0.8%.

Aqueous nutrient solutions were prepared for analysis by adding 1 ml to a scintillation vial containing 10 or 15 ml of either a modified Bray solution (Bray, 1960) or a commercial emulsifier fluor (Insta-Gel, Packard Instrument Co., Inc.).

Methanol extracts of plant tissues were prepared for analysis by adding 1 ml to a counting vial containing 10 ml of Insta-Gel fluor.

Radioactivity was determined with either a Nuclear-Chicago Model 701A ambient counter and 181D scaler or a Beckman LS-150 system. Counting efficiency correction was determined using internal standardization or channels-ratio quench correction.

Autoradiographic Analysis. Plant tissues, frozen with Dry Ice immediately upon harvest, were freeze-dried, and then mounted on filter paper, pressed, and exposed to Kodak No-Screen X-ray film for varying periods.

Culture of Plants. Seedlings of corn (*Zea mays*, Pioneer 3956 Hybrid) and sorghum (*Sorghum vulgare*, Northrup King Mini Milo 50A Hybrid) were grown in modified Hoagland No. 2 solutions containing 2-8 ppm of iron supplied as the EDTA chelate (Hoagland and Arnon, 1938). The 1- and 2-month-old corn and sorghum plants were contained and aerated in foil-covered pint or gallon jars in a growth chamber (Model CEL 37-14, Sherer-Gillette Co.) under a 12-hr photoperiod with a light intensity of approximately 26,900 lx. The temperature was maintained at 27° during the day and at 21° at night. The relative humidity was about 40%. Plants older than 2 months were maintained under greenhouse conditions.

Design of Studies. *Phytotoxicity Studies.* Corn seedlings were grown, three per culture, in untreated nutrient solutions for 4 weeks and then transferred to nutrient solutions containing 0, 0.1, 10, or 100 ppm of unlabeled 4-aminopyridine. There were five three-plant cultures (replications) per treatment, except for the 100-ppm treatment where four cultures were used. After 1 week, the plants were harvested and sectioned and the fresh shoot weights used as a measure of phytotoxic response.

Table I. Fresh Shoot Weights of 1-Month-Old Corn Seedlings Grown 1 Week in Solutions Containing 4-Aminopyridine

Concn, ppm	No. of plants	Mean wt of shoots and std dev, g
Control	15	12.11 ± 1.88
0.1	15	11.67 ± 2.99
1.0	15	13.00 ± 3.52
10.0	15	12.75 ± 2.79
100.0	12	12.58 ± 2.64

Ninety sorghum seedlings were grown also, three per culture, in untreated solutions for 4 weeks and then transferred to solutions containing 0, 10, or 100 ppm of 4-aminopyridine. After 1 week, one group of 36 plants was harvested. To evaluate possible latent toxic effects of the chemical to sorghum, two additional groups of 27 plants each were removed from the treated cultures after 1 week and transferred to untreated solutions for additional periods of 1 or 2 weeks, and then harvested. At harvest, all plants were sectioned into shoots and roots; fresh weights were recorded for the shoots, and both shoots and roots were air-dried and weighed.

Nutrient Culture Translocation Studies. One-Month-Old Corn. Twenty-four corn seedlings were grown in untreated nutrient solutions. After 4 weeks, 18 were transferred (three per culture) to solutions containing 10 ppm (3.2 μCi) of 4-[^{14}C]aminopyridine, and the remaining six were maintained as controls. The solutions were sampled at 1, 3, 5, and 7 days after transfer and analyzed for radioactivity by liquid scintillation spectrometry. After 7 days, all plants were harvested, sectioned, rinsed with water and acetone, frozen, and retained for analysis by Schöniger combustion-liquid scintillation spectrometry (six treated plus two controls) or autoradiography (three treated and a control). The tissues were rinsed with water and acetone to reduce the possibility of ^{14}C surface contamination resulting from volatilization of the parent chemical and/or radiolabeled metabolites from the nutrient solutions. The remaining nine treated and three controls were retained for a later degradation study using tlc-autoradiography (Starr and Cunningham, 1974).

Two-Month-Old Corn. Corn plants were grown in nutrient solutions until about 2 months of age; three of the more uniform plants, which were forming tassels at this stage of growth, were selected and transferred singly to nutrient solutions containing about 10 ppm (6.1 μCi) of 4-[^{14}C]aminopyridine. After 7 days, the three treated plants and a control were harvested, rinsed with water and acetone, sectioned into approximate 12-in. lengths, frozen, and retained for liquid scintillation analysis.

Three-Month-Old Corn. Two plants bearing small ears were grown in nutrient solutions for 3 weeks; one was then transferred to a nutrient solution containing 5 ppm (25.5 μCi) of 4-[^{14}C]aminopyridine and the other was retained as a control. After 7 days, the plants were harvested and sectioned, and all tissues (including the ears with silk and husks removed) were rinsed with water and acetone, frozen, and retained for liquid scintillation analysis.

