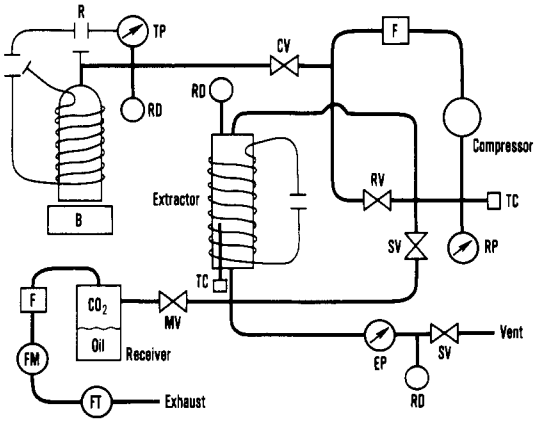

Figure 1. Supercritical CO_2 extraction apparatus: (B) balance, (R) relay, (TP) tank pressure, (RD) rupture disk, (CV) check valve, (F) gas filter, (TC) thermocouple, (RP) regulated pressure, (RV) regulating value, (SV) shutoff valve, (MV) micro metering valve, (FM) instantaneous flow meter, (FT) flow totalizer, and (EP) extractor pressure.

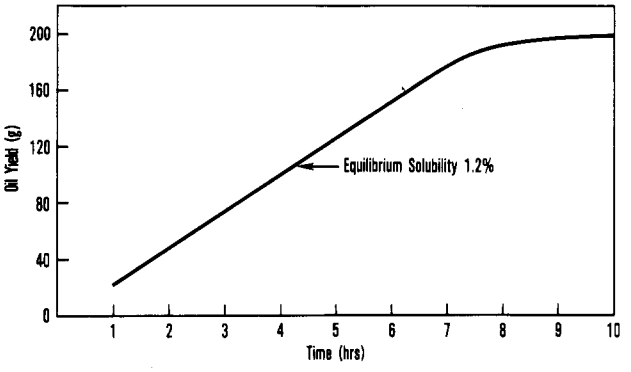

Figure 2. Extraction of soybean oil with supercritical CO_2 at 5000 psi and 50 °C.

eluting the triglyceride to a considerable extent before elution of the pigments and other unsaponifiables. We also found that increasing the extraction pressure in increments of 1000 psi, from 2500 to 9500 psi, and at a constant temperature of 50 °C gave oils of increasing color intensity. These observations are contrary to those of Stahl et al. (1980), who reported that oil fractions collected at low pressure were dark and turbid whereas those taken at high pressure were clear and lighter colored.

Work is continuing on the extraction and refining of soybean oil by conventional and supercritical methods. Organoleptic studies on both oil and meal will be reported in a future paper. Other edible and nonedible oilseeds, seed oils, and other plant materials also are being examined.

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Inhibition of Larval Growth in *Spodoptera frugiperda* by Sublethal Dietary Concentrations of Insecticides

Sublethal concentrations of some insecticides inhibited larval growth of the fall armyworm *Spodoptera frugiperda*. Fenvalerate, permethrin, endosulfan, SD-35651, and CGA-72662 inhibited growth by more than 39% at 12 days, while chlorpyrifos, methyl parathion, sulprofos, precocene, and piperonyl butoxide were less inhibitory. Toxaphene, carbaryl, thiodicarb, methomyl, amitraz, chlordimeform, and imidazole produced only a transitory growth reduction. Aldicarb, profenofos, CGA-19255, diflubenzuron, and SKF-525-A had no sublethal effect while methiocarb and methoprene increased growth. Dose–response relationships were determined for fenvalerate, methyl parathion, SD-35651, and CGA-72662.

There is much interest in chemicals which might be applied to protect crops by deterring insect feeding. This strategy aims to prevent damage to the plant rather than to kill the insect and might have advantages in reduced risks of pest resistance and side effects on beneficial insects. Candidate chemicals for this use include natural products such as warburganal (Nakanishi, 1977) and synthetic chemicals such as tryclazol and other organotins (Ascher, 1980).

