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Received for review January 15, 1954. Accepted March 16, 1954. Published by permission of Yuan-Chi Tang, President of the Taiwan Fertilizer Co., Ltd.

CHELATES

Absorption and Translocation of Ethylenediaminetetraacetic Acid by Sunflower Plants

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Chelating agents are coming into widespread use in agriculture for the prevention and cure of iron-deficiency chlorosis in plants. Little, however, is known about absorption, translocation, and metabolism of these materials by plants. This investigation was carried out in order to gain some information on absorption and translocation of ethylenediaminetetraacetic acid, the most widely used of the chelates. Solution culture experiments with sunflower, employing the split-root technique, indicate that iron is absorbed by one portion of the split root growing in nutrient solutions adjusted to pH 7.0, but is not utilized, resulting in iron-deficiency chlorosis. When ethylenediaminetetraacetic acid was supplied through the other portion of the split root, excellent plant growth was obtained, indicating that the chelate made iron available to all portions of the plant. It would be of considerable help for agricultural use to know whether the chelate transports iron to the root surface where only the iron is absorbed, or whether the whole chelated molecule is absorbed by the roots of plants.

Ethylenediaminetetraacetic acid (EDTA) and its metal salts are in widespread use both commercially and experimentally in plant nutrition and in plant and animal biochemistry. Since Jacobson (*4*) first reported the use of ferric dipotassium ethylenediamine tetraacetate (Fe-EDTA) as a satisfactory source of iron for plants growing in solution cultures, there has been considerable impetus behind the use of ethylenediaminetetraacetic acid and similar materials by other workers. Weinstein (*12*) found that ferric disodium ethylenediamine tetraacetate was a good nutrient source of iron for sunflower plants grown in culture solutions adjusted to pH 7.0. Although iron supplied as ferrous sulfate was absorbed by the plants, it was not utilized at this pH. High nutrient levels of manganese did not induce symptoms of iron deficiency in sunflower plants supplied with iron as ferric disodium ethylenediamine tetraacetate, whereas plants supplied with iron as ferrous sulfate exhibited advanced symptoms of iron-deficiency chlorosis. This suggests that plants absorb the chelated iron molecule and transport it to the site of enzyme synthesis, where the iron is released through enzymatic decomposition of the chelate and is replaced by hydrogen or other cations.

Ethylenediaminetetraacetic acid and other chelating materials are now being extensively applied to soils for control of iron-deficiency chlorosis in many field and ornamental crops (*1, 5–8, 11, 13*).

Wallace and North (*10*) have presented data indicating that ethylenediaminetetraacetic acid is absorbed and metabolized by plants. By supplying corn seedlings with ferric disodium ethylenediamine tetraacetate containing an isotopically labeled nitrogen atom, they found radioactivity in a number of nitrogen fractions. Further evidence that ethylenediaminetetraacetic acid is absorbed by plants is presented in this paper.

Materials and Methods

An experiment designed to provide evidence relative to absorption and translocation of ethylenediaminetetraacetic acid by sunflower plants was carried out in solution culture. The seeds (*Helianthus annuus* L.) were planted in flats of washed quartz sand on August 11, 1953. On September 1 a series of eight sunflower plants was set up in a split-root technique in solution cultures. Each culture consisted of two 2-liter borosilicate glass beakers with a wooden cover assembly so constructed as to support one plant and to allow aeration of solutions (Figure 1). Plants were supported in an upright position by wooden dowels attached to the cover assembly, which was coated with Tygon varnish. Root systems of the sunflower seedlings were separated into two equal portions, one portion being placed in each of two containers. An additional series of plants was grown in eight conventional solution cultures. Each culture consisted of a 1-gallon wide-mouthed glass jar accommodating two seedlings. Although these plants were subjected to the same nutrient treatments, the root systems were not separated, the cultures being used as controls for each of the split-root treatments.

