Table V. Effect of the Formulation on Total Uptake of Croneton and Its Metabolites from the Soil by *Clementine* Trees

croneton formulation	residues in	residues, ppm: days after soil treatment 10	25	50
10% granular[a]	mature leaves	1.9		4.1
	young terminals	0.3	0.6	
	whole fruit		0.2	0.1
50% emulsifiable concentrate[a]	mature leaves	2.4		7.5

[a] Equivalent to 30 g of AI/tree.

were 0.2 and 0.1 ppm, respectively, which was much lower than in the leaves.

ACKNOWLEDGMENT

The authors express their thanks to E. Dunkelblum for the analysis of croneton sulfone and phenol sulfone on GLC–MS and to L. Sorokski for her skillful technical assistance.

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Received for review March 31, 1978. Accepted October 31, 1978. Contribution No. 129-E, 1978 series.

Fate and Efficacy of Acephate after Application to Plants and Insects

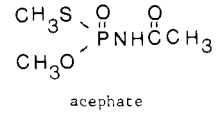
Don L. Bull

A single foliar application of ^{14}C-labeled acephate was absorbed rapidly by cotton leaves (>50% in 24 h), and unabsorbed residues were essentially depleted in 48 h. The absorbed acephate was metabolized by the leaves to small amounts (ca. 9% of dose) of the insecticide methamidophos and to lesser amounts (<5% combined) of at least four other products. Two of the latter four products were tentatively identified as *O,S*-dimethyl phosphorothioate and *S*-methyl acetylphosphoramidothioate. Absorbed acephate and/or its metabolites were rapidly translocated throughout the plant including the fruit. However, with normal application methods, any such translocation of toxicants that might occur is apparently insufficient to kill pests that feed on new growth or fruit. Acephate was considerably more toxic to third-stage tobacco budworms, *Heliothis virescens* (F.), than to adult boll weevils, *Anthonomous grandis* Boheman, in tests with topical applications to the insects and with bioassays of treated cotton foliage. Some evidence obtained in studies of the absorption and metabolism of ^{14}C-labeled acephate by the two species suggests that the metabolic conversion of the chemical to methamidophos in tobacco budworms may contribute to the observed differences in susceptibility between species.

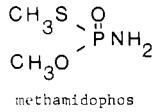
The organophosphorus insecticide acephate (*O,S*-dimethyl acetylphosphoramidothioate) is registered by the Environmental Protection Agency for use in controlling a broad spectrum of arthropod pests of plants. This chemical is recommended for use in cotton production but little is known of its fate after application to plants or of its effects on key pests of that crop. The present report describes the absorption, translocation, and metabolism of acephate in cotton plants and its fate and efficacy after treatment of two important pests of cotton.

MATERIALS

Chemicals. Radioactive (two different samples labeled with ^{14}C at the *S*-methyl position, 3.0 and 4.8 mCi/mmol) and nonradioactive acephate, as well as certain of its theoretical toxic [methamidophos (Monitor), *O,S*-dimethyl phosphoramidothioate] and nontoxic (I, *O,S*-dimethyl phosphorothioate; II, *S*-methyl acetylphosphoramidothioate) metabolites, were provided by Chevron Chemical Co., Richmond, CA. For all tests, ^{14}C-labeled acephate was diluted with sufficient nonradioactive material to produce a final specific activity of ca. 10 000 cpm/μg.



acephate



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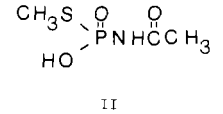

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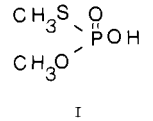

I

Plants and Insects. Cotton plants used were Stoneville 213 variety grown in the standard way either in the greenhouse or field. Tobacco budworms, *Heliothis virescens* (F.), used in the test were taken from laboratory cultures of (1) an insecticide-susceptible (S) strain maintained through many generations without introduction of new stock and (2) an insecticide-resistant (R) strain tested as the F_1 generation from parental stock collected in cotton fields that were under heavy insecticide treatment. Adult boll weevils, *Anthonomus grandis* Boheman, used in the test were obtained from the Robert T. Gast rearing facility at Mississippi State, MS.

