Absorption of Monofluoroacetate-2-^{14}C Ion and Its Translocation in Sugarcane

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Between 5 and 10% of the radioactivity absorbed from ammonium monofluoroacetate-2-^{14}C solutions applied to leaf axils and to nutrient solution of growing sugarcane plants was translocated upward to the leaves; the remainder was adsorbed at the application site in the leaf axil or on the roots. Total radioactivity in plants grown in treated nutrient culture decreased 12-fold over a 3-month period, mainly by desorption (with or without metabolic decomposition of the compound) from the roots. The unabsorbed radioactivity vaporized from the nutrient solution within 30 days. Maximum transport within the plants occurred to the rapidly expanding spindle and newer leaf laminar tissue, not to the meristematic region or the stalk.

Monofluoroacetic acid, FCH_2COOH, is a constituent of certain poisonous species of the *Dichapetalum* and other families (Marais, 1944; Peters, 1952, 1953, 1954; Peters and Shorthouse, 1964). Poisoning of animals feeding on these plants appears to result not from the ingested fluoroacetate ion, but from the more toxic monofluorocitric acid metabolically derived from fluoroacetic acid. The highly toxic acute reaction in animals led to commercial production of sodium monofluoroacetate (Compound 1080) and monofluoroacetamide (Compound 1081) as poisons for rats and other undesirable wildlife. Although extremely toxic to warm-blooded animals (LD_{50} values range from 5 mg. per kg. for the rat, 0.06 to 0.2 for dogs and cats, to 4 to 15 for primates) and therefore a considerable potential hazard to man, domestic animals, and desirable wildlife, sodium monofluoroacetate is often suitable and preferred for use (under controlled conditions) in more remote areas. In such areas it has potential for protection of desirable ranch or range animals from predators, and some agricultural crops from invasions of rats and mice.

In wasteland borders of sugarcane fields in Hawaii, sodium monofluoroacetate has been applied experimentally, as a component at 0.5% by weight of a grain bait, and at a rate of 8 to 10 pounds of bait per acre. In Hawaii three species of rats—*Rattus norvegicus*, *R. rattus*, and *R. exülans*—cause considerable damage and crop loss in sugarcane by gnawing mature stalks. The primary chewing may be extensive, but the major loss occurs as secondary souring and rotting from the invasion of disease organisms and insects. A suitable bait, distributed in a safe manner near crop fields by aircraft or ground unit, offers a possible means for controlling field rodents.

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In connection with these experimental applications, we studied the ability of the monofluoroacetate ion to penetrate and translocate in sugarcane foliage from application in the leaf axils, and the uptake of monofluoroacetate through sugarcane roots and its distribution in the plant. The movement and persistence of radioactivity supplied by ammonium monofluoroacetate-2-^{14}C are described in this paper. No attempt was made to study the metabolic fate of the compound.

Monofluoroacetic acid is a relatively volatile organic acid of boiling point 165° C. The free acid vaporizes from exposed nonadsorptive surfaces, but shows a high degree of adsorption to organic matter in soils and to cellulose and plant surfaces.

Comparatively little is known of the behavior of monofluoroacetic acid or its salts or amide in either plants or soils. Ramsey and Clifford (1949) stated that Compound 1080 probably remains unchanged when added to plant tissues and foods, but they cited no data and, in any case, were concerned with foods in warehouse storage.

Horiuchi studied monofluoroacetamide ("fussol" or Compound 1081) effects on mice (Horiuchi and Kumasawa, 1956), insecticidal sprays on tea and citrus foliage (Horiuchi, 1955, 1957, 1959), aqueous culture solutions absorbed by roots of cotton plants (Horiuchi and Yoshimura, 1958), and incorporation in soil (Horiuchi, 1960). He reported that spray residues disappeared from citrus leaves (orange) within 14 days; slight amounts of foliar organic fluorine were found in tea leaves at 28 days, but there was no increase in inorganic fluoride. While the disappearance of the applied compound from leaves was presumably due either to volatilization or to excretion of absorbed chemical from the roots, monofluoroacetamide absorbed from nutrient solution by cotton roots accumulated rapidly in the leaves to about 5% of the total applied, then decreased in the leaves after 7 days, while concentration in the roots reached a maximum. Organic fluorine was excreted from the roots until it approached control

levels, but this fluorine was not in the form of fluorine-containing organic acids; total fluorine in the culture solution decreased rapidly, possibly by volatilization of the amide, the free acid after hydrolysis, or the excreted metabolic product. Horiuchi proposed fluoromethane as a possible plant metabolite, and presented evidence that the C—F bond was not broken to release inorganic fluoride. Soil microorganisms, probably gram-negative bacteria, broke the C—F bond to form inorganic fluoride.

