

Table VI. Total Operator Exposure for Complete Herbicide Application Sequence<sup>a</sup>

application system	inhalation exposure, $\mu\text{g}/\text{kg}$	dermal deposition, $\mu\text{g}/\text{kg}$	total exposure, $\mu\text{g}/\text{kg}$
conventional tank fill + boom spray + disc incorporation	0.37	982.3	982.7
conventional tank fill + spray/harrow combination	0.55	987.3	987.8
closed system (Chemprobe) + spray/harrow combination	0.55	10.6	11.2

<sup>a</sup> Exposure values based on application to 20-acre plots.

pected during normal use of this herbicide.

The total exposure for any application system can be calculated by adding the values for individual operations. Several possibilities are shown in Table VI. Note that the use of the closed-system tank fill does not reduce the total inhalation exposure but significantly reduces the dermal deposition. The later result is believed to be mainly due to the use of neoprene gloves (as specified in label instructions) on top of the cotton gloves. Therefore, the most

important conclusion derived from this study is that the total operator exposure to diallate during herbicide applications can be reduced by almost 2 orders of magnitude by the use of neoprene gloves and a closed system for tank-fill operations.

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#### LITERATURE CITED

- Davies, J. E. *Residue Rev.* 1980, 34.  
 Durham, W. F.; Wolfe, H. R. *Bull. W.H.O.* 1962, 26, 75.  
*Fed. Regist.* 1977, 42 (153), 40009.  
 Louis, R. G.; Brown, A. R.; Jackson, M. D. *Anal. Chem.* 1977, 49, 1668.  
 Simon, C. G.; Bidleman, T. F. *Anal. Chem.* 1979, 51, 110.  
 Turner, B. C.; Glotfelty, D. E. *Anal. Chem.* 1977, 49, 7.  
 USDA "The Biologic and Economic Assessment of Diallylate". Submitted to EPA on Sept 12, 1977; USDA Agricultural Research: Washington, DC 20250, 1977; Technical Bulletin 1620.  
 Webb, P. "Bioastronautics Data Book"; NASA, National Technical Information System, U.S. Department of Commerce: Springfield, VA 22151, 1964.

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## Fall Armyworm Control and Residues of Methomyl on Coastal Bermuda Grass

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Methomyl, monocrotophos, and acephate effectively controlled fall armyworm larvae in stands of Coastal Bermuda grass, but carbaryl and trichlorfon were ineffective. Residues of methomyl, the compound selected for detailed studies, declined rapidly with time after application, and by 7 days about 7% of the initial deposit remained regardless of the rate of application. Methomyl loss during dehydration in a natural gas dryer was about 54%. The pelletizing process caused an additional loss of about 14%. Thus, the total loss of methomyl during processing of green hay to pellets amounted to about 68%. Losses of residues during air curing of hay in the field amounted to about 37%.

Coastal Bermuda grass [*Cynodon dactylon* (L.) Pers.] is a warm-season forage grass of major importance in the southeastern United States. With good management practices 2 to 4 times as much dry matter per unit area can be produced with Coastal than with common Bermuda grass (Dobson et al., 1974). It is grazed by cattle, cut and cured for hay, or cut, dehydrated, and ground for use in feed mixes for cattle, hogs, and poultry; the meal is also pelletized for animal feed.

A devastating infestation of fall armyworm (*Spodoptera frugiperda* J. E. Smith) developed on Coastal Bermuda grass in southeastern North Carolina in Aug and Sept 1975. The population of larvae was so great that severe defoliation and losses in yield occurred. There were moderate outbreaks in at least 2 years since 1975. Other southern states frequently have such infestations.

Reports of ineffectiveness of carbaryl (1-naphthyl *N*-methylcarbamate) and trichlorfon [dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate], commercial products

which are registered for control of fall armyworms on Bermuda grass, and the need for control prompted us to initiate field studies on the comparative effectiveness of carbaryl, trichlorfon, methomyl [*S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate], and monocrotophos [dimethyl phosphate ester with (*E*)-3-hydroxy-*N*-methylcrotonamide] in the fall of 1975. The studies were designed to evaluate control of fall armyworm larvae by ground and aerial applications and to supply samples of green, cured, and pelletized Coastal Bermuda grass for residue analyses. Portions of this work were reported briefly in abstract form (Campbell and Sheets, 1976).