Some commercial insecticides might act in part through repellency or other sublethal effects as recently demonstrated with carbaryl (Young and McMillian, 1979) and chlordimeform (Lund et al., 1979). These discoveries have led us to investigate the growth inhibiting effects of sub-

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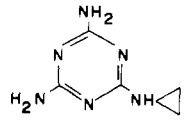
lethal concentrations of a broad spectrum of insecticides.

In this report 24 insecticides and 2 insecticide synergists were tested for sublethal effects on growth in the fall armyworm *Spodoptera frugiperda*. The compounds were impregnated into food by a modification of the method of McMillian et al. (1969) and compared to chinaberry leaf extract which they found to inhibit larval growth. Independently, a study using methods very similar to those described below was recently published with flavinoids inhibiting growth of *Heliothis zea* (Ellinger and Waiss, 1980).

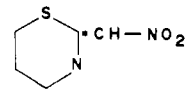
MATERIALS AND METHODS

Colonies of *S. frugiperda* known as Bradenton, Tifton, and Starkeville were obtained from the U.S. Department of Agriculture, Southern Grain Insects Laboratory, and were used interchangeably. These larvae were uniformly susceptible to permethrin (LD_{50} = 0.03–0.08 μg/larva) and 10–20-fold resistant to methomyl (LD_{50} = 1.0–1.2 μg/larva) and to methyl parathion (LD_{50} = 1.5–2.4 μg/larva) by a standard topical test (Brazzel, 1970). Adults were held at 27 °C and 50% relative humidity under a 14-h photoperiod and provided a syrup solution; larvae were fed the diet described below.

Insecticides were technical grade as provided by manufacturers; most were >95% pure, and doses were calculated on the active ingredients. SKF-525-A is 2-[(diethylamino)ethyl]-2,2-diphenylpentanoate; CGA-19255 is 6-azido-*N*-cyclopropyl-*N'*-ethyl-1,3,5-triazine-2,4-diamine; CGA-72662 is *N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine,



CGA-72662



SD-35651

and SD-35651 is tetrahydro-2-(nitromethylene)-2*H*-1,3-thiazine.

Chinaberry leaves from *Melia azedarach* were ground over sodium sulfate, Soxhlet extracted by chloroform, and concentrated in vacuo at 45 °C to produce a 5.3% yield of resin.

Compounds were incorporated into the diet of Greene et al. (1976) which was modified to increase the amounts of agar, casein, and water by 96, 33, and 5.9%, respectively. Dry ingredients except agar were milled to pass a 600-μm sieve and mixed. Fifteen milligrams of the test compound in methylene chloride was added to a slurry of 52 g of diet in methylene chloride in a 300 mL round-bottom flask. After the mixture was stirred, the solvent was removed by Buchi rotavapor at 45 °C. The impregnated powder was air-dried overnight and then vigorously blended into 245 mL of a warm 2.18% (w/w) agar solution and poured into 30-mL plastic cups to solidify. This resulted in 20 cups of diet impregnated with 50 ppm of the test compound. One newly hatched larva was placed in each of 10 cups, and this was repeated on a second day for a total of 20 replicates per treatment. Live larvae were weighed at 6 and 12 days; dead and pupating larvae were recorded but were not included in the weight analysis. Most compounds which caused mortality at 50 ppm were tested at 10-fold lesser concentrations until 90% survival resulted. An experiment consisted of six test compounds, a chinaberry leaf control, and a solvent control. Analysis of variance was computed in a general linear model contrast routine to test the results for significance. In separate experiments the dosage–response relationships of four compounds were determined by using probit analysis to compute median inhibitory concentrations, i.e., the concentration which reduced mean growth to half that of the control group.