One-Month-Old Sorghum. Thirty-six sorghum seedlings were grown in nutrient cultures for 1 week; 30 of these plants were then transferred (three per culture) to solutions containing 5 ppm (2.4 μCi) of 4-[^{14}C]aminopyridine, and the other six were kept as controls. The treated plants were divided into five groups of six plants each. One group each was harvested at 1 hr, 1 day, or 7 days. The remaining two groups were transferred to untreated solutions, grown an additional 1 or 7 days, and then harvested. At harvest, each group of six plants was sectioned, rinsed with water and acetone, frozen, and retained for analysis

Table II. Fresh and Dry Weights of 1-Month-Old Sorghum Plants Grown 1 Week in Cultures Containing 4-Aminopyridine

Concn, ppm	No. of plants per concn	Add. time grown in untreated solutions, weeks	Mean wt of tissues and std dev, g: Shoots Fresh	Shoots Dry	Roots dry
0	12	0	3.38 ± 1.29	0.40 ± 0.15	0.67 ± 0.23
10			3.39 ± 1.21	0.54 ± 0.14	0.78 ± 0.18
100			3.97 ± 1.12	0.54 ± 0.14	0.94 ± 0.10
0	9	1	6.01 ± 1.52	0.97 ± 0.38	1.35 ± 0.14
10			6.04 ± 1.31	1.13 ± 0.30	1.41 ± 0.23
100			7.77 ± 2.57	1.01 ± 0.40	1.42 ± 0.22
0	9	2	14.01 ± 3.94	1.72 ± 0.59	2.68 ± 0.65
10			16.03 ± 4.83	1.74 ± 0.68	2.19 ± 0.69
100			15.33 ± 6.86	1.74 ± 0.83	2.31 ± 0.70

Table III. Distribution of Radioactivity in 1-Month-Old Corn Tissues and Nutrient Solutions after Plants Grown 1 Week in Triple-Plant Cultures Containing 10 ppm of 4-[^{14}C]Aminopyridine

Sample	Mean[a] radioact.: dpm per plant (×10^4)	ppm[b] per plant	% init. application: Per plant	Per culture
Plants				
Shoots	10.1	1.43	1.4	4.1
Roots	144.0	84.63	19.4	58.2
Rinses of roots and culture jars				20.9
Nutrient solutions				4.6
Total recovery				87.8

[a] Mean values for six plants (two cultures). [b] Calculations based on fresh weight and all radioactivity assumed present as parent compound.

Table IV. Distribution of Radioactivity in 1-Month-Old Sorghum Plants Grown in Triple-Plant Cultures Containing 5 ppm of 4-[^{14}C]Aminopyridine

Time grown in treated solutions	Add. time grown in untreated solutions	Tissue	Mean radioact.: dpm[a]/section (×10^4)	ppm[b]/section	% init. application
1 hr	0	Shoots	1.2	4.21	0.7
		Roots	2.9	11.29	1.8
1 day	0	Shoots	0.4	1.00	0.2
		Roots	6.2	23.73	3.6
7 days	0	Shoots	2.3	1.77	1.4
		Roots	69.0	54.87	42.9
7 days	1 day	Shoots	3.0	1.73	1.9
		Roots	64.3	36.42	39.5
7 days	7 days	Shoots	3.3	1.37	1.9
		Roots	27.7	16.33	15.6

[a] Mean values for three plants (one culture). [b] Calculations based on fresh weight and all radioactivity assumed present as parent compound.

by Schöniger combustion-liquid scintillation spectrometry (three plants) or autoradiography (one plant), or retained frozen (two plants) for the degradation study (Starr and Cunningham, 1974).

Three-Month-Old Sorghum. Six 3-month-old plants, with heads in various stages of development, were transferred (two per culture) to nutrient solutions containing 5 ppm of 4-[^{14}C]aminopyridine (24 μCi); two plants of similar development were kept as controls. The solutions in the four cultures were sampled at 0 and 7 days and analyzed for radioactivity by liquid scintillation spectrometry. At 7 days, all plants were sectioned about 1 in. above the culture jars and the tissues rinsed with water and acetone. The heads were removed intact, the stalks, including leaves, were cut into about 8-in. lengths, and all tissues were frozen. For analysis, the heads and stalk sections were blended with 80% methanol and filtered under vacuum. The extracts and the extracted tissues, after air-drying and combustion, were analyzed by liquid scintillation spectrometry.