#### EXPERIMENTAL SECTION

The study consisted of small-plot experiments in 1975, 1976, and 1977, which were treated with ground equipment, and large plots in 1975, which received aerial applications. Samples from all experiments were analyzed for residues of methomyl.

**Small-Plot Experiments.** In 1975, plots 7.5 by 15 m were laid out in Scotland County, NC, on a dense, well-established stand of Coastal Bermuda grass showing moderate to severe fall armyworm damage. Treatments were methomyl at 0.28, 0.56, and 1.1 kg/ha, monocrotophos

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at 0.28, 0.56, and 1.1 kg/ha, trichlorfon at 0.56 and 1.1 kg/ha, carbaryl at 1.1 and 2.2 kg/ha, and an untreated control. The treatments were arranged in a randomized block design with three replications. The insecticides as diluted commercial formulations were applied Sept 30, 1975, at 230 L/ha with a small, manually operated, ground sprayer mounted on bicycle wheels and having an effective spray width of 3.6 m. Water was the diluent. The grass had not been cut for about 1 month and was 15–25 cm tall at the time of treatment.

Counts of live and dead fall armyworms were made on all plots 1 and 3 days after application by counting the larvae on the ground and on the grass within five randomly selected sites 30 by 30 cm in size. A sample of green hay weighing 0.5–1 kg was clipped from six randomly selected squares (30 by 30 cm) for residue analysis. Control plots and all methomyl plots were sampled for residue analysis immediately after application and at 1, 3, 7, and 14 days after application. A sample also was clipped on days 1, 3, and 7 from a 0.6 by 1 m area and allowed to air cure before residue analysis. Different sites were selected for worm counts and for residue samples each sampling day. Green samples were packed in insulated boxes with solid carbon dioxide for transport to the laboratory. After being cured in the field for 1–4 days, air-dried samples were transported to the laboratory at ambient temperature, and all samples were stored at  $-18^{\circ}\text{C}$ .

The 1976 field experiment was treated July 26 on an infested site in Roberson County, NC. Treatments were the control, methomyl, monocrotophos, acephate (*O,S*-dimethyl acetylphosphoramidothioate), and carbaryl at 0.28, 0.56, and 1.1 kg/ha. Plot design, application methods, and sampling were the same as those described for the 1975 small-plot experiment.

The experiment in 1977 was identical with the 1976 test except the location was changed (Scotland County in 1977), the date of application was different (Aug 16, 1977), and air-cured samples were not taken in 1977.

**Large-Plot Tests for Aerial Application.** Plots for aerial applications were established at two locations about 13 km apart in Scotland County, NC. The grass was at the same growth state as that used for the ground application. There were three plots at one site (McLaurin field) and four plots at the other (McCormick field). Plots were 0.6 ha in size and were sprayed by fixed-wing aircraft on Sept 30, 1975. The unreplicated treatments were as follows: McLaurin field, methomyl at 0.28, 0.56, and 1.1 kg/ha; McCormick field, methomyl at 0.28, 0.56, and 1.1 kg/ha and trichlorfon at 1.1 kg/ha.

Adequate untreated areas were available at each site for control sampling. Water was used as the diluent. Volumes of solution applied were as follows:

insecticide	rate, kg/ha	diluent, L/ha
methomyl	0.28	9.4
	0.56	19
	1.1	38
trichlorfon	1.1	9.4

Counts of live and dead larvae were made at 1 and 3 days after application on five 30 by 30 cm sites in each of three areas of the large plots. Different sites were counted each day. Counts of armyworm numbers are reported as averages of the three areas.

Large samples of about 200 kg of green hay were harvested with conventional equipment from all aerially treated methomyl plots at 1, 3, and 7 days after application. The grass was dried in a natural gas furnace with the temperature of the drying head about  $425^{\circ}\text{C}$  in normal operation. The grass passed through the dryer in about

3 min. The time for passage through the dryer varied with the moisture content and volume of grass. The dried grass passed from the dryer into the hammer mill and then into the pellet mill. The pellet mill was operated at about  $200^{\circ}\text{C}$ . The time between harvest in the field and processing into pellets varied from 1 to 2 h. Samples of green chopped hay, dried hay, and pellets were packed in solid carbon dioxide on the site and transported to the laboratory for residue analysis.