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Table I. Fate of ^{14}C-Labeled Acephate after Application to Surfaces of Individual Leaves of Field-Grown Cotton[a]

hours post-treatment	distribution, % of dose			
	external rinse	internal extract	unextractable	lost
0	100.0			
2	70.7	22.1	1.3	5.9
4	57.0	27.7	2.4	12.9
8	42.0	35.0	3.6	19.4
24	17.9	47.9	7.0	27.2
48	5.5	39.2	9.0	46.3
96	1.8	29.7	16.9	51.6

[a] Dose was 100 μg/leaf; data are averages of three replicates.

Analytical Methods. In studies of the fate of ^{14}C-labeled acephate in plants and insects, the radioactivity in different samples was measured via conventional liquid scintillation techniques. After appropriate volume reduction under vacuum, the radioactive materials in different solvent extracts were analyzed with two-dimensional development on thin-layer chromatography (TLC) plates precoated with silica gel (Silplate F-22, 0.25-mm gel thickness; Brinkmann Instruments, Westbury, NY). Solvent systems consisted of (A) 6:5:2, isopropyl alcohol–benzene–ammonium hydroxide and (B) 10:2:4, benzene–diethylamine–methanol. Identifications were based on the coincidence of radioactive areas located by exposing plates to X-ray films with authentic standards that were visualized by exposure to iodine vapor. Radiocarbon that could not be extracted from certain insect and plant tissues, and all of that in plants used in translocation studies, was determined with an oxygen combustion procedure (Bull and Ivie, 1976).

TEST PROCEDURES AND RESULTS

Absorption and Photodecomposition. The fate of foliar applications of ^{14}C-labeled acephate was determined after uniform manual distribution of an aqueous solution (100 μg in 100 μL of 0.1% Triton X-100 per leaf) over the upper surfaces of individual, fully expanded leaves of field-grown cotton. In each test, triplicate samples of treated leaves were collected at the specified times posttreatment; unabsorbed radioactivity was recovered by rinsing leaves thoroughly with methanol, and then absorbed materials were extracted by homogenizing rinsed leaves with a mixture of acetone and water (9:1, v/v). Solids separated by centrifugation were reextracted twice and then dried for combustion analyses. The external rinses and (combined) internal extracts were radioassayed and analyzed separately as described.

The results (Table I) indicated that the applied ^{14}C-labeled acephate disappeared rapidly from surfaces of leaves (>80% loss in 24 h). Levels of radiocarbon accumulated rapidly in leaves, as evidenced by a >50% recovery of the applied dose in internal extracts after 24 h, and then declined at later times posttreatment. After 96 h, ca. 17% of the dose was bound in unextractable form by leaf tissue and ca. 52% was lost presumably to volatilization and translocation.

Analyses of unabsorbed radioactive materials indicated there was little apparent photodecomposition of ^{14}C-labeled acephate (Table II) to products that could be detected and identified with the methods used. The parent compound was predominant in most extracts and only minor amounts (<5% of dose) of the known degradation products I and II and unidentified radioactivity [unknown(s) A] were detected at any time posttreatment (See Table III for R_f values on TLC.)

Table II. Degradation of ^{14}C-Labeled Acephate on the Surfaces of Treated Cotton Leaves[a]

hours post-treatment	% of dose as indicated compound			
	unknown A	I	II	acephate
0	0.0	1.8	1.0	97.2
2	0.3	2.0	2.2	66.2
4	0.2	2.0	2.7	52.1
8	0.2	1.6	1.9	38.3
24	0.2	2.2	1.7	13.8
48	0.1	1.0	1.2	3.2
96	0.0	0.6	0.6	0.6

[a] Data represent TLC analyses of external rinse from tests described in Table I.

Table III. Fate of ^{14}C-Labeled Acephate in Field-Grown Cotton after Treatment of Leaves by Petiole Injection[a]

nature of radioactivity	TLC R_f[b]		% of dose at indicated days posttreatment				
	A	B	0	1	3	7	14
acephate	0.24	0.71	93.5	79.7	61.6	38.3	16.6
methamidophos	0.76	0.79	0.6	5.6	8.5	9.1	5.3
I	0.15	0.60	0.5	0.4	0.8	0.8	0.7
II	0.10	0.53	0.5	0.5	1.2	1.3	1.1
unknown 1	0.00	0.00	0.1	0.4	0.7	1.1	0.9
unknown 2	0.05	0.07	0.0	0.3	0.7	1.1	0.7
unextractable			4.8	12.9	17.1	24.4	30.8
lost			0.0	0.2	9.4	23.9	43.9

[a] Dose was 100 μg/leaf; data are averages of six replicates. [b] Solvent systems: (A) isopropyl alcohol–benzene–NH_4OH, 6:5:2, v/v, and (B) benzene–diethylamine–methanol, 10:2:4, v/v.

