eral detoxication system varies in importance with the age and sex of the bees apparently resulting in significantly different carbaryl persistence which, in turn, leads to clear differences in carbaryl toxicity.

N-Hydroxycarbaryl was identified with R_f values in a two-dimensional thin-layer chromatography system. Locke (1972) has shown *N*-hydroxycarbaryl could be confused with carbaryl in one direction (ether–hexane) and with 5-hydroxycarbaryl in the other (methylene chloride–acetonitrile). Until spectral and synthetic confirmation is obtained, therefore, the finding of *N*-hydroxycarbaryl should be viewed with caution.

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Degradation of 4-Aminopyridine-^{14}C in Corn and Sorghum Plants

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Acetone-soluble radiolabeled metabolites, as determined by thin-layer chromatographic procedures, were not detected in roots and shoots of young corn plants (*Zea mays*) cultured 7 days in nutrient solutions containing 10 ppm of 4-aminopyridine-^{14}C. Autoradiograms of thin-layer plates containing acetone extracts of young sorghum plants (*Sorghum vulgare*), 2 weeks after treatment, showed some degradation of the labeled compound, with three similar major metabolites detected in both roots and shoots. The majority of ^{14}C extracted from these tissues was suggested to be present as the parent chemical, or possibly as 4-aminopyridine released during hydrolysis of sugar and/or amino acid conjugates. Less radioactivity was extracted from sorghum tissues maintained 7–14 days in nutrient solutions than from those kept in cultures 1 hr to 7 days, suggesting that some of the radiolabeled parent compound was bound or incorporated into the tissues of those plants cultured the longer periods. Although these data indicate that additional studies are necessary, the work suggests that 4-aminopyridine absorbed and translocated by corn and sorghum plants is not degraded in appreciable quantities to extractable, nonconjugated products, potentially more toxic than the parent chemical.

The avian frightening agent, 4-aminopyridine, has proved effective for reducing blackbird damage to ripening corn (De Grazio *et al.*, 1971); 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. Previous studies with ^{14}C-labeled 4-aminopyridine, as described in a recent paper by Starr and Cunningham (1974), have shown that corn and sorghum plants absorb and translocate the chemical and/or its metabolites from treated nutrient solutions, that the degree of uptake is dependent upon plant age, and that the majority of the radioactivity is present in the roots and lower vegetative shoot tissues of the plants.

When an organic compound is translocated to storage areas within a plant, it may be subjected to degradation processes that can detoxify the material or, in some instances, increase its toxicity (Mitchell *et al.*, 1960). This study was conducted to evaluate the extent of breakdown of absorbed and translocated 4-aminopyridine-^{14}C in young corn and sorghum plants grown for short periods in treated nutrient cultures.

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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-aminopyridine-^{14}C was purchased from the International Chemical and Nuclear Corporation. The sample was recrystallized with unlabeled 4-aminopyridine before use and was determined to be sufficiently radiopure 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.

Culture and Processing of Plants. Test plants were seedlings of corn (*Zea mays,* Pioneer 3956 hybrid) and sorghum (*Sorghum vulgare,* Northrup King mini milo 50A hybrid) retained from the earlier translocation study (Starr and Cunningham, 1974). The seedlings were maintained in foil-covered pint jars containing modified Hoag-

Figure 1. Autoradiograph of cellulose tlc plate containing purified acetone extracts of 1-month sorghum plants grown in 5 ppm of radiolabeled 4-aminopyridine for varying periods. Sample applications containing 1×10^{-3} to 7×10^{-3} μCi per spot (left to right): shoots; 4-aminopyridine-^{14}C standard; roots; 4-aminopyridine-^{14}C standard.

Table I. Radiolabeled Degradation Products Detected in Sorghum Shoot and Root Extracts of Plants Grown Varying Periods in Solutions Containing 4-Aminopyridine-^{14}C

Radiolabeled component	R_f	Mean % of radioact. recovd from two cellulose tlc plates	
		Shoots	Roots
1	0.85 (parent compd)	93.3	90.0
2	0.68	3.6	4.4
3	0.58	0.7	1.1
4	0.26	0.7	0.5
Miscell. minor components occurring between spots 1 + 2 and 3 + 4		1.7	4.0

land No. 2 solutions with 2–8 ppm of iron supplied as the EDTA chelate (Hoagland and Arnon, 1938). These plant cultures were held in a growth chamber (Sherer-Gillette Co. Model CEL 37-14) with a 12-hr photoperiod, light intensity of about 26,900 lx, day temperature of 27°, night temperature of 21°, and relative humidity of about 40%.