**Residue Analysis.** At the time the field experiments were initiated in 1975, the decision was made to pursue a tolerance for methomyl through the Interregional Research Project 4 with national offices at New Brunswick, NJ. Thus, samples from methomyl plots and from methomyl-treated grass that was dehydrated for processed animal feed were analyzed for residues.

The method of analysis was similar to that developed by Pease and Kirkland (1968). For green samples 10 g of mixed, chopped grass was placed in a Soxhlet thimble and extracted for 3 h with 200 mL of a chloroform-methanol solution (1:1 v/v) at 10 cyclings/h. The volume of the crude extract was reduced to 2–3 mL under vacuum, and 50 mL of 0.1 N sodium hydroxide was added to the flask. The contents were swirled and then heated uncovered in a  $100^{\circ}\text{C}$  water bath for 15 min. The flask was stoppered and heated for 15 min more to allow the oxime to form. The contents were cooled in a water bath and neutralized with 1 N sulfuric acid, and the oxime was separated by extraction 3 times with 50-mL portions of methylene chloride using gentle shaking. Then, 0.2 mL of triethylamine was added to the oxime solution, and the flask was placed on a water bath at  $65^{\circ}\text{C}$ . The solution was concentrated to 2–3 mL and brought to volume for gas chromatography.

The residues were quantitated on a Tracor Model 222 gas chromatograph equipped with a flame photometric detector operated in the sulfur mode. Two columns were used at different times during the course of the study. One was 122 cm by 2 mm (i.d.), U-shaped glass packed with 10% OV-101 on Supelcoport (80–100 mesh). The temperatures of the inlet, column oven, and detector were 190, 100, and  $185^{\circ}\text{C}$ , respectively. Gases and flow rates were hydrogen 200 mL/min, air 100 mL/min, and nitrogen 69 mL/min. The other column was 92 by 0.64 cm (i.d.), U-shaped glass packed with 5% Carbowax 20M on Gas-Chrom Q (60–80 mesh). The temperatures of the inlet, column oven, and detector were 200, 180, and  $180^{\circ}\text{C}$ , respectively. Gas flow rates were hydrogen 50 mL/min, air 80 mL/min, and nitrogen 85 mL/min.

Known amounts of methomyl were added to untreated samples of green grass, dry grass, or pellets and to aqueous solutions (spikes) before extraction, and the fortified samples were analyzed to determine the efficiency of the procedure (Table I). Residue levels in experimental samples were not corrected for incomplete recovery.

**Statistical Analysis.** Analyses of variance were performed on counts of dead armyworms, on the percent control values from all experiments where counts were made (the small-plot experiments in 1975 and 1977 and the aerial tests in 1975), and on residue data from all experiments except the 1975 small-plot test. LSDs are reported for counts of dead larvae, for percentages of control of larvae, and for residues in ppm.

## RESULTS AND DISCUSSION

The infestations of fall armyworms on Coastal Bermuda grass in 1975 and in 1977 in southern North Carolina provided the opportunity to evaluate the effect of several insecticides on fall armyworm mortality. The data show

Table I. Recoveries of Methomyl Added to Untreated Coastal Bermuda Grass or Solvent Immediately before Extraction<sup>a</sup>

experiment	grass or solvent	fortification level, ppm	no. of samples	recovery, %	
				range	av
1975 ground application	green grass	0.5-50.0	15	53-88	77
	solvent	0.5-50.0	15	51-92	64
	air-cured grass	0.5-50.0	15	46-90	66
1975 aerial application	solvent	0.5-50.0	15	50-76	60
	green grass	5.0-50.0	14	45-90	61
	solvent	5.0-50.0	14	50-76	60
	dehydrated grass	0.25-5.0	5	55-87	65
	solvent	0.25-5.0	5	50-79	62
	pellets	0.25-2.5	5	54-84	75
1976 ground application	solvent	0.25-2.5	5	51-83	68
	green grass	10.0-100.0	5	60-98	83
	solvent	10.0-100.0	5	66-100	77
	cured grass	2.0-25.0	3	83-96	88
1977 ground application	solvent	2.0-25.0	3	82-103	94
	green grass	0.25-100.0	6	64-96	77
	solvent	0.25-100.0	6	56-75	67

<sup>a</sup> Fortified samples and solvents were converted to the oxime. Quantitation was based on the oxime standard.