Metabolism. Fully expanded leaves of field-grown cotton were each treated by petiole injection (Bull et al., 1967) with a solution of 100 μg of ^{14}C-labeled acephate in 10 μL of water. Then triplicate samples were collected at the specified times posttreatment and extracted and analyzed as described. The results (Table III) indicated that recoveries of the parent compound declined steadily to a level of ca. 17% of the dose after 14 days. In the leaves, acephate was metabolized to the toxic product methamidophos, which accumulated to a peak level of 9.1% of the dose after 7 days, and to minor concentrations of I and II and two unidentified products. Unextractable radioactivity accumulated to ca. 31% of the dose after 14 days, and ca. 44% was lost (presumably to translocation).

Translocation. The apparent rapid movement of ^{14}C-labeled acephate and/or its metabolites from treated leaves was investigated further by using whole field-grown cotton plants. Small fruiting plants were selected for uniformity of size and development, and then several main-stem leaves were each treated by petiole injection with a solution of 100 μg of ^{14}C-labeled acephate in 10 μL of water. At 1 and 3 weeks posttreatment, treated plants were collected and divided into several subsamples of different plant parts. The samples were weighed, dried 48 h in an oven at 40–50 °C, weighed again, and ground to a fine powder in a Wiley mill, and then suitable aliquots were analyzed for radiocarbon content by oxygen combustion.

The results (Table IV) established that much of the radioactive material injected into leaves was subsequently translocated throughout the plant. After 3 weeks, ca. 56% of the recovered radioactivity was found in plant parts other than the treated leaves; most (ca. 70%) of this translocated radiocarbon was associated with fruiting forms. A major portion of the radioactive material that accumulated in the fruit was in the bract (0.5–2.1 ppm)

Table IV. Translocation of ^{14}C-Labeled Acephate after Treatment of Individual Leaves of Field-Grown Cotton by Petiole Injection[a]

plant part	% of applied radiocarbon recovered at indicated weeks posttreatment	
	1	3
TL[b]	74.8	31.3
FB above TL		
stem	0.4	0.9
leaves	0.5	1.3
fruit[c]	3.0	26.3
FB below TL		
stem	<0.1	0.5
leaves	0.1	0.9
fruit	<0.1	0.2
stalk above TL	1.5	4.3
stalk remaining		2.2
root	1.1	1.1
terminal leaves	0.4	0.6
terminal fruit	0.2	
fallen fruit		1.0
total recovery	82.2	70.6

[a] Dose was 100/μg of leaf; seven leaves treated/plant. [b] TL, treated leaves; these were attached to the main stem, the first near the base of the plant and the last near the top. FB, fruiting branches; those designated "above TL" were each attached to the main stem immediately above each of the seven treated leaves; those designated "below TL" represent all such branches located below the first TL near the base of the plant. [c] All fruit at 1 week were squares; most were bolls at 3 weeks.

Table V. Fate of ^{14}C-Labeled Acephate at 2 Days after Injection into a Stem of a Field-Grown Cotton Plant[a]

plant part[b]	wet wt, g	μg in extract[c]	μg in residue[c]	% of extracted radioactivity as-		
				unkn 1	acephate	methamidophos
leaves	6.7	152.0	8.5	1.0	93.5	5.5
bracts	2.7	102.9	11.5	1.0	92.5	6.5
bolls	15.1	57.5	8.3	2.0	83.5	14.5
blooms	3.4	3.7	1.2			
squares	1.7	0.5	0.3			
stem	3.5		6.6			

[a] Dose was 400 μg; data are from a single test. [b] Bracts were combined from seven fruiting forms: three squares, two blooms, and two bolls (one large, one small, both immature). Stem was dried, milled, and combusted. [c] Microgram equivalents of ^{14}C-labeled acephate.

and calyx (3.3–6.2 ppm), but appreciable quantities (2.9–5.1 ppm) were also found in the seed. At 1 and 3 weeks posttreatment, ca. 82 and 71%, respectively, of the applied dose was recovered. That portion of the dose unaccounted for may have been lost by volatilization of ^{14}C-labeled acephate or radioactive products of its decomposition (either naturally or during drying).