There appears to be no evidence for the formation of monofluorocitric acid in plants. Baker's yeast formed fluorofatty acids, probably butyric and hexanoic, but no fluorocitrate (Aldous, 1963).

Several authors have reviewed the effects of inorganic fluoride on plants and soils (Bartholomew, 1935; Bollard and Butler, 1966; Lundegårdh, 1966), and the amount of available F^- in soil for uptake by the bean plant has been measured (Applegate *et al.*, 1960).

EXPERIMENTAL

Foliar Absorption. Four-month-old sugarcane plants of variety H 49-5 were treated with 0.5 ml. of ammonium monofluoroacetate-2-^{14}C solution, supplying 50 μg. of F or 250 μg. of the salt. (The solution was obtained from International Chemical and Nuclear Corp. as a concentrated aqueous solution of 5 mc. per mmole.) The solution was applied to the leaf axil (dewlap) of leaf 3 (counting from the top); excess liquid ran into the adjacent dewlap area of leaves 4 and 5. Laminar tissue samples 0.5 inch wide by 4 inches long, beginning one fourth of the distance from the leaf tip to the axil, were cut periodically for radioactive assay. These samples, and portions of the entire plant taken at the end of a 1-month period, provided the data in Tables I and II. All counts were made with a GM gas flow counter.

Root Absorption. Ammonium monofluoroacetate-2-^{14}C was added to an aerated standard nutrient solution containing eighteen 4-month-old sugarcane plants, variety H 49-5, to provide a total of 1000 μg. of F (5000 μg. of the salt). An additional 26,400 μg. of unlabeled sodium monofluoroacetate (5000 μg. of F) were added to the nutrient medium. The plants remained in the radioactive solutions for 35 days, with water added as necessary. They were then placed in fresh nutrient without radioactivity until completion of the 3-month experiment. Control plants were grown in aerated nutrient solution. Radioactive assay of the culture solutions with time is shown in Table III; sugarcane samples taken at 0.5, 1, 1.5, and 3 months were counted as separate chopped dried portions of leaf, stalk, and root. These data are shown in Figures 1, 2, and 3.

RESULTS AND DISCUSSION

Foliar Application. Sugarcane leaves absorbed monofluoroacetate-2-^{14}C ion and translocated a small propor-

Table I. Radioactivity of Sugarcane Leaf Lamina and Sheath Sections after Applications of Ammonium Monofluoroacetate-2-^{14}C to Axils of Leaves 3, 4, and 5

Interval, Days	Original Leaf No.	Final Leaf No.	Radioactivity, Net C.P.M./G. Dry Weight	
			Lamina	Sheath
2	3	3	636	...
10	3	4	2,071	...
16	Spindle	1	6,734	...
30	...	Spindle	3,407	...
30	...	1	2,738	647
30	Spindle	2	11,316	798
30	1	3	1,012	1,960
30	2	4	3,146	18,608
30	3	5	556	40,935
30	4	6	436	33,243
30	5	7	1,310	22,213

Table II. Radioactivity of Chopped Dried Portions of Sugarcane Plants, One Month after Application of Ammonium Monofluoroacetate-2-^{14}C to Axils of Leaves 3, 4, and 5

Plant Part	Radioactivity, Net C.P.M./G. Dry Weight
Spindle	2402
Leaf 1	
Total laminar tissue	2596
Midrib	1036
Sheath	647
Leaf 2 (original spindle)	
Total laminar tissue	3931
Midrib	2539
Sheath	798
Growing point	1484
Upper stalk[a]	3422
Lower stalk[a]	1267
Bagasse fiber from upper stalk	3385
Bagasse fiber from lower stalk	1379
Juice from upper stalk	5036[b]
Juice from lower stalk	1912[b]
Secondary shoots (suckers)	
Secondary A (26 g. fresh wt.)	
Lamina, leaf 1	737
Midrib, leaf 1	444
Sheath, leaf 1	Trace
Growing point plus stalk	167
Secondary B (1.4 g. fresh wt.), entire plant	4279

[a] Upper stalk portion contains green leaves, lower stalk contains dried senescent leaves.
[b] Net c.p.m./ml. of expressed juice reduced to dryness.

