Miyazaki *et al.* were known, establishes the identity of the metabolites with certainty and suggests that the metabolic sequence proposed by Miyazaki *et al.* is a common one in mammals.

Residues in Blood, Urine, and Tissues. Table II shows the residues of diazinon and hydroxydiazinon found in the blood, urine, and tissues of the sheep killed after 48 hr.

The proportion of hydroxydiazinon found in the blood was surprisingly high, varying from about 25 to 70% of the diazinon content. It was much lower in the tissues, from about 1.5% in fat to about 12% in the liver, and the proportions of II and III were clearly lower still, although these compounds were not determined quantitatively. The compounds were distributed among the tissues according to their relative polarities. Thus, diazinon and metabolite III were largely concentrated in fat (the concentration of diazinon in the fat was over 100 times its concentration in the blood). The more polar hydroxydiazinon was more evenly distributed, with only about five times as much in the fat as in the blood, and the most polar of the compounds, metabolite II, was most prominent in the urine.

There is little information about the toxicity of these metabolites to mammals. Miyazaki *et al.* (1970) determined the oral toxicity to mice of several of the compounds they detected but mentioned only the value for hydroxydiazinon (120 mg/kg). This is close to the figure (82 mg/kg) quoted by Martin (1968) for the toxicity of diazinon. On the other hand, Sawicki (1971) found that hydroxydiazinon was considerably more toxic than diazinon to a susceptible strain of houseflies (the LD_{50}'s were, respectively, 0.12 and 0.30 μg per female fly when topically applied in acetone as 1-μl drops). Evidently the toxicity of these cholinesterase-inhibiting metabolites to other species cannot be inferred from the available data and the possible hazards of these residues cannot yet be assessed.

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Distribution of Picloram Residues in Sugarcane

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[^{14}C]Picloram absorbed through the roots of sugarcane plants (a hybrid of *Saccharum* spp.) accumulated in the leaves. Residues retranslocated from older to younger leaves without detectable radioactivity in either the apical meristem of the primary stalk or the stalk. With the depletion of the supply to the roots over a period of 13 weeks, the leaf residues reached a maximum and began to decline, with a portion of the radioactivity lost from the plant in the senescent abscised leaves. Picloram had a constant linear inhibiting effect on the elongation of leaf tissue from the primary spindle.

Two patterns of residue distribution in sugarcane from the root absorption of labeled herbicides have been described previously (Hilton *et al.*, 1970). The contact herbicide, pentachlorophenol (PCP), adsorbed strongly on the root surfaces with no ^{14}C appearing in the leaves and with only 5% of the recoverable activity in the stalk at the end of 8 weeks. In contrast, ^{14}C-containing residues from the ring-labeled *s*-triazine herbicides, atrazine and ametryne, moved freely upward through the stalk to accumulate in the leaves. The roots retained 25 to 35% of the recovered radioactivity; stalk residues were low, about 3 to 4% for atrazine and 6 to 7.5% for ametryne over a 13-week absorption period. Movement of radioactive products to the leaves appeared to take place through the xylem system with transpired water. The regular senescence and abscission from the stalk of the older lower leaves resulted in the removal of the major part of the residue deposits.

Plants treated with PCP and the ring-labeled *s*-triazine herbicides lost substantial amounts of total radioactivity with time, even when the triazine residues from the abscised leaves were added to those remaining in the growing plant. The loss of PCP was attributed to its volatility; sugarcane appears to be able to break the *s*-triazine ring quite readily with the production of volatile compounds from the roots and leaves (Goswami, 1972).

In this study we have used carboxyl-labeled picloram as an example of a herbicide known to be xylem- and

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phloem-mobile in many plants (*e.g.*, Isensee *et al.*, 1971) and to have considerable persistence in the parent form. Movement of other phloem-active herbicides, such as 2,4-D, has been demonstrated in sugarcane only from foliar application (Burr *et al.*, 1956). Since picloram in the field has shown little apparent phytotoxicity to established grasses, including sugarcane, we wished to determine the pattern of uptake and distribution in the plant and to measure its effect on the growth of the spindle of the primary shoot. In addition to the usual plant sections (roots, stalk, green attached leaves, and dry abscised leaves) we examined the apical meristematic tissue of the primary stalk and the suckers (secondary basal shoots), with and without expanded leaves, as possible sites of accumulated ^{14}C from picloram.