In further attempts to characterize the distribution of translocated radioactive materials, large quantities (400 μg in 20 μL of water) of ^{14}C-labeled acephate were injected into the main stems of fruiting branches (each bearing fruit in varying stages of development), and then the different plant parts were extracted and analyzed at 2 days posttreatment. The injection was made in the base of the stem at a point ca. 3 cm from the stalk. Results of the test (Table V) provided additional evidence of the movement of radioactive material into the fruit; much of this was recovered from bracts (ca. 29% of the recovered radiocarbon was in bracts accumulated from all fruit), but appreciable quantities were also found in immature bolls

Table VI. Rate of Absorption of ^{14}C-Labeled Acephate by Third-stage Tobacco Budworms (TBW) and Adult Boll Weevils (BWv)

hours posttreatment	insect	distribution, % of dose			% recovery
		external	internal	excreta	
0	S-TBW	99	1		100
	R-TBW	99	1		100
	BWv	92	8		100
2	S-TBW	71	8	12	91
	R-TBW	70	7	13	90
	BWv	67	15	6	88
4	S-TBW	62	6	19	87
	R-TBW	71	11	14	96
	BWv	61	16	11	88
8	S-TBW	40	12	23	75
	R-TBW	52	11	20	83
	BWv	48	18	16	82

(ca. 14% in whole bolls) and lesser amounts in blooms (ca. 1%) and squares (ca. 0.1%). Analyses of different extracts with TLC indicated that the metabolism of acephate in leaves and bracts was comparable to that discussed previously for injected leaves. It is noteworthy that most (83.5%) of the radioactivity extracted from immature bolls was acephate and that 14.5% was methamidophos. About 90% of the injected radioactivity was recovered from the different subsamples.

Absorption and Metabolism by Insects. Adult boll weevils (15–20 mg/each) and third-stage S and R tobacco budworm larvae (25–30 mg/each) were each treated with 1 μg of ^{14}C-labeled acephate in 1 μL of acetone by topical application to the dorsum. Then the insects were confined without food at 25 °C—individually in glass vials (budworms) or in groups of ten in 125-mL erlenmeyer flasks (weevils). At specified times posttreatment, treated insects (10/replicate per time, six replicates) were rinsed five times with successive 5-mL aliquots of acetone to recover unabsorbed radioactive material (external). Radioactive material excreted by treated insects or lost by physical contact was recovered from holding containers by first scrubbing with a small amount of water and then rinsing with acetone (excreta). After removal of unabsorbed radioactive material, insects were homogenized as described for cotton leaves with a mixture of acetone and water (internal); the latter two extracts were analyzed by TLC as described.

Test results (Table VI) indicated that the rate at which topically applied ^{14}C-labeled acephate disappeared from the external surfaces of insect bodies was ca. the same for both species at all times posttreatment. It appeared that there were some minor differences between S and R tobacco budworms in the diminution of unabsorbed radioactivity and that the rate was slower with the R strain; however, differences in total recovery of applied materials tend to negate any definite conclusions. The data indicate that there was a more rapid accumulation of internal radioactivity in weevils than in tobacco budworms. Greater amounts of radioactive material were recovered from the containers previously occupied by treated tobacco budworms but, with the procedure used, it was impossible to separate excreted radioactivity from that rubbed off through contact of treated insects with inner surfaces of containers.

TLC analyses of pooled extracts from the absorption tests indicated that absorbed acephate was metabolized to small amounts of methamidophos in tobacco budworm larvae but apparently was unchanged in adult boll weevils. The amounts of methamidophos detected in internal

extracts of S and R tobacco budworms were essentially the same, ca. 1% of the dose at 2 h posttreatment and 2% after 8 h. Only trace amounts of products I and II (<1% of dose) were detected in any of the internal and excreta extracts; these extracts consisted almost entirely of acephate.