Complete nutrient solutions, with the exception of additions of iron and ethylenediaminetetraacetic acid, were

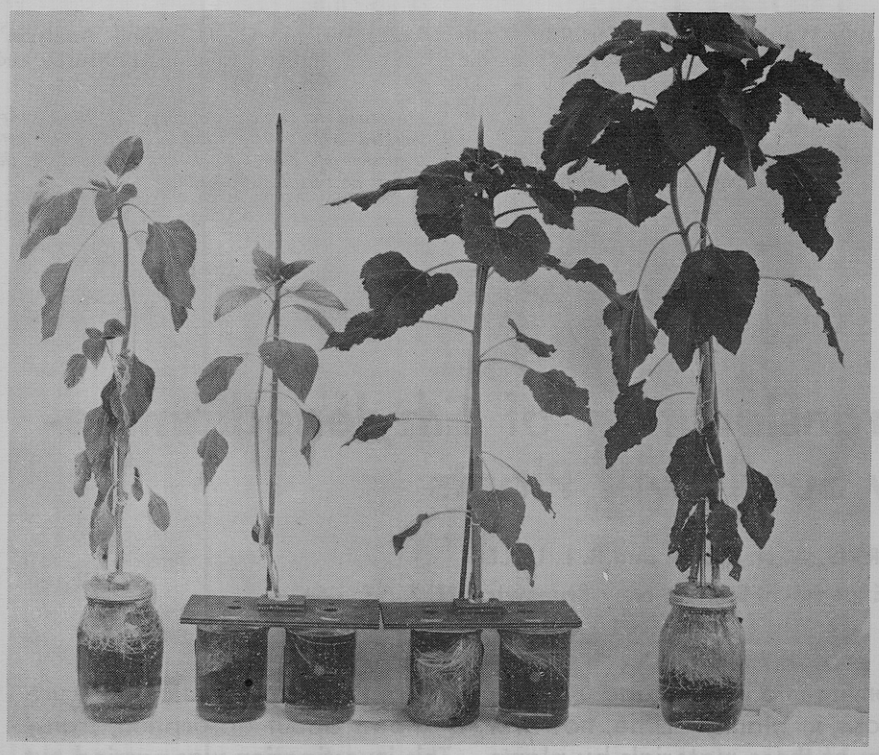

Figure 1. Sunflower plants grown in split-root and complete-root solution cultures at pH 7.0

Left. Supplied with 0.5 p.p.m. of iron as ferrous sulfate, no disodium ethylenediamine tetraacetate
Left Center. Left compartment supplied with 0.5 p.p.m. of iron as ferrous sulfate; right compartment with no iron and no disodium ethylenediamine tetraacetate
Right Center. Left compartment supplied with 0.5 p.p.m. of iron as ferrous sulfate, right compartment with 5.0 p.p.m. of disodium ethylenediamine tetraacetate
Right. Supplied with 0.5 p.p.m. of iron as ferrous sulfate and 5.0 p.p.m. of disodium ethylenediamine tetraacetate

supplied to all cultures. Nutrient solutions were formulated from reagent-grade salts and distilled water. The solutions for both series of plants were adjusted to either pH 5.0 or 7.0. The pH 5.0 nutrient solution was composed of the following macronutrient salts: 0.001*M* potassium dihydrogen phosphate, 0.002*M* potassium sulfate, 0.002*M* magnesium sulfate heptahydrate, and 0.005*M* calcium nitrate tetrahydrate; the pH 7.0 nutrient solution contained: 0.001*M* potassium dihydrogen phosphate, 0.0012*M* potassium sulfate, 0.002*M* magnesium sulfate heptahydrate, 0.005*M* calcium nitrate tetrahydrate, and 0.0008*M* potassium hydroxide. Micronutrient salts were supplied to both nutrient solutions as follows: 0.25 p.p.m. of manganese as manganese sulfate monohydrate, 0.10 p.p.m. of zinc as zinc sulfate heptahydrate, 0.10 p.p.m. of boron as boric acid, 0.01 p.p.m. of copper as cupric sulfate pentahydrate, and 0.01 p.p.m. of molybdenum as sodium molybdate dihydrate.

In the split-root series, all nutrient solutions in the left containers were supplied with 0.5 p.p.m. of iron as ferrous sulfate. No iron was added to nutrient solutions in the right containers. Half of these cultures were supplied with 5.0 p.p.m. of disodium ethylenediamine tetraacetate (Na_2EDTA), which represents slightly more disodium ethylenediamine tetraacetate than would be necessary to chelate 0.5 p.p.m. of iron. In the complete-root culture series, all nutrient solutions contained 0.5 p.p.m. of iron as ferrous sulfate, and half of the cultures contained 5.0 p.p.m. of disodium ethylenediamine tetraacetate (Table I). These solutions were completely renewed every 2 days.