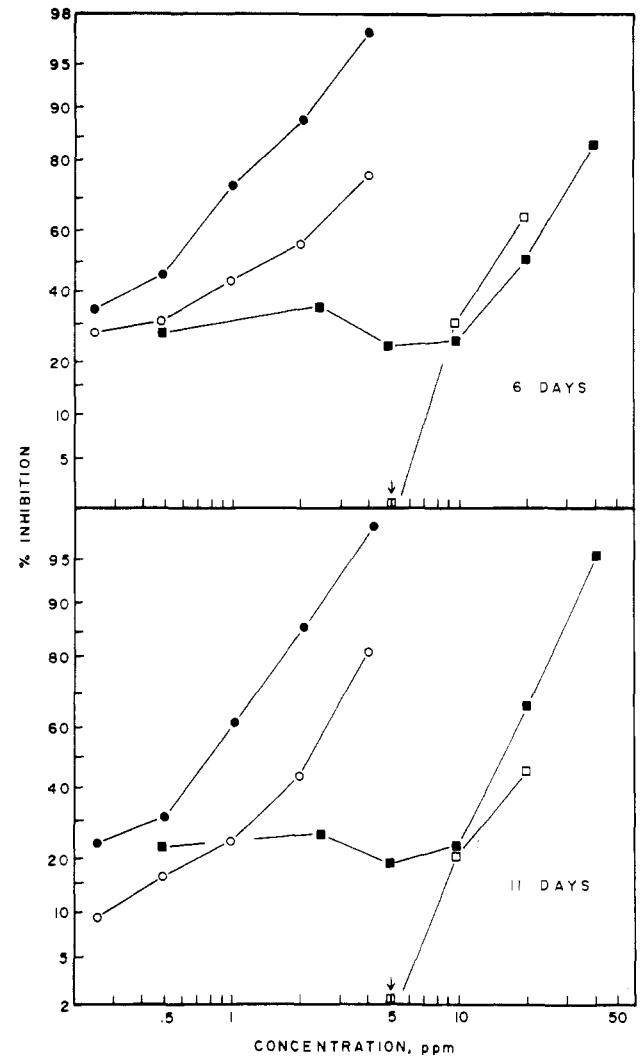


Figure 1. Relationship of inhibition of growth in *S. frugiperda* larvae and sublethal concentration in the diet of the insecticides fenvalerate (●), methyl parathion (○), CGA-72662 (■), and SD-35651 (□). Each point represents mean percent inhibition calculated from weights of surviving larvae from 40 first instars placed on the diet.

RESULTS AND DISCUSSION

Of the compounds tested, 19 (73%) inhibited larval growth at either 6 or 12 days (Table I). At 6 days growth was reduced by 15 insecticides, and at 12 days growth was reduced by 12 compounds and increased by 2. Control mortality at 6 days was 6.9% with no appreciable mortality after 6 days though nearly one-third pupated before day 12. Chinaberry reduced initial growth by 52% and later growth by 26%. These compounds fell into eight general categories and at least one chemical from each group reduced growth.

Pyrethroid insecticides fenvalerate and permethrin were potent in reducing growth at sublethal concentrations as is evident when they are compared to the chinaberry extract. Three organochlorine insecticides had significant effects although DDT was lethal at the concentration tested. Carbamate insecticides reduced growth only at 6 days with older larvae overcoming inhibition so that 40% pupated before day 12. Unlike the carbamates which inhibit growth at the early stages of larval development, three of the organophosphates reduced growth only after 12 days. Trichlorfon which caused 35% initial mortality re-

Table I. Inhibition of Larval Growth in Surviving *S. frugiperda* Larvae Fed the Insecticide-Impregnated Diet (Exposure Begun with 20 Unfed First Instars)