Comparative Toxicity of Acephate and Methamidophos. The relative toxicity of the two insecticides to tobacco budworm larvae and adult boll weevils was evaluated by treating the insects topically and also by confining them on treated cotton foliage where they were exposed to the chemicals in two ways—by ingestion and by contact.

For tests of topical applications, third-stage S and R tobacco budworms and adult boll weevils were treated with graded doses of each chemical and held 72 h on untreated food for observations of mortality. Acetone solutions of the chemicals were applied to the dorsum of each insect with a micrometer-driven syringe calibrated to deliver the desired concentration in 1 μL volume. Control insects were treated only with acetone. Ten insects were treated with each dose, and tests were replicated at least three times; dosage mortality data were calculated by using a computer programmed for probit analyses. (Methyl parathion was used as a standard in these tests since it is the product used most in commercial cotton production.)

For tests of feeding or contact toxicity, potted cotton plants from a greenhouse were sprayed with aqueous preparations of the insecticides at rates equivalent to those used in the field. At the specified times posttreatment, 12 leaves from each treatment were collected and placed individually in ventilated glass containers. Then four leaves from each treatment at each sample time were bioassayed with third-stage tobacco budworm larvae (three/container) and adult boll weevils (ten/container). For most studies, the leaves bioassayed were those present on the plant during treatment. However, both leaves and fruit that developed after treatment were also bioassayed to determine whether toxicants were translocated there from treated foliage in concentrations sufficient to kill the pests. Control insects were held on untreated leaves for each time. Mortality was observed over a 72-h period; tests were replicated three times.

Results of tests of topical application (Table VIIA) indicated that acephate was substantially more toxic to both strains of tobacco budworm larvae than to adult boll weevils. Methamidophos was ca. 75 times more toxic than acephate to weevils but was generally less toxic than acephate to tobacco budworms. Differences in the toxicity of the two compounds to the S and R strains of tobacco budworm were less (1.6- to 2.8-fold) than the differences in toxicity of methyl parathion (8.7-fold). Bioassays of treated plants (Table VIIB) provided somewhat similar results; activities of acephate and methamidophos against tobacco budworms were ca. the same at all times posttreatment, but methamidophos treatments were definitely more effective against boll weevils. Only the foliage present at the time of treatment was toxic to the insects in the bioassays; no mortality was observed when they were fed or exposed to new leaves or fruit that developed subsequent to insecticide application. (Even when multiple applications (two–three) of acephate were made at 5-day intervals, any retranslocation of toxicants to new leaves or fruit that may have occurred was insufficient to kill the pests.) Moreover, during the course of the tests with treated potted plants, natural infestations of greenhouse white flies, *Trialeurodes vaporariorum* (Westwood), avoided treated foliage but fed freely on leaves that developed subsequent to insecticide applications.

Table VII. Insecticidal Activity of Acephate and Methamidophos

A	topical toxicity, LD_{50} at 72 h posttreatment					
	boll weevil		tobacco budworm (S)		tobacco budworm (R)	
	μg/g	slope	μg/g	slope	μg/g	slope
acephate	937.7	1.6	13.3	1.1	36.7	0.7
methamidophos	12.6	1.3	23.7	0.9	37.7	0.8
methyl parathion	12.6	2.4	3.0	2.5	26.0	0.8

B	% mortality at 72 h after exposure to treated foliage[a]			
	acephate		methamidophos	
days posttreatment	boll weevil	tobacco budworm (S)	boll weevil	tobacco budworm (S)
0	47.5	100.0	97.5	100.0
3	12.5	83.3	32.5	83.3
7	5.0	25.0	20.0	58.3
14	0.0	33.3	22.5	50.0
21	0.0	0.0	5.0	0.0

[a] Chemicals were applied at a rate equivalent to 1.12 kg/ha; no mortality was observed in the untreated controls.

DISCUSSION

These studies clearly demonstrated that acephate applied as a foliar spray was readily absorbed by cotton plants. Due possibly to its brief residual life on foliar surfaces, the applied acephate apparently was not extensively degraded via photodecomposition. Within the cotton plant, as in pine seedlings (Werner, 1974), the chemical was metabolized by cleavage of the amide bond of the molecule to form appreciable amounts of the insecticide methamidophos. Lesser amounts of nontoxic metabolites I and II were produced by cleavage of the P–N bond of acephate (or methamidophos) and the P–O methyl linkage, respectively. It is also possible that some cleavage of the P–S–methyl linkage of acephate occurred, as was found in houseflies, *Musca domestica* L. by Kao and Fukuto (1977). Since the P–S–methyl moiety of the acephate molecule was the site of the radiocarbon label, such a reaction might account for at least some of the decline in radiobalance during the latter phases of both plant and insect studies.