In an additional experiment, eight 3-month-old plants (two per culture) were transferred to solutions containing 5 ppm (24 μCi) of 4-[^{14}C]aminopyridine; two additional plants were retained as controls. After 7 days the plants were harvested; the heads were removed, the seeds stripped off, and the seeds and stripped heads (rachis plus branches and racemes) were rinsed with water and acetone, and retained for analysis by liquid scintillation spectrometry. The seeds were air-dried and combusted in triplicate 100-mg samples. The fresh stripped heads were blended in 80% methanol and filtered under vacuum, and these extracts analyzed. The residual tissues were not analyzed, since the first experiment with sorghum heads showed satisfactory removal of radioactivity of methanol extraction (radioactivity in treated tissues was only slightly greater than that in controls).

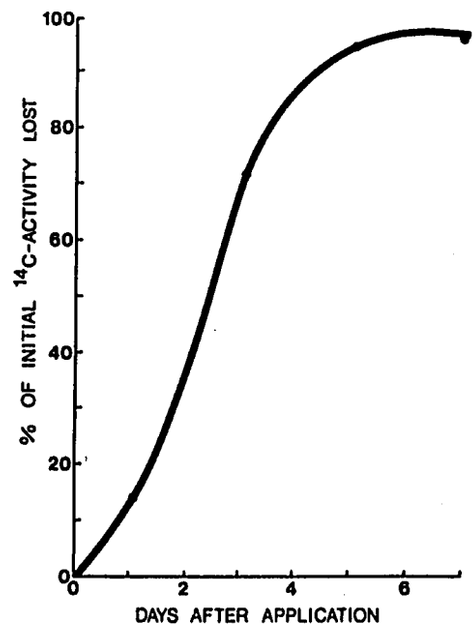

Figure 1. Rate of loss of radioactivity from 4-[^{14}C]aminopyridine-treated cultures containing 1-month-old corn plants.

Table V. Distribution of Radioactivity in 2- and 3-Month-Old Corn Plants Grown 1 Week in Single-Plant Cultures Containing 4-[^{14}C]Aminopyridine

Concn in growth medium, ppm	Plant age, months	Plant section	Radioact. dpm/section (×10³)	Radioact. ppm[a]/section	Radioact. % init. application
10	2[b]	Stalk plus leaves (lower 12 in.)	646.8	4.33	4.93
		Stalk plus leaves (upper 12 in.)	5.3	0.03	0.04
		Stalk section			
		1 (lower 12 in.)	293.0	1.40	0.52
		2 (2nd 12 in.)	21.2	0.07	0.04
		3 (3rd 12 in.)	3.1	<0.01	<0.01
		4 (4th 12 in.)	ND[d]		
		5 (5th 12 in.)	ND		
		Ear	ND		

[a] Calculations based on fresh weight and all radioactivity assumed present as parent compound. [b] Mean value of three plants. [c] One plant. [d] Not detected.

Table VI. Distribution of Radioactivity in 3-Month-Old Sorghum Plants Grown 1 Week in Single-Plant Cultures Containing 5 ppm of 4-[^{14}C]Aminopyridine

Plant section	Radioact. dpm[a]/section (×10⁴)	Radioact. ppm[b]/section	Radioact. % init. application
Stalk plus leaves (lower 8 in.)	58.89	5.06	1.11
Stalk plus leaves (upper 8 in.)	0.44	0.04	0.01
Uppermost internode of stalk	0.01	<0.01	<0.01
Head (intact)	0.72	0.05	0.01
Head			
Seeds	0.34[c]	0.05	0.01
Stripped head	0.04[c]	0.02	<0.01

[a] Mean values for six plants unless otherwise indicated. [b] Calculations based on fresh weight and all radioactivity assumed present as parent chemical. [c] Mean values for eight heads.

Soil Translocation Studies. One-Month-Old Corn. Four representative soils were used (for composition, see Table VII) to evaluate the effects of soil composition on the availability of labeled 4-aminopyridine for absorption and upward translocation into young, vigorously growing plants.

For each soil type, four modified foil-covered chromatographic columns (15 cm long, 4 cm diameter) were each filled with 160 g of air-dried, screened soil. Single 10-day-old corn seedlings were transferred to the columns and grown 10 days in a growth chamber under the same climatic conditions as used in the nutrient culture translocation studies with 1-month-old corn. The surface of the soil in three columns in each group was then treated uniformly with 1 ml of a methanol solution containing 2.5 μCi of 4-[^{14}C]aminopyridine. This treatment resulted in a concentration of about 10 ppm, based on uniform distribution. Control columns were each treated with 1 ml of methanol. The columns were kept in the growth chamber for 8 additional days, and each soil was watered every 1 or 2 days with enough nutrient solution or water to produce a runoff of about 10 ml. Plants were then sectioned about 1 in. above the soil surface, rinsed with water and acetone, air-dried, macerated, combusted, and analyzed for radioactivity by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Phytotoxicity Studies. One-month-old corn and sorghum plants cultured for 7 days in nutrient solutions containing 0.1–100 ppm of 4-aminopyridine maintained a healthy appearance. This result, together with the slightly increased fresh and dry tissue weights of the treated plants (Tables I and II) and an increase in the volume of water transpired, suggests that the chemical may have stimulated growth. There was, however, too much variation in the tissue weights among individual seedlings for the apparent increase to be statistically significant.