Table II. Control of the Fall Armyworm on Coastal Bermuda Grass with Ground Application of Insecticides in Scotland County, NC, in 1975 and 1977

insecticide	rate of application, kg/ha	1975						1977					
		av no. of dead larvae <sup>a</sup>			av % control			av no. of dead larvae <sup>a</sup>			av % control		
		day 1	day 3	av	day 1	day 3	av	day 1	day 3	av	day 1	day 3	av
methomyl	0.28	36.0	40.0	38.0	85.9	93.3	89.6	14.0	14.3	14.2	96.1	97.4	96.8
	0.56	43.7	43.3	43.5	95.6	96.7	96.2	20.7	18.3	19.5	100.0	100.0	100.0
	1.12	69.7	55.7	62.7	93.9	100.0	97.0	21.3	17.7	19.5	98.6	100.0	99.3
monocrotophos	0.28	64.0	54.3	59.2	89.8	98.5	94.2	28.0	21.3	24.7	95.1	98.9	97.0
	0.56	59.7	81.7	70.7	93.8	99.2	96.1	24.3	18.7	21.5	98.9	100.0	99.5
	1.12	70.0	71.7	70.8	97.9	99.2	98.6	21.3	19.3	20.3	100.0	100.0	100.0
carbaryl	1.12	13.7	4.7	9.2	21.8	15.7	19.0	0.3	2.3	1.3	1.2	6.9	4.1
	2.24	8.0	4.3	6.7	9.8	9.4	9.8						
trichlorfon	0.56	5.0	5.0	5.0	9.3	13.2	11.3						
	1.12	6.3	8.7	7.5	10.0	26.2	18.2						
acephate	0.28							5.0	11.7	8.3	30.1	56.6	43.4
	0.56							16.3	21.7	19.0	76.8	100.0	88.4
	1.12							23.3	17.3	20.3	86.6	100.0	93.3
control	0	0	0	0	0	0	0	0	0	0	0	0	0
LSD <sub>0.05</sub>		23.2	26.3	22.1	9.1	9.9	5.3	10.5	7.9	8.6	10.3	9.5	7.3

<sup>a</sup> Average number of fall armyworm larvae counted in five 30 by 30 cm sites. Different sites were counted on day 1 and day 3. Data for carbaryl applied at 0.28 and 0.56 kg/ha were omitted from the table.

that methomyl and monocrotophos at rates of 0.28-1.12 kg/ha and acephate at 0.56 and 1.12 kg/ha effectively controlled larvae of all ages present on sites used for the small-plot experiments in 1975 and 1977 (Table II). Carbaryl and trichlorfon, the two insecticides that were labeled for fall armyworms on Bermuda grass when this research was initiated, were both ineffective.

A natural infestation of fall armyworms was present at the time the site for the 1976 test was selected. A rapid population decline occurred soon after, apparently caused by disease, and at the time of application larvae were scarce on the site. Therefore, biological data were not obtained in 1976.

At the time the aerial tests were sprayed in 1975, the decision had been made to pursue registration of methomyl for fall armyworm on Bermuda grass. Hence, the aerial applications were restricted to methomyl except for one rate of trichlorfon at one site. Trichlorfon was ineffective, providing less than 2% control. In contrast, methomyl, applied by air as an aqueous spray, gave good control at 0.56-1.12 kg/ha at both locations (Table III). The larvae were larger (2.5-4.5 cm in length) at the McLaurin location than at the McCormick field (<2 cm in length), and methomyl was somewhat less effective on the large larvae with only 26% mortality (McLaurin location) at the 0.28 kg/ha