Eto et al. (1977) reported that active intermediates with potent anticholinesterase activity were formed by chemical (and perhaps biochemical) oxidation of acephate and methamidophos. From certain chemical evidence, these authors concluded that the product formed by activation of methamidophos was the sulfoxide derivative. Biological activation is thought to be essential to the ultimate insecticidal activity of these two chemicals; both are poor anticholinesterase agents in vitro but have good in vivo inhibitory activity (Suksayretrup and Plapp, 1977). However, in the present study, no direct evidence of the formation of such metabolites was found.

Within the plant, acephate and/or its metabolites were translocated from treated leaves to all parts of the plant, including the fruit. When acephate was injected directly into a fruiting branch, significant amounts of the chemical and its toxic metabolite methamidophos were subsequently found in the fruiting structures. The bracts contained the

major portion of products translocated to fruit and, although appreciable amounts of acephate and methamidophos were identified in large immature bolls, very little of these products accumulated in the buds either before or during bloom. Indeed, in some other studies (unreported) the movement of translocated materials into the fruiting forms appeared to be influenced by the developmental activity underway when the chemicals became available. Thus the source to sink distribution pattern detected in cotton suggests that acephate may have at least some limited symplastic (Crisp, 1972) systemic activity. That is, at least under certain conditions, it can move to some extent with the assimilate stream in the phloem tissue and accumulate in the fruit. However, more practical tests in the greenhouse with cotton plants treated by foliar application indicated that any translocation of toxicants to fruit was insufficient to kill major chewing pests such as boll weevils and tobacco budworms and that translocation to new growth was insufficient to afford protection against either chewing or sucking pests.

The tests comparing the topical toxicity of acephate and methamidophos to S and R tobacco budworms indicated S insects were only moderately more susceptible to the two phosphoramidothioate insecticides. Studies of the absorption of acephate by these insects indicated that, although the rates were quite similar, the absorption by R insects may have been slightly slower, as was found by Szeicz et al. (1973) in comparable studies with other insecticides. There was very little biotransformation of absorbed acephate by larvae of either strain, a finding which agrees with similar studies of S and R houseflies by Suksayretrup and Plapp (1977) but is quite unlike those reported in similar comparative studies of tobacco budworms (Whitten and Bull, 1970, 1974, 1978; Bull and Whitten, 1972; Williamson and Schechter, 1970). (These latter studies demonstrated that R insects had a substantially enhanced capability to detoxify a variety of different structural types of organophosphorus and other insecticides.) However, the present results may help explain the similarities in toxicity of acephate and methamidophos to the two strains of tobacco budworm and they do lend support to speculation by Plapp (1972) that the two chemicals have certain structural characteristics that somehow circumvent the resistance mechanisms of tobacco budworms.

The case of the boll weevil was quite different. Acephate had low insecticidal activity against adult boll weevils, but methamidophos was highly toxic (ca. 75-fold difference at LD_{50} level). Since absorption tests demonstrated that acephate was absorbed rapidly by boll weevils, its low toxicity may be attributable to the apparent inability of the insect to metabolize the chemical to methamidophos. The work of Eto et al. (1977) demonstrated that the biologically active intermediate produced by oxidation of methamidophos was a considerably more potent anticholinesterase agent than the comparable activation product of acephate.

These studies thus have provided some explanation of why acephate in field use is effective against *Heliothis* spp. but not against the boll weevil. Also, this work has shown that the compound has no significant plant systemic properties following conventional application; therefore, it is apparent that its insecticidal activity is manifested through direct contact with the insects or through their feeding on foliage to which the chemical was applied.

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Received for review August 4, 1978. Accepted October 30, 1978. This work was done in cooperation with Texas A&M University, Texas Agricultural Experiment Station, College Station, TX. This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended. Also, mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the U.S. Department of Agriculture.