Plants were harvested on September 28, after a nutrient treatment period of 28 days. Plants were fractionated into leaves, stems, and roots. In the split-root series, roots from each container were kept separate. Aliquots of each fresh fraction were dried in a forced-draft oven at 70° C. for the determination of per cent dry weight. Similar aliquots were frozen for subsequent determination of water-soluble, water-insoluble, and total iron.

Iron fractions were determined by a modification of the method described by Crummett (*2*). The frozen tissues were partially thawed and wrapped in 6-inch squares of fine-meshed muslin, and the sap was expressed under a pressure of 2000 pounds per square inch. The press cake was loosened, washed with 10 ml. of distilled water, and expressed again. This was done three times. The combined juice and washings were filtered through Whatman No. 4 filter paper. The sum of the water-soluble iron in the filtrate and water-insoluble iron in the press cake constitutes total iron. Iron was determined by the method of Toth *et al.* (*9*).

Prior to harvest, catalase activity measurements were carried out on terminal leaves of plants in split-root cultures at pH 7.0 with and without disodium ethylenediamine tetraacetate. Catalase activity was determined by the perborate titration method of Feinstein (*3*). Activity measurements were made in a water bath at 25° C., using leaf tissue homogenates diluted 1 to 50 with deionized water.

Results

Mild symptoms of iron deficiency, manifested by slight interveinal mottling, were apparent in plants supplied disodium ethylenediamine tetraacetate at pH 7.0 within 7 days. Slight chlorosis was also evident on leaves of the plant in one split-root culture supplied with disodium ethylenediamine tetraacetate at pH 5.0, but these symptoms soon disappeared. Tips of roots of plants in both split-root and complete-root cultures with no chelate at pH 7.0 were slightly swollen and yellow, symptoms characteristic of iron deficiency in sunflower plants. In the split-root series (pH 7.0, no disodium ethylenediamine tetraacetate), these symptoms were evident in roots growing in both nutrient containers. All other plants grew normally.

After 11 days, chlorosis on plants in split-root and complete-root cultures with no disodium ethylenediamine tetraacetate at pH 7.0 was very pronounced. Leaves on the upper halves of the plants were almost entirely chlorotic with the exception of the veins, which remained green. Roots of plants in both containers in the split-root cultures were light brown, and poorly developed,

Table I. Nutrient Treatments

Split-Root Cultures		*Complete Cultures*
Left container	*Right container*	
pH 5.0 + $FeSO_4$	pH 5.0	pH 5.0 + $FeSO_4$
pH 5.0 + $FeSO_4$	pH 5.0 + EDTA	pH 5.0 + $FeSO_4$ + EDTA
pH 7.0 + $FeSO_4$	pH 7.0	pH 7.0 + $FeSO_4$
pH 7.0 + $FeSO_4$	pH 7.0 + EDTA	pH 7.0 + $FeSO_4$ + EDTA

Table II. Weights of Sunflower Plants Grown in Complete-Root Cultures

(Adjusted to pH 5.0 or 7.0 with and without disodium ethylenediamine tetraacetate)

Treatment	Plant Fraction	Av. Fresh Wt./Plant, G.	% Increase in Fresh Wt. Due to EDTA	Av. Dry Wt./Plant, G.	Av. % Dry Wt.
pH 5.0	Leaves	72.0		7.99	11.10
+ $FeSO_4$	Stems	180.5		13.30	7.37
	Roots	59.5		3.53	5.93
		312.0		24.82	
pH 5.0	Leaves	64.5		9.37	14.53
+ $FeSO_4$	Stems	196.3		15.26	7.77
+ EDTA	Roots	63.8		3.38	5.30
		324.6	4	28.01	
pH 7.0	Leaves	28.3		4.10	14.49
+ $FeSO_4$	Stems	90.0		6.14	6.82
	Roots	30.8		1.82	5.91
		149.1		12.06	
pH 7.0	Leaves	53.0		8.53	16.09
+ $FeSO_4$	Stems	170.8		13.95	8.17
+ EDTA	Roots	46.8		3.09	6.60
		270.6	81	25.57	

and exhibited characteristic swelling and pigmenting of root tips. Of special interest was the response of plants in split-root cultures supplied with disodium ethylenediamine tetraacetate at pH 7.0. These plants were about twice as large as those in split-root cultures not supplied with the chelate at pH 7.0. In the split-root cultures with disodium ethylenediamine tetraacetate at pH 7.0, the portions of the root systems in the containers supplied with ferrous sulfate were white and very well developed, while the portions in the compartment supplied with disodium ethylenediamine tetraacetate exhibited very early symptoms of iron deficiency which became no more severe. A view of these plants is shown in Figure 1.