Twelve 1-month-old corn plants were analyzed (nine treated and three controls). The treated plants were grown in nutrient cultures containing 10 ppm (3.2 μCi) of 4-aminopyridine-^{14}C for 7 days, then harvested, sectioned into roots and shoots, rinsed with water and acetone, and retained frozen for analysis. The controls were grown in untreated cultures for 7 days and processed similarly.

Twelve 1-month-old sorghum plants were analyzed (ten treated and two controls). The treated plants were cultured in nutrient solutions containing 5 ppm (2.4 μCi) of 4-aminopyridine-^{14}C. Two plants each were harvested 1 hr, 1 day, and 7 days after transfer to treated cultures. At 7 days, the remaining plants were transferred to untreated nutrient solutions; two were harvested after 1 day and two after 7 days. At harvest, plants were sectioned into roots and shoots, rinsed with water and acetone, frozen, and retained for analysis. Two control plants, maintained in untreated cultures for 14 days, were harvested and processed similarly.

Tlc–Autoradiographic Analysis. The 4-aminopyridine was separated on commercial thin-layer plates (Eastman Kodak Co.) containing cellulose or alumina adsorbents with a fluorescent indicator. Two mobile phase systems were used. System 1 was an 80:16:4 (v/v/v) mixture of isopropyl alcohol, distilled water, and ammonium hydroxide (about 29% ammonia) for use with cellulose plates; this was a modification of previously described systems (Miller and Hall, 1961; International Chemical and Nuclear Corp., 1968). System 2 was a mixture of acetone and ammonium hydroxide (97:3, v/v) that was later developed by Hazleton Laboratory personnel (unpublished report, 1970) for use with alumina plates.

The solvent front was allowed to ascend 10 to 15 cm up the plates at a temperature of 25–26°. The minimum detectable level (uv, 254 nm) for 4-aminopyridine was about 100 ng; the best resolution of 4-aminopyridine-^{14}C and degradation products was obtained with the cellulose plates and system 1. Rate of flow values of 0.85 and 0.67 were obtained for 4-aminopyridine on cellulose and alumina plates, respectively.

Liquid Scintillation Analysis. Acetone extracts of plant tissues were analyzed for ^{14}C activity by adding 1 ml to a counting vial containing 15 ml of a commercial emulsifier-fluor (Insta-Gel, Packard Instrument Co., Inc.).

To determine efficiency of the acetone extraction technique, ^{14}C was also determined in the corn and sorghum tissue residues remaining after extraction. These residues were air-dried and oxidized by Schöniger oxygen flask combustion. Between 50 and 100 mg of each sample was combusted in duplicate, and the combustion products absorbed in 15 ml of a solution containing 12% (v/v) of scintillation grade ethanolamine in methanol. A 5-ml aliquot of this solution was withdrawn and added to a vial containing 15 ml of a scintillation solution (6 g of PPO plus 75 mg of POPOP per liter of toluene), and the radioactivity was determined.

Carbon-14 activity contained on tlc adsorbents was determined by removing about 1-cm^2 portions and counting these samples in 10-ml volumes of a thixotropic gel preparation containing 4% Cab-O-Sil in Insta-Gel fluor (Snyder and Stephens, 1962; Snyder, 1964).

Radioactivity was determined with a Beckman LS-150 liquid scintillation system. Counting efficiency correction was determined with internal standardization or channels-ratio quench correction.

Corn Studies. Corn tissues for tlc–autoradiography were extracted by blending with acetone; the extracts were filtered and concentrated under vacuum (40–45° water bath temperature), and the pigments were removed by the method of Peterson (1971). These concentrates were each applied to cellulose and alumina tlc plates and developed in the solvent systems, and the plates were then exposed to Kodak No-Screen X-ray film for 9–33 days.

Sorghum Studies. Because of the low specific activity of the radiolabeled compound available for use (0.15 mCi/mmol), two composite samples were prepared for the shoots; this involved combining the 1-hr, 1- and 7-day, and the 8- and 14-day tissues. A similar procedure was followed for the roots. These four composites were then blended in acetone and filtered, and the solutions evaporated under vacuum (40–45° water bath temperature) and nitrogen.