MATERIALS AND METHODS

Uniform-sized, small sugarcane plants, hybrid H50-7209, established individually in covered, glazed porcelain crocks containing 3.0 l. of aerated Hoagland nutrient solution, were treated at 6 weeks of age (about 1 ft in height with six to eight expanded leaves) with approximately 1.2 mg (4.8 μCi) of [^{14}C]picloram per plant. The radioactive compound in water was added to the nutrient solution as a single increment. The nutrient was maintained at a constant volume and the radioactivity was monitored over a 13-week period. Elongations of the spindle of treated and untreated plants were measured daily, using a pin-perforation technique in which a pin was inserted through the spindle at the first visible dewlap. Elongation from a single perforation could be measured for a 2-week period before another puncture was necessary (Mongelard and Mimura, 1971).

Radioactivity in the plants and nutrient solution was measured by liquid scintillation spectrometry. Plants were harvested after 1, 2, 4, 8, and 13 weeks' continuous growth in the radioactive solution. Component parts consisting of roots, stalk, green leaves, senescent abscised leaves (dry leaf trash), primary apical meristem, and basal suckers, with and without expanded leaves, were coarsely chopped, vacuum-dried in a desiccator over $CaCl_2$, and ground in a laboratory Wiley mill to pass a 40-mesh screen. Combustion analysis with a Parr oxygen bomb was used to measure ^{14}C residues in the various plant parts. Carbon dioxide was absorbed in KOH solution placed inside the bomb; $BaCO_3$ was precipitated in scintillation counting vials, washed, dried, and dispersed by ultrasonic vibration in a toluene scintillation liquid containing Cab-O-Sil for counting (Hilton *et al.*, 1972). The average recovery from the procedure when known amounts of [^{14}C]picloram were added to dried fiber was 84.1 ± 3.9%.

RESULTS AND DISCUSSION

Radioactivity in the aerated nutrient solution decreased exponentially with time when growing plants were present. Without plants, ^{14}C from picloram in the aerated nutrient solution remained constant. Initially the losses from the nutrient could be accounted for as plant residues; from 4 weeks on, the cumulative losses of ^{14}C from the nutrient were greater than the apparent residues taken into the plants, increasing to a difference of 28% in 13 weeks (Table I). Although there is no ready explanation, two possibilities exist: either there was a metabolic degradation of the picloram molecule with a loss of ^{14}C as $^{14}CO_2$, or the dilution of the ^{14}C residues caused by a threefold increase in plant weight between the 1- and 21% at 1 week, 18% at 2 weeks, 10% at 4 weeks, and 12% at 8 essary to reach the limit of detection, resulting in an apparent loss in the amounts detected. The latter argument would be most plausible for residues evenly distributed throughout the plant at concentrations near the detection limit. Thus, the limit of 0.19 ppm on fresh weight, using the combustion technique, implies that a 693-g plant at 13 weeks could contain 132 μg of undetected [^{14}C]picloram. This amount represents a maximum dilution loss of 13% based on 998 μg removed from the nutrient solution, leaving about 15% loss still unaccounted for. Similar calculations at earlier time periods indicated even greater potential dilution losses when the amounts taken into the plants were less in comparison with the plant weights: 21% at 1 week, 18% at 2 weeks, 10% at 4 weeks, and 12% at 8 weeks. The calculations as well as recoveries in Table I make it obvious that residues were not distributed throughout the plant at concentrations which remained undetected. Growth dilution did not account for the losses

Table I. Root Uptake and Distribution of [^{14}C]Picloram in Sugarcane

Component sample	Duration of treatment, weeks	Picloram in sample, μg	Picloram recovery, %
Nutrient solution	1	1050[a]	83.1
	2	933	70.7
	4	592	44.5
	8	360	27.7
	13	264	20.9
Green leaves	1	86	6.8
	2	402	30.5
	4	455	34.2
	8	584	45.0
	13	519	41.0
Dry leaf trash	1	ns[c]	ns
	2	ns	ns
	4	ns	ns
	8	26	2.0
	13	50	4.0
Primary apical meristematic tissue	1	ns	ns
	2	ns	ns
	4	ns	ns
	8	ns	ns
	13	ns	ns
Stalk	1	ns	ns
	2	ns	ns
	4	ns	ns
	8	ns	ns
	13	ns	ns
Roots	1	15	1.1
	2	26	1.9
	4	19	1.4
	8	27	2.1
	13	79	6.3
Suckers (secondary shoots) with leaves	1	11	0.89
	2	*b*	
	4	11	0.80
	8	*b*	
	13	*b*	
Suckers (secondary shoots) without leaves	1	ns	ns
	2	ns	ns
	4	ns	ns
	8	ns	ns
	13	ns	ns
Total recovery	1	1162	91.9
	2	1361	103.1
	4	1077	80.9
	8	997	76.8
	13	912	72.2

[a]Initial solutions contained 1264, 1320, 1330, 1299, and 1262 μg of picloram. [b]No secondary shoots present with expanded leaves. [c]Denotes that the plant part had no significant radioactivity when compared with that from untreated tissue.