Table III. Radioactivity from Ammonium Monofluoroacetate-2-^{14}C Added to Nutrient Solution Containing Sugarcane Plants

Interval,[a] Days	Radioactivity, Net C.P.M./Ml.	
	Untreated control	Treated
0.12	...	1122
2	...	141
4	12	154
7	9	43
10	14	24
15	19	35
28	11	15
35	11	10
35 + 0.17[b]	0	0
35 + 7[b]	0	0
35 + 42[b]	0	0

[a] pH ranged from 7.2 to 7.8 during initial 35-day period.
[b] Nutrient medium replaced with fresh untreated nutrient at 35 days.

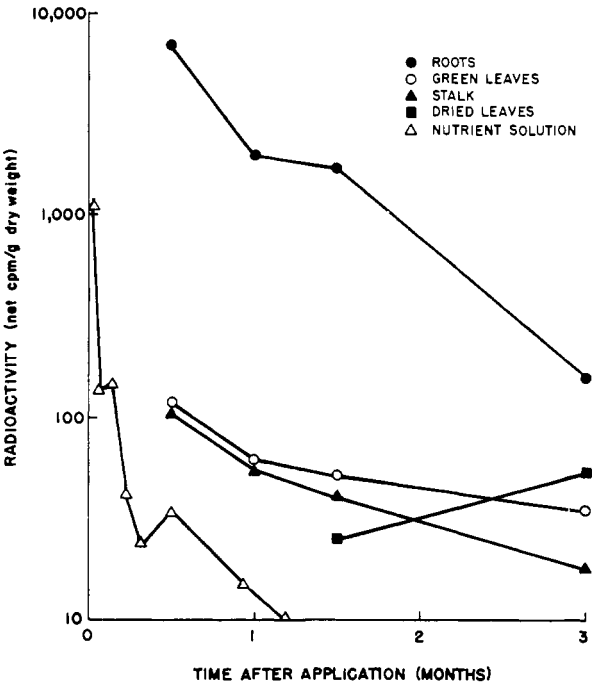

Figure 1. Apparent change with time of radioactivity in plants grown in nutrient solution treated with ammonium monofluoroacetate-2-^{14}C

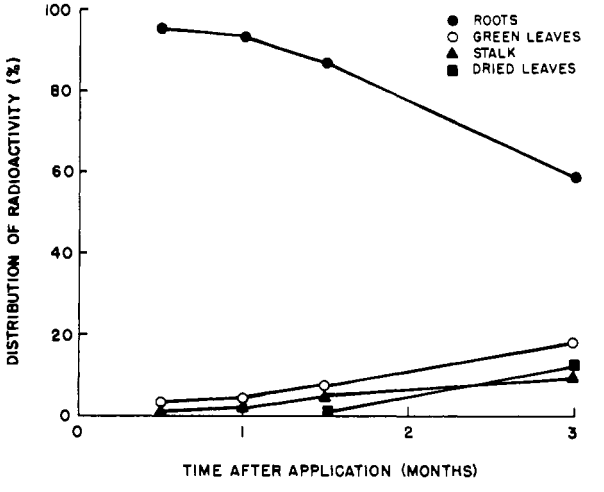

Figure 2. Apparent change with time in distribution of radioactivity in various parts of plants grown in nutrient solution treated with ammonium monofluoroacetate-2-^{14}C

tion of the radioactivity into new leaf tissue. Approximately 10% of the total radioactivity appeared in tissues younger than those of the application area; little translocation took place from the treated leaf sheath into the blade of the same or lower leaves (Table I). Uptake and movement of the applied radioactivity were restricted, by either poor absorption into the leaf or the high degree of adsorption to the sheath tissue. Most of the translocated activity appeared in the laminas of the younger, rapidly expanding leaves, with less activity in the midribs and relatively little in the leaf sheaths except at or near the point of origin (Table II). From the data it appeared that radioactivity either was translocated from older to younger