Table III. Control of the Fall Armyworm with Aerially Applied Methomyl at Two Locations in Scotland County, NC, in 1975

location	rate of application, kg/ha	av no. of dead larvae <sup>a</sup>			av % control		
		day 1	day 3	av	day 1	day 3	av
McLaurin field	0.28	15.0	11.3	13.2	37.8	26.2	32.0
	0.56	32.7	37.7	35.2	68.6	97.5	80.0
	1.12	56.0	50.7	53.4	89.3	100.0	94.6
	0	0	0	0	0	0	0
McCormick field	0.28	18.7	21.3	20.0	62.3	95.4	78.8
	0.56	30.7	24.3	27.5	85.5	98.2	91.8
	1.12	21.7	22.0	21.8	94.9	100.0	97.4
	0	0	0	0	0	0	0

<sup>a</sup> Average number of fall armyworm larvae counted in five 30 by 30 cm sites. Different sites were counted on day 1 and day 3.

rate compared to about 95% mortality of small larvae (McCormick location) 3 days after application.

Residues of methomyl on green Coastal Bermuda grass immediately after application, averaged for the 3 years, were 11.1, 21.6, and 53.4 ppm, respectively, for the 0.28, 0.56, and 1.12 kg/ha rates (Table IV). The levels declined

Table IV. Residues of Methomyl in Green Coastal Bermuda Grass As Affected by Rate of Application and Time (Small-Plot Experiments)<sup>a</sup>

time after application, days	rate of application, kg/ha	1975 residue		1976 residue		1977 residue		av residue, ppm
		ppm	%	ppm	%	ppm	%	
0	0.28	9.8	100	12.9	100	10.7	100	11.1
	0.56	23.5	100	22.1	100	19.9	100	21.6
	1.12	63.0	100	47.8	100	49.4	100	53.4
1	0.28	5.7	58	4.72	37	4.52	42	4.98
	0.56	16.6	71	9.12	41	13.6	68	13.1
	1.12	47.1	75	24.1	50	20.1	41	30.4
3	0.28	1.5	15	3.01	23	1.12	10	1.88
	0.56	4.0	17	5.96	27	1.46	7	3.81
	1.12	12.3	20	11.1	23	4.23	18	9.21
7	0.28	1.2	12	0.69	5	0.48	4	0.79
	0.56	2.3	10	1.49	7	1.07	5	1.62
	1.12	7.0	11	2.01	4	2.01	4	3.67
14	0.28	0.08	0.8	0.20	2	0.20	2	0.16
	0.56	0.79	3	0.33	1	0.46	2	0.53
	1.12	1.30	2	0.92	2	1.37	3	1.20
LSD <sub>0.05</sub>				1.52		5.30		

<sup>a</sup> Except for one sample that contained 0.06 ppm, residues on samples from untreated controls were <0.05 ppm, the lowest detectable limit. The 1975 data were not analyzed statistically because there were several missing values. Values for 1975, 1976, and 1977 are based on average moisture contents of 68.4, 67.6, and 68.0%, respectively. In 1976, rain amounting to 2.2 cm fell on the sixth day after application, and in 1977, 6.9 cm fell on the second day after application. Otherwise, only trace amounts of rain fell during the experimental periods.

Table V. Residues of Methomyl on Air-Cured Coastal Bermuda Grass As Affected by Rate of Application and Time between Treatment and Harvest (Small-Plot Experiments)<sup>a</sup>

time after application, days	rate of application, kg/ha	1975 residue, ppm	1976 residue, ppm
1	0.28	14.5	6.48
	0.56	23.5	17.4
	1.12	58.9	30.8
3	0.28	3.8	0.68
	0.56	7.6	1.56
	1.12	13.3	4.42
7	0.28	2.4	1.27
	0.56	3.8	2.05
	1.12	9.2	3.55
LSD <sub>0.05</sub>		6.8	3.91

<sup>a</sup> Values for untreated controls in 1975 varied from <0.2 to 0.4 ppm; in 1976 all were <0.05 ppm. Values for 1975 and 1976 are based on average moisture contents of 15 and 13%, respectively. Only trace amounts of rain fell during the sampling period in 1975; in 1976, rain amounting to 2.2 cm fell on the sixth day after application while the samples cut on day 3 were still in the field.

rapidly with time after application, and by 7 days about 7% of the initial deposit remained regardless of the rate applied.

The data were not combined over years for statistical analysis, but from a review of the data in Table IV one can conclude that major differences are probably related to rainfall. For example, a heavy rain (6.9 cm) fell on day 2 in 1977, and average residues at day 3 were lowest on 1977 samples. By day 14 differences in levels between years had largely disappeared (Table IV). Day 14 residues varied between about 1 and 3% (of the amount present at day 0) for all rates in the 3 years.