To recognize possible losses of radiolabeled products that occurred during cleanup, aliquots of the crude concentrated extracts, together with 4-aminopyridine-^{14}C standards and 4-aminopyridine-^{14}C spiked control samples, were each applied to cellulose and alumina tlc plates and developed, and autoradiograms were prepared from the plates by exposing them to Kodak No-Screen X-ray film for 7 weeks.

The crude shoot and root extracts were then each combined into a single solution, one for each time period (1 hr to 7 days and 8 to 14 days); the pigments were removed by the liquid–liquid partition method described by Peterson (1971); these purified extracts were evaporated to just dryness on a water bath (45–50°) under nitrogen. The residues were redissolved in a mixture of acetone and methanol (1:1). The purified solutions and 4-aminopyridine-^{14}C standards were each applied to cellulose and alumina tlc plates and processed as described for the corn samples. After development of autoradiograms of those plates containing the purified extracts, the areas on the developed plates containing ^{14}C were removed by scraping for counting by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Developed autoradiograms of both the cellulose and alumina tlc plates containing purified corn extracts revealed only single spots for the shoot and root applications, with R_f values the same as those of the 4-aminopyridine-^{14}C standards and spiked samples. Analysis of the acetone extracts and tissue residues showed that the extraction procedure removed 83.5–95.0% of the ^{14}C activity.

Autoradiograms prepared from crude sorghum extracts showed considerable tailing but suggested that 4-aminopyridine-^{14}C was present in both shoots and roots. Analysis of the crude acetone extracts and tissue residues revealed some difference with time in the amount of acetone-extractable ^{14}C present. Extraction of root tissues removed about the same amount of radioactivity from the earlier as from the later samples (87.5% for the 1-hr, 1-day, and 7-day composite *vs.* 90% for the 8- and 14-day composite) but removed more ^{14}C from the earlier sample of shoot tissues (99.5% for the 1-hr, 1-day, and 7-day composite *vs.* 80.7% for the 8- and 14-day composite). This suggests that some of the radiolabeled parent compound, or derived radioactivity of a polar character, was bound or incorporated in the tissues of those plants maintained in the nutrient solutions for the longer periods.

An autoradiogram prepared from the purified sorghum extracts (Figure 1) showed degradation of the translocated 4-aminopyridine within 2 weeks, with three similar major metabolites in both the shoot and root tissues (Figure 1, Table I); about 90–93% of the extractable radioactivity was suggested to be present as the parent chemical, or possibly as 4-aminopyridine resulting from the hydrolysis (during the cleanup procedure) of sugar and/or amino acid conjugates.

These preliminary data suggest that 4-aminopyridine absorbed and translocated by corn and sorghum plants 1–2 weeks following treatment is not degraded in appreciable quantities to acetone-extractable, nonconjugated products potentially more toxic than the parent chemical. However, this study indicates that additional work is warranted regarding possible conjugate formation of 4-aminopyridine with plant sugars and/or amino acids.

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Pathway of Nitro Reduction of Parathion by Spinach Homogenate

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The nitro reduction of parathion to aminoparathion by spinach homogenate under anaerobic conditions proceeds *via* hydroxylaminoparathion as intermediate metabolite. Hydroxylaminoparathion was stable in the acidified reaction medium and therefore detectable by thin-layer chromatography. However, in the alkaline medium, it underwent autoxidation to nitrosoparathion, which was detectable by both thin-layer chromatography and gas chromatography. Nitrosoparathion itself was not demonstrated as an intermediate metabolite, but the one added to the subject reaction system was easily reduced to aminoparathion *via* hydroxylaminoparathion. It was also converted rapidly to hydroxylaminoparathion by NADPH. These results suggest the possibility that nitrosoparathion may act as one of two consecutive intermediate metabolites.

Our previous study demonstrated nitro reduction of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) or reduction of the nitro group of the organophosphorus insecticide to the amino group, in the presence of spinach homogenate containing NADP, G-6-P, and FAD under anaerobic conditions, whereby the gas chromatogram revealed a peak suggestive of an intermediate metabolite (Suzuki and Uchiyama, 1974). The investigation reported herein was undertaken to isolate and identify the intermediate metabolite in the nitro reduction of parathion. The report also describes the findings noted for the pathway of nitro reduction of parathion by spinach homogenate.

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EXPERIMENTAL SECTION

Preparation of Spinach Homogenate and Reaction