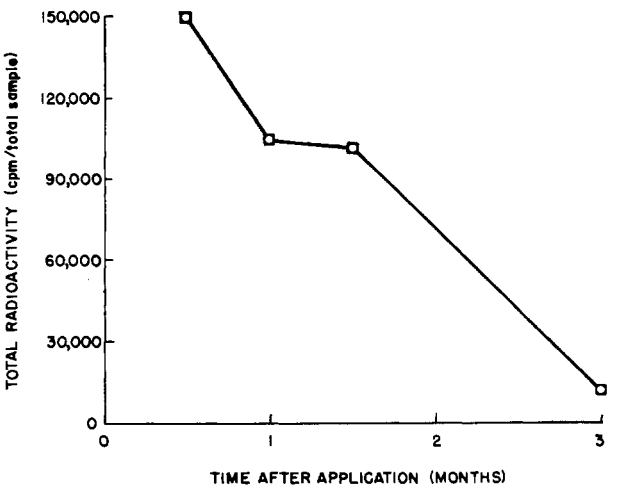

Figure 3. Apparent change with time of total radioactivity of plants grown in nutrient solution treated with ammonium monofluoroacetate-2-^{14}C

leaves after reaching a maximum in each leaf, or was continuously supplied from the source. The growing point did not show particularly high activity, but, in the grass plant, it is located well inside and below the expanding leaf tissue (spindle and first few leaves), although above the site of treatment in these experiments. Fiber and juice from younger stalk portions contained more radioactivity than fiber and juice from older tissue; secondary suckers from the base of the original plant were radioactive, with more activity in the youngest tissue.

In summary, a portion of the active carbon atom, probably with the attached fluorine atom, moved from the site of application in the leaf axil to the lamina of the new leaf, but with a high degree of retention at the treated site. The experiments did not indicate whether monofluoroacetic acid is the translocated substance, but it appears that the F-^{14}C bond remains in both plant and animal systems.

Root Application. Radioactivity in the nutrient medium containing growing sugarcane plants decreased rapidly with time (Table III). Only 10% of the original count remained after 5 days, and 1% at the end of 35 days when the radioactive solution was replaced with fresh untreated solution. There was no apparent leakage of radioactivity from the treated plants into the fresh untreated solution. There is considerable evidence of volatility: Radioactivity in the plants is less than can be accounted for as loss from the nutrient; stock solutions of ammonium monofluoroacetate-2-^{14}C or of nutrient solution with added ammonium monofluoroacetate-2-^{14}C lost radioactivity on standing in open containers or on heating; and when the untreated control plants were placed in proximity to treated plants, the radioactivity of the control plants increased to an equilibrium level above background activity and then decreased. The pH range of 7.2 to 7.8 is not high enough to prevent hydrolysis and loss of the free acid.

Most of the radioactivity removed by plants from the nutrient solution remained in the roots (Figures 1 and 2). The early pattern of absorption from solution suggests a strong and relatively complete adsorption by the roots, with translocation of 5 to 8% of the total radioactivity into the

leaves and stalk. The radioactivity bound to the roots decreased rapidly after the source in the nutrient medium was depleted, but even after 3 months (including almost 2 in fresh untreated nutrient) the roots retained 60% of the radioactivity remaining in the plant. During the 3-month period the total apparent radioactivity of the plants decreased from an average of 150,000 to 12,000 c.p.m., with losses in all plant parts except the lower dead leaves. The above-ground portions decreased only from a total of 6700 c.p.m. at 15 days to 4900 at 90 days. During this time the weights of the treated plants increased 300%. It is possible that the losses of radioactivity in the leaf and stalk—which appear to be approximately 27%—represent only increased self-absorption by the plant fiber. On the other hand, root losses were real, with a 45-fold decrease in counts per minute per gram for a growth weight increase of only twofold.

The data for root uptake suggest a strong adsorption of monofluoroacetate ion on the roots, with only minor translocation to the leaves and stem. This is followed by a slower desorption from the roots and by vaporization, with or without metabolism of the monofluoroacetate to more volatile compounds.

The high degree of adsorption of monofluoroacetate to leaf and root tissue, as well as to other cellulosics such as filter paper, was entirely unexpected in view of its water solubility and volatility. It can be assumed that monofluoroacetate would remain adsorbed to fibrous bait components, probably would not be washed off the bait formulations by moderate rainfall, and would not readily leach into soils, especially into those with considerable organic content.

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