Nutrient Culture Translocation Studies. To determine nonplant losses of radioactivity, 0- and 7-day samples were taken of aerated nutrient and water solutions treated with 10 ppm of 4-[^{14}C]aminopyridine and maintained without plants. Liquid scintillation analysis showed that about 15% of the loss of radioactivity at 7 days could be attributed to nonplant factors such as volatilization and/or microbial degradation.

Young Plants. One-month-old corn plants grown in solutions treated with 10 ppm of 4-[^{14}C]aminopyridine had absorbed 19.4% of the radioactivity by 7 days and translo-

Table VII. Radioactivity in 1-Month-Old Corn Plants Grown 10 Days in Alkaline Soils Treated with 10 ppm of 4-[^{14}C]Aminopyridine

Soil type	Soil composition[a] pH (paste)	Organic matter, %	Sand, %	Silt, %	Clay, %	Mean[b] radioact. dpm (×10³)	Mean[b] radioact. ppm[c]	Mean[b] radioact. % init. application
Loam	7.6	2.5	59	30	11	1.15	0.06	0.02
Loam	7.7	4.0	61	19	20	1.49	0.06	0.03
Sandy clay loam	7.6	1.9	47	27	26	4.76	0.21	0.09
Loamy sand	7.8	2.9	77	16	7	14.76	0.66	0.28

[a] Soils analyzed by Soils Testing Laboratory, Colorado State University, Ft. Collins, Colo. [b] Mean values for three plants per soil type. [c] Calculations based on fresh weight and all radioactivity assumed present as parent chemical.

cated 1.4% of it to the shoots (Table III). During the same period, radioactivity in the nutrient solutions in which these plants were grown decreased from an average of 86.2% on day 1 to 4.6% on day 7 (Figure 1). Autoradiograms of treated plants showed that most of the radioactivity was concentrated in the roots, lower stem, and leaf sections nearest the stalk, but that some activity was present throughout the plant, including the meristematic tissues.

One-month-old sorghum growing in solutions treated with 5 ppm of the compound (Table IV) absorbed 1.8% of the radioactivity and translocated 0.7% of it to the shoots within 1 hr; after 7 days in these treated solutions, the roots contained 42.9% and 1.4% had been translocated to the shoots. When roots of 7-day-treated plants were placed in untreated nutrient solutions, radioactivity leached from the roots to the cultures, indicating that much of the radiolabeled material was present in a free form (Table IV). Autoradiograms of treated plants showed that radioactivity was present in the stems 1 hr after treatment, but only the 7- and 14-day plants showed ^{14}C in the leaf blades, concentrated along the center veins.

Older Plants. In 2- and 3-month-old corn plants grown 7 days in treated cultures, most of the translocated chemical was in the lower 12 in. of each plant (Table V). There was no evidence of radioactivity in the husks, silk, or ear of a 3-month-old plant, and detectable ^{14}C activity in the vegetative portion was less than in the 2-month-old plants.

In 3-month-old sorghum (Table VI), the pattern of radioactivity in the vegetative portions was similar to that in corn; most was concentrated in the lower sections and only trace amounts appeared in the uppermost internodes. However, unlike the corn ear, both the seeds and the stripped heads (rachis, branches, and racemes) contained detectable radioactivity (Table VI). Radioactivity in the heads varied widely (range <0.01–0.08 ppm) and apparently was related to vigor of growth as evidenced by fresh head weights. Separate analyses of seeds and stripped heads also showed considerable variation; radioactivity in the seeds ranged from 38 to 100% of that in the total head.

Soil Translocation Studies. The effect of soil composition on the availability of 4-[^{14}C]aminopyridine for absorption by 1-month-old corn plants is shown in Table VII. Although composition had an effect on the amount of chemical available for absorption, the total amount actually absorbed by the plants from any of the four soils was very small (maximum, 0.28% of the 10 ppm applied).

CONCLUSIONS

The nutrient culture studies showed that 4-[^{14}C]aminopyridine was readily absorbed by roots of corn and sorghum plants but that the quantity of radioactivity translocated into the upper vegetative tissues appeared to be inversely proportional to plant age. Small quantities of radioactivity were also detected in seeds of 3-month-old sorghum grown 7 days in treated cultures. However, shoots of young corn grown in soil treated with 4-[^{14}C]aminopyridine contained only small quantities of radioactivity, illustrating the tenacity with which 4-aminopyridine and/or metabolites are adsorbed onto soil colloids. Thus, it is unlikely that detectable quantities of the chemical would be present in plants grown under field conditions because of soil absorption of chemical and the extremely low application rates used.

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