		larval weight					
		day 6			day 12		
chemical	dose, ppm	no.	control, mg	treated, mg	no.	control, mg	treated, mg
controls (8 experiments)	0.0	149	36.9 ± 2.0		91	417 ± 16.3	
chinaberry leaf extract	50.0	154	36.9	17.7[a]	133	417	309[a]
Pyrethroid Insecticides							
fenvalerate	5.0	20	52.5	2.6[a]	19	451	37[a]
permethrin	5.0	18	52.1	8.4[a]	16	330	182[a]
Organochlorine Insecticides							
DDT	50.0	8	52.5	5.7[a]	8	451	139[a]
endosulfan	50.0	18	52.5	13.8[a]	18	451	275[a]
toxaphene	50.0	20	52.5	43.1[a]	13	451	525
Carbamate Insecticides							
aldicarb	53.0	20	23.5	19.6	5	413	454
carbaryl	53.0	15	23.5	16.0[a]	4	413	407
methiocarb	50.0	15	27.2	19.4	11	365	516[b]
methomyl	50.0	19	52.1	38.2[a]	15	330	373
thiodicarb	50.0	18	52.1	33.5[a]	16	330	314
Organophosphorus Insecticides							
chlorpyrifos	0.5	20	38.3	38.2	20	585	478[a]
methyl parathion	0.5	19	38.3	51.0[b]	15	585	390[a]
profenofos	0.5	19	38.3	33.2	18	585	535
sulprofos	0.5	19	38.3	39.8	15	585	455[a]
trichlorfon	5.0	13	12.8	6.0[a]	12	501	321[a]
Formamidine Insecticides							
amitraz	50.0	19	60.9	43.8[a]	17	461	378
chlordimeform	50.0	20	52.5	40.4[a]	15	451	435
Heterocyclic Insecticides							
SD-35651	5.0	14	12.8	5.8[a]	14	501	246[a]
imidazole	50.0	20	52.1	29.7[a]	20	330	337
Insect Growth Regulators							
CGA-19255	50.0	19	52.5	47.4	18	451	432
CGA-72662	50.0	18	60.9	5.5[a]	15	461	34[a]
diflubenzuron	0.05	20	25.5	22.5	18	416	359
methoprene	50.0	18	27.2	30.2	18	365	693[b]
	5.0	19	25.5	24.2	19	416	463
precocene	50.0	20	60.9	16.6[a]	20	461	387[a]
Insecticide Synergists							
piperonyl butoxide	50.0	20	60.9	45.6	19	461	353[a]
SKF-525-A	50.0	19	60.9	61.6	18	461	415

[a] Significantly below control mean, $p < 0.05$. [b] Significantly above control mean, $p < 0.05$.

duced growth in survivors while profenofos had no effect. Formamidine insecticides chlordimeform and amitraz caused a transient reduction in larval growth similar in effect to the carbamates. Of two heterocyclic insecticides, SD-35651 was more active than imidazole; though initial mortality was observed in this experiment, in later tests SD-35651 was not lethal. Insect growth regulators CGA-72662 and precocene were growth inhibitors though no morphological abnormalities were observed. Diflubenzuron, most toxic, killing all larvae at 0.5 ppm, was not inhibitory at 0.05 ppm. Methoprene at 50 ppm typically increased growth to abnormally large larvae which molted to larval–pupal intermediates; at the subeffective concentration of 5 ppm, no effect was observed. The synergist piperonyl butoxide reduced growth at 12 days while SKF-525-A had no effect. Pupation resulted in reduced numbers of observations in the carbamates, trichlorfon, amitraz, CGA-19255, and SKF-525-A; other missing observations were due to mortality.

Dosages of four compounds were related to inhibition of growth (Figure 1); the relationship was most distinct with fenvalerate, methyl parathion, and SD-35651, while with CGA-72662 a 30% response was observed over a 20-fold dilution. Median inhibitory concentrations (and probit-line slopes) of these compounds at 6 days were 0.5 (1.8), 1.2 (1.1), 15.3 (4.1), and 16.5 ppm (1.8) and at 11 days were 0.7 (2.2), 1.9 (1.7), 20.4 (3.4), and 13.5 ppm (2.8), respectively, omitting two lower concentrations of CGA-72662 in the computations. Initial mortalities in this experiment at the highest and next to highest concentrations for each compound were 10 and 2.5% (fenvalerate), 85 and 20% (methyl parathion), 5 and 0% (SD-35651), and 17.5 and 10% (CGA-72662).

The mechanisms by which insecticides inhibited growth could include repellency, disruption of feeding physiology, or other chronic toxicity possibly related to insecticidal action. These mechanisms are under investigation, but results are preliminary at this time. Prime candidates for further research are the experimental insecticides SD-35651 and CGA-72662 which possess remarkable insecticide chemistry. Analogues of CGA-72662 were larvicidal in houseflies (DeMilo et al., 1981). These aminotriazines as well as the tremendous variety of triazinyl herbicides which have been synthesized deserve further evaluation as plant protection agents.