Harvest data are shown in Tables II and III. Examination of the data shows the following.

In the complete-root culture series at pH 5.0, addition of disodium ethylenediamine tetraacetate resulted in only slightly higher fresh- and dry-weight values, indicating that at this pH value the plants obtained sufficient iron from ferrous sulfate.

In the split-root series at pH 5.0, addition of disodium ethylenediamine tetraacetate also resulted in only slightly higher fresh- and dry-weight values.

At pH 7.0, addition of disodium ethylenediamine tetraacetate to complete-root cultures resulted in an 82% increase in fresh weight and a 112% increase in dry weight over plants not supplied with the chelate, indicating that at this pH value the plants obtained sufficient iron from ferric disodium ethylenediamine tetraacetate, but not from ferrous sulfate.

At pH 7.0, addition of disodium ethylenediamine tetraacetate to split-root cultures resulted in a 560% increase in fresh weight and a 420% increase in dry weight over plants not supplied with the chelate.

In general, the per cent-dry-weight values of leaf and stem tissues of all plants supplied with disodium ethylenediamine tetraacetate were higher than those of plants not supplied with the chelate.

Data on water-soluble, water-insoluble, and total iron are shown in Tables IV and V.

In the complete-root series at pH 5.0, addition of disodium ethylenediamine tetraacetate did not significantly alter iron content of the various tissue fractions.

In the split-root series at pH 5.0, addition of disodium ethylenediamine tetraacetate resulted in an increase in the water-soluble iron content of all tissue fractions.

At pH 7.0, addition of disodium ethylenediamine tetraacetate resulted in a significant increase in the water-soluble content of iron in leaf, stem, and root tissues. Of special interest is the large increase in water-soluble iron content of leaves and roots in the container that was supplied with disodium ethylenediamine tetraacetate.

Catalase activity measurements were carried out on terminal leaves of plants growing in split-root cultures at pH 7.0. Results are shown in Table VI.

Results of catalase activity measurements and iron determinations indicate that, when no chelate was added at pH 7.0, there was a very low catalase activity in spite of the fact that the total iron content of these tissues was very high.

Discussion

It appears that disodium ethylenediamine tetraacetate or a decomposition product is readily absorbed through the roots of sunflower plants. This is indicated in the split-root series both by the increase in growth and by the higher water-soluble iron content of tissues supplied with disodium ethylenediamine tetraacetate at pH 7.0 when compared with those not supplied with the chelate. Preliminary experiments indicated that iron impurities in the chelate, nutrient salts, and distilled water employed were not sufficient to support normal growth of sunflower plants. Plants supplied with as much as 0.1 p.p.m. of iron as ferric disodium ethylenediamine tetraacetate in nutrient solutions at pH 7.0 resulted in severe leaf chlorosis. Water-soluble iron is

Table III. Weights of Sunflower Plants Grown in Split-Root Cultures

(Adjusted to pH 5.0 and 7.0 with and without disodium ethylenediamine tetraacetate)

Treatment			Av. Fresh Wt./Plant,	% Increase in Fresh Wt.	Av. Dry Wt./Plant	Av. %
Left container	Right container	Plant Fraction	G.	Due to EDTA	Fraction, G.	Dry Wt.
pH 5.0	pH 5.0	Leaves	78.0		12.08	15.49
+ $FeSO_4$		Stems	259.0		21.95	8.47
		Left root	60.0		3.39	5.65
		Right root	9.3		0.58	6.24
			406.3		38.00	
pH 5.0	pH 5.0	Leaves	89.5		15.17	16.95
+ $FeSO_4$	+ EDTA	Stems	244.0		23.24	9.52
		Left root	21.5		1.16	5.40
		Right root	58.5		3.39	5.79
			413.5	2	42.96	
pH 7.0	pH 7.0	Leaves	20.8		2.81	13.51
+ $FeSO_4$		Stems	39.0		3.21	8.23
		Left root	12.0		1.37	11.42
		Right root	6.0		0.61	10.17
			77.8		8.00	
pH 7.0	pH 7.0	Leaves	105.5		10.25	14.46
+ $FeSO_4$	+ EDTA	Stems	292.0		24.39	8.35
		Left root	86.0		5.00	5.81
		Right root	32.0		2.16	6.75
			515.5	563	41.80	