Residues of methomyl on samples of air-cured grass generally were higher (Table V) than those for corresponding samples of green uncured grass (Table IV). If residues for green grass and those for air-cured hay are converted to the same moisture base, the loss of methomyl during air curing can be estimated. Such calculations show that, although most residue values reported in Table V for

Table VI. Methomyl Residues at Different Stages of Processing Coastal Bermuda Grass<sup>a</sup>

time between application and harvest, days	rate of application, kg/ha	green hay, ppm	cured hay, ppm	milled hay, ppm	pellets, ppm
1	0.28	5.4	5.5	7.4	4.5
	0.56	12.8	28.0	25.6	15.8
	1.12	51.1	45.2	39.2	35.5
3	0.28	0.9	0.8	0.9	0.7
	0.56	2.6	2.4	2.8	2.0
	1.12	5.9	6.6	6.2	4.8
7	0.28	0.4	0.7	0.4	0.4
	0.56	1.1	0.8	1.0	0.7
	1.12	1.9	2.3	2.2	1.8
LSD <sub>0.05</sub> time × rate		12.5	N.S.	11.0	9.1

<sup>a</sup> Each value is an average of four analyses (two subsamples from two aerial plots). The residues on green, cured, milled, and pelletized Coastal Bermuda grass are based on average moisture contents of 64.9, 10.1, 5.41, and 6.66%, respectively.

air-cured grass are greater than corresponding values for uncured (green) grass (Table IV), the actual amount of methomyl present on the grass dropped during air curing. Losses varied from about 6 to 92%. Consistently high losses (92, 90, and 85%, respectively, for the 0.28, 0.56, and 1.12 kg/ha rates) occurred on grass harvested on day 3 and air cured in 1976. These losses were far greater than all others for air curing, the next closest percentage loss being 71, and were caused in part by the 2.2 cm of rain that fell while the samples were in the field.

Methomyl residues on green hay, cured hay, milled hay, and pellets of Coastal Bermuda grass, based on the average percent moisture in the different forms, did not vary greatly (Table VI). Calculations show, however, that the dehydration process in the natural gas furnace reduced the actual amount of methomyl present. The average loss during dehydration was about 54%. Milling did not appear to alter the amount of methomyl, but pelletizing caused an additional loss of about 14% (or 27% of that present on dried hay), making the total loss from green hay to pellets average about 68%. The temperature in the

pellet mill was about 200 °C so the decline in methomyl levels was expected.

The average loss of methomyl residues during air curing was about 39% if the effect of rainfall is eliminated or about 46% if values for those samples that were rained upon are included. Thus, dehydration and pelletizing collectively had a much greater effect on residues than air curing.

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#### LITERATURE CITED

- Campbell, W. V.; Sheets, T. J. *J. Elisha Mitchell Sci. Soc.* **1976**, *92* (2), 68.  
 Dobson, S. H.; Kimbrough, E. L.; Baird, J. V.; Burns, J. C.; et al. *N.C. State Agric. Ext. Serv. Circ.* **1974**, No. 451, 1-15.  
 Pease, H. L.; Kirkland, J. J. *J. Agric. Food Chem.* **1968**, *16*, 554.

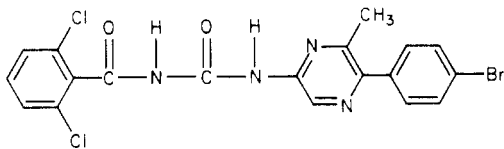
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## Fate of an Insect Growth Regulator EL-494 in Soybean Callus Tissue, Soybean Plants, and Gypsy Moth Larvae