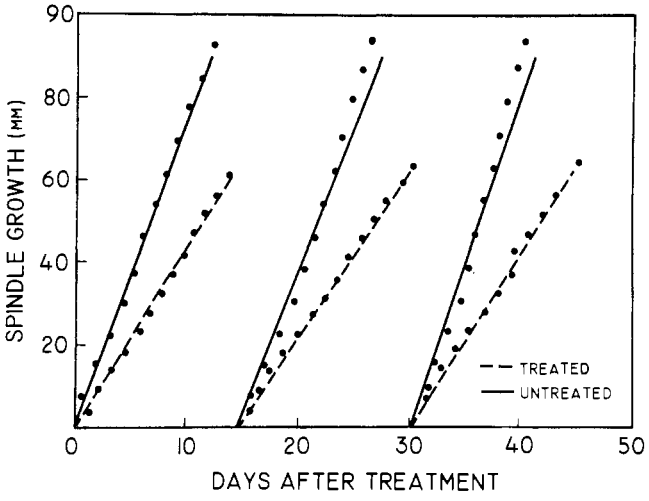

Figure 1. Inhibition by picloram of the growth of the sugarcane spindle.

of ^{14}C measured over the 13-week time period. Most of the weight increase resulted from the growth of the primary stalk, which had no detectable residues at any time.

[^{14}C]Picloram was readily absorbed through the roots and transported to the leaves. Stalk concentrations were below the detectable limit and there was no accumulation. Root residues remained low, with a possible increase only at 13 weeks. The primary apical meristem contained no detectable radioactivity, nor did the basal suckers until the leaves expanded and began to function. Picloram reduced tillering (formation of secondary shoots) of treated plants.

Residues in the leaves at 8 and 13 weeks, including those in the older abscised leaves, were nearly constant at 46% of the added ^{14}C. With the substantial depletion of the picloram from the nutrient solution, it appeared that the maximum intake had been reached and a balance established between plant radioactivity and the unexplained losses of ^{14}C. Small but apparently increasing portions of the leaf activity remained in the abscised dry leaf trash of the last two sampling periods. Without movement of the picloram in the phloem, residues in the green leaves should remain after senescence and abscission, provided they are not volatile. Xylem-mobile herbicide residues, such as those from atrazine and ametryne, accumulated in the abscised leaves in 13 weeks to an extent of 69.4% from atrazine and 61.3% from ametryne based on recoverable radioactivity (Hilton *et al.*, 1970). [^{14}C]Picloram in the abscised leaves at the same time comprised 7.8% of the plant radioactivity. These data support the assumption of phloem mobility of picloram in sugarcane, in which the herbicide was retranslocated from older to younger leaves with maximum nutrient-mobilizing capacity. The transfer was incomplete, possibly because of a slow transformation of [^{14}C]picloram to an immobile form, and would result in time in a substantial loss of residues from the plant. Gas chromatographic analysis of sugarcane residues showed that the translocated substance was the picloram molecule.

Spindle growth measurements showed a constant inhibition of the rate of elongation as a result of the picloram treatment (Figure 1). The reduction of spindle growth may have occurred because of inhibition of root growth or of new leaf expansion. The constant growth suppression for the 6-week observation period agreed better with the nearly constant root residues than with changing nutrient or leaf residues. This hypothesis is also in general agreement with field observations that picloram in soil inhibited root and shoot emergence and growth of young sugarcane plants. The field results have been interpreted as an inhibition of root growth as a function of picloram concentration in the soil, since transplanting the vegetative cuttings to fresh soil allows normal growth to occur. From the present data it appeared unlikely that picloram directly affected the apical meristem, since no detectable residues were present. In the absence of contrary data we believe the growth inhibition to be related to picloram in or surrounding the roots rather than to residues in the leaves. In contrast to some other plants (Isensee *et al.*, 1971) the accumulation of picloram in the newer photosynthetic leaves suggests that they represent the region of greatest metabolic activity. If this is true, it follows that the residues in leaves do not have the phytotoxicity for sugarcane of those in or around the roots.

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