Table IV. Iron Content of Tissues of Sunflower Plants Grown in Complete-Root Cultures

(Adjusted to pH 5.0 or 7.0 with and without disodium ethylenediamine tetraacetate)

Treatment	Plant Fraction	Water-Soluble Iron, P.P.M.	Water-Insoluble Iron, P.P.M.	Total Iron, P.P.M.
pH 5.0 + $FeSO_4$	Leaves	10.1	56.3	66.4
	Stems	6.0	8.8	14.8
	Roots	26.6	288.0	314.6
pH 5.0 + $FeSO_4$ + EDTA	Leaves	10.7	65.2	75.9
	Stems	5.5	17.7	23.2
	Roots	21.4	247.7	269.1
pH 7.0 + $FeSO_4$	Leaves	5.8	63.9	69.7
	Stems	14.6	16.2	30.8
	Roots	21.8	515.5	537.3
pH 7.0 + $FeSO_4$ + EDTA	Leaves	16.3	90.8	107.1
	Stems	14.6	11.3	25.9
	Roots	38.3	246.1	284.4

considered here as an indication of the amount of reserve or available iron for enzyme incorporation, and not as a measure of metabolically active iron. The term "metabolically active iron" must be reserved for that fraction that is present in enzyme molecules at any given time. However, low catalase activity values of chlorotic leaf tissues of plants grown in split-root cultures at pH 7.0 with no added chelate indicate that when soluble-iron content is low, catalase activity is also low, and plants suffer from a true iron deficiency.

The exact nature of the activation of precipitated or inactivated iron in plant tissues by ethylenediaminetetraacetic acid is not known. It is believed, however, that in the split-root series at pH 7.0, iron is absorbed by one portion of the root system, but it is inactivated after entry into the plant, which makes it unavailable for incorporation into enzyme molecules. When disodium ethylenediamine tetraacetate is supplied through the other portion of the root system, it apparently chelates this inactivated iron and makes it available for metabolic use. It is possible that a certain amount of iron is released to enzyme porphyrins by decomposition of the chelate by other enzyme systems. There also appears to be a transfer of chelate from one side of the root system to the other with subsequent mobilization of iron. Some of this soluble chelated iron is apparently then transported back to the other side of the root system.

Summary

An experiment was carried out to determine if disodium ethylenediamine tetraacetate is absorbed by sunflower plants. A series of plants with split-root systems was grown in solution cultures at pH 5.0 and 7.0. In both pH series, the left nutrient compartment contained a complete nutrient solution with iron supplied as ferrous sulfate. The right nutrient compartment also contained a complete nutrient solution but with no iron, although half of these were supplied with disodium ethylenediamine tetraacetate. A similar series of plants was grown in conventional solution cultures at pH 5.0 and 7.0. All cultures contained a complete nutrient solution with iron supplied as ferrous sulfate. Half of these cultures were supplied with disodium ethylenediamine tetraacetate.

Harvest data indicated that in both the complete-root and split-root series at pH 5.0, addition of disodium ethylenediamine tetraacetate resulted in only a slight increase in fresh- and dry-weight yields. At pH 7.0, addition of disodium ethylenediamine tetraacetate resulted in marked increases in yields.

Determinations of water-soluble, water-insoluble, and total iron show that, in general, there was a greater increase in iron content of all plants supplied with disodium ethylenediamine tetraacetate at pH 7.0 than at pH 5.0.

Catalase activities of terminal leaves of plants growing in split-root cultures at pH 7.0 indicated that when no disodium ethylenediamine tetraacetate was added, there was very low catalase activity in spite of the fact that total iron content of these tissues was high.

Ethylenediaminetetraacetic acid or a decomposition product is apparently readily absorbed through roots of sunflower plants. It is believed that absorbed ethylenediaminetetraacetic acid chelates inactivated iron in the plant and transports it to the site of enzyme synthesis.