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The metabolism of EL-494 [*N*-[[[5-(4-bromophenyl)-6-methyl-2-pyrazinyl]amino]carbonyl]-2,6-dichlorobenzamide], a potential insecticide which inhibits chitin synthesis, was investigated in soybean plant, soybean callus tissue, and gypsy moth larvae. Plants were incubated with [<sup>14</sup>C]benzoyl-labeled EL-494 for 14 days. Callus tissues were incubated with [<sup>14</sup>C]benzoyl- and 2-[<sup>14</sup>C]pyrazinyl-labeled EL-494 for varying times (3, 6, 12, and 24 days). Gypsy moth larvae (fifth and sixth instars) were incubated with [<sup>14</sup>C]benzoyl-labeled EL-494 for 7 days. Only two [<sup>14</sup>C]benzoyl-labeled metabolites were found in the plant tissue or the gypsy moth larvae, and these were 2,6-dichlorobenzamide (6.1-16.2% in plant tissue and 5.6-8.3% in gypsy moth larvae) and 2,6-dichlorobenzoic acid (0.3-2.0% in plant tissue and ~0.3% in gypsy moth larvae). 2-Amino-5-(4-bromophenyl)-6-methylpyrazine was the only [<sup>14</sup>C]-pyrazinyl-labeled metabolite of EL-494 found in the plant extracts. The metabolic degradation products generated by the soybean callus tissue were qualitatively and quantitatively similar to those of whole plants. Gypsy moth larvae eliminated ~27-31% of the [<sup>14</sup>C]EL-494 by excretion.

EL-494 [*N*-[[[5-(4-bromophenyl)-6-methyl-2-pyrazinyl]amino]carbonyl]-2,6-dichlorobenzamide; I] is a



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new chemical suggested for possible use as an insecticide.

It has molt-inhibiting properties and has been shown to have insecticidal properties against the southern armyworm, *Spodoptera eridania* (Cramer), tobacco budworm, *Heliothis virescens* (F.), fall armyworm, *Spodoptera frugiperda* (J. E. Smith), yellow fever mosquito, *Aedes aegypti* (L.), and housefly, *Musca domestica* (L.) (Lilly Research Laboratory, 1977). Retnakaran (1979) found that EL-494 was more active than Dimilin against the spruce budworm, *Choristoneura fumiferana* (Clemens), in laboratory tests. The EC<sub>50</sub>, determined by diet tests, was 0.205 ppm for the third, 0.249 for the fourth, 0.287 for the fifth, and 0.486 for the sixth instars. In greenhouse tests he demonstrated that EL-494 was resistant to leaching and UV degradation and the compound remained active on potted balsam fir trees, *Abies balsamea* (L.), and white spruce trees, *Picea glauca* (Moench), for at least 15 days. In laboratory tests EL-494 reduced the survival of the pink

bollworm larvae, *Pectinophora gossypiella* (Saunders), more than several other chitin synthetase inhibitors including Dimilin (Flint et al., 1978). Abdel-Monem et al. (1980) demonstrated that EL-494 affected the molting process of the gypsy moth larvae, *Lymantria dispar* (L.), at 0.39-0.88 ppm when larvae were fed an EL-494-treated diet for one stadium. It was more effective at longer feeding regimes. They also found that the incorporation of [<sup>14</sup>C]glucose into chitin was reduced (77%) relative to controls when larvae were fed diets containing 20 ppm of EL-494.

The purpose of this investigation was to determine the fate of EL-494 in plants and in larvae of gypsy moth, *L. dispar*. Soybean plants and soybean cotyledon callus tissue were employed for metabolism studies because of their extensive use in similar studies (Mumma and Hamilton, 1979).

#### MATERIALS AND METHODS

**Tissue Culture Technique and Incubation.** Soybean (*Glycine max* (L.) Merrill var. Acme) cotyledon callus stock cultures were grown on an agar-solidified medium (Miller, 1963) with 3% sucrose,  $\alpha$ -naphthaleneacetic acid (20 mg/L), and kinetin (0.5 mg/L) added. The stock cultures were maintained under low-intensity fluorescent light [0.5  $\mu$ Einstein/(m<sup>2</sup>·s)] at 25 °C and were routinely subcultured aseptically on the same medium once a month.

Two-week-old callus clumps (four clumps per flask and three flasks per treatment) were injected with labeled EL-494 (~200  $\mu$ g) dissolved in 60  $\mu$ L of chloroform (5  $\mu$ L/clump). The labeled EL-494 ([<sup>14</sup>C]benzoyl, specific activity 12.33  $\mu$ Ci/mg; 2-[<sup>14</sup>C]pyrazinyl, specific activity

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