ACKNOWLEDGMENT

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Absorption and Translocation of 4-(Trifluoromethyl)chlorobenzene in Soil and Crops

Water containing 1 mg/L 4-(trifluoro[^{14}C]methyl)chlorobenzene (TFCB) was supplied to pot cultures of three grass (*Zea mays* L.; *Festuca rubra* L.; *Lolium multiflorum* L.) and three legume (*Vicia sativa* L.; *Trifolium perenne* L.; *Medicago sativa* L.) species. The chemical was absorbed by soil and subsequently translocated to plant leaves at increasing amounts for maize to ryegrass, clover, alfalfa, red fescue, and vetch. Legumes showed a high capacity of degradation of the contaminant, suggesting their utilization to reclaim soil and water contaminated by TFCB.

4-(Trifluoromethyl)chlorobenzene (TFCB), an intermediate in the synthesis of the herbicide trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) has been detected in groundwater at a concentration of 1 mg L^{-1} as a result of the incorrect disposal of discharges at a chemical factory in the country north of Vicenza, Italy. The groundwater was used to feed the public aqueduct, and the level of contamination made the water unacceptable for human consumption.

Since the water was used also for irrigation, it is important to determine the mobility of the contaminant in soil and crops. Data on the entry and degradation patterns of certain dinitroaniline herbicides into plants have been described (Parka and Soper, 1977; Probst and Tepe, 1969). However, extrapolation of such data to TFCB was not possible. Therefore, experiments were designed in which water containing ^{14}C-labeled TFCB was applied to pot cultures and the radioactivity was determined in the soil and in the roots, stems, and leaves of the crops.

MATERIALS AND METHODS

Several clay pots (10 × 10 × 14 cm) were prepared, each containing 2 kg of soil. This was representative of the soil found in the contaminated zone, a fluvisol, according to the FAO classification, with the following characteristics: pH 7.2, clay 8.5%, silt 17.9%, sand 73.6%, $CaCO_3$ 2.5%, and organic matter 1.3%.

Three grass (*Zea mays* L.; *Festuca rubra* L.; *Lolium multiflorum* L.) and three legume (*Vicia sativa* L.; *Trifolium perenne* L.; *Medicago sativa* L.) species were sown.

Plants were grown in a controlled environment at 27 °C, 80% humidity, and 14-h light/10-h dark cycle and watered through a hole in the bottom of the pots. After 20 days from sowing, the pots were dipped in a 1-cm layer of water containing 1 mg L^{-1} (trifluoro[^{14}C]methyl)chlorobenzene (TFCB). Since at this concentration loss by volatilization was negligible, at the end of the absorption period no radioactivity was detected in soil and plants of the control pots. The radioactive TFCB prepared by the Radiochemical Centre (Amersham) had 11 μCi/mg specific activity and 92% radiochemical and chemical purity.

Table I. Vertical Distribution of (Trifluoromethyl)chlorobenzene in the Soil after 24-Hour Contact with Water Containing 1 mg L^{-1} Contaminant[a]

soil layer, mm	(trifluoromethyl)-chlorobenzene, mg/kg of soil
0-15 (bottom)	1.32 ± 0.048
15-30	0.65 ± 0.041
30-45	0.31 ± 0.013
45-60	0.28 ± 0.026
60-75	0.26 ± 0.025

[a] Data are the means of three experiments ± standard errors.

Two replications (one pot each) were used in completely randomized design, and the entire study was repeated 3 times.

After 1, 7, 24, 60, and 240 h (see Tables I–IV), the plants were separated from the soil and rapidly washed, and roots, stems, and leaves were sampled. The soil was horizontally cut in 15-mm slices, which were extracted with ethanol to evaluate the absorbed radioactivity.

Plant material was homogenized with an Ultraturrax apparatus, and the homogenate was fractionated into ethanol-soluble and ethanol-insoluble fractions. Radioactivity was evaluated in the soil extract and in each fraction of the plant homogenate by means of a Tricarb spectrometer, with Instagel as the scintillation mixture. The ethanol-soluble fraction was submitted to thin-layer chromatography at −20 °C, using silica gel plates and heptane as the solvent. The low temperature of operation was chosen in order to prevent the volatilization of TFCB. Spots were removed from the plate, after identification by

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