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Table V. Iron Content of Tissues of Sunflower Plants Grown in Split-Root Cultures

(Adjusted to pH 5.0 or 7.0 with and without disodium ethylenediamine tetraacetate)

Treatment		Plant Fraction	Water-Soluble Iron, P.P.M.	Water-Insoluble Iron, P.P.M.	Total Iron, P.P.M.
Left container	Right container				
pH 5.0 + $FeSO_4$	pH 5.0	Leaves	16.6	59.1	75.7
		Stems	6.1	7.8	13.9
		Left root	61.4	514.8	576.2
		Right root	14.4	24.9	39.3
pH 5.0 + $FeSO_4$	pH 5.0 + EDTA	Leaves	21.2	48.8	70.0
		Stems	9.0	11.8	20.8
		Left root	86.0	1303.3	1389.3
		Right root	16.7	57.6	74.3
pH 7.0 + $FeSO_4$	pH 7.0	Leaves	10.6	93.0	102.1
		Stems	6.5	5.8	12.3
		Left root	25.2	326.0	351.2
		Right root	5.5	28.4	33.9
pH 7.0 + $FeSO_4$	pH 7.0 + EDTA	Leaves	22.1	52.2	74.3
		Stems	11.0	13.6	24.6
		Left root	52.0	316.8	368.8
		Right root	28.5	96.6	125.1

Table VI. Catalase Activities of Terminal Leaf Tissues of Sunflower Plants

(Grown in split-root solution cultures at pH 7.0 with and without disodium ethylenediamine tetraacetate)

Treatment		Catalase Activity per Mg. Fresh Wt.	% of Control (+ EDTA)
Left container	Right container		
pH 7.0 + $FeSO_4$	pH 7.0	0.0263	39.5
pH 7.0 + $FeSO_4$	pH 7.0 + EDTA	0.0665	100.0

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Received for review January 9, 1954. Accepted March 12, 1954. Work carried out under grant-in-aid cosponsored by Refined Products Corp., Lyndhurst, N. J., and Niagara Chemical Division, Food Machinery Corp., Middleport, N. Y. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Soils, New Brunswick.

Organic Phosphates Good Bait Poisons for DDT-Resistant Flies; Herbicide Absorption

INSECTICIDE BAITS

Dry Sugar Baits for the Control of Houseflies

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Extensive tests were conducted in 1953 by the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine to evaluate the effectiveness of dry sugar baits for the control of houseflies resistant to DDT and other chlorinated hydrocarbon insecticides. In laboratory tests sugar baits containing only 0.1% of malathion, Diazinon, or Bayer L 13/59 gave 99% kills of flies in 16 hours. Higher concentrations gave faster kills. Baits stored for 1 month showed no loss of toxicity. In practical tests in open dairy barns and poultry houses applications of 100 grams of the bait per 2500 to 5000 square feet of floor area usually gave reductions of 90% or higher within 4 hours. Five applications per week of baits containing 0.5 and 1.0% of the toxicants maintained highly effective control of flies under adverse conditions. As flies feed on individual grains of sugar, the bait can be scattered so sparsely that animals would be unlikely to eat much of it. The bait is inexpensive, easily prepared, and easily applied from a jar with holes in the lid.

WIDESPREAD RESISTANCE to the chlorinated hydrocarbon insecticides has intensified research on the development of new insecticides and other means of controlling houseflies, *Musca domestica* L. At the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine the greatest emphasis has been placed on attractant baits. Studies during 1952 and 1953 indicated that baits consisting of an attractant and toxicant in water provided effective control of houseflies when exposed in metal pans or applied lightly on hard-surfaced floors of barns with a garden sprinkling can (*1*, *2*). However, the exposure of large quantities of poison bait in pans might be too hazardous for practical use, and spoiled baits create a disposal problem. The sprinkling-can method of applying baits caused unsightly staining of hard-surfaced barn floors, and the treatments were not highly effective on absorbent surfaces, such as earthen floors.

In the course of studies with liquid baits it was observed that flies were attracted to and fed on the dry residue on barn floors for several hours after the bait had been applied. This observation suggested that a dry bait might be effective against flies and at the same time overcome the objections to liquids. In subsequent laboratory tests it was found that dry granulated sugar was eaten more readily than aqueous sugar solutions, and combined with certain phosphorus insecticides was effective in killing houseflies. Although baits are still effective after storage for 28 days, daily application of small amounts of sugar minimizes danger to small animals and eliminates residual hazards. The most promising combinations of sugar and toxicants were tested extensively for the control of resistant flies in dairy barns and poultry houses in the vicinity of Orlando. This paper presents the results of the laboratory and field investigations.

Laboratory Studies

Tests were conducted to compare the effectiveness of dry granulated-sugar baits containing Diazinon [*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) thiophosphate], malathion, or Bayer L 13/59 (a dialkyl phosphonate). The toxicants were mixed with the sugar at concentrations of 5, 2, 1, 0.5, and 0.1% and ground in a mortar. Technical