the heating times. Differences in fat and dry matter were minute since losses were prevented except for small amounts through refrigeration. The major difference may be noted in the group of birds fed endrin; these differences no doubt reflect the slower growth of these chickens.

Residues formed in the controls (raw), and in the cooked and heated tissues are summarized in Table III. All data were calculated on a dry matter basis to provide a base of comparison. While variation between birds was large, certain trends seem apparent. There was some reduction in residue concentration in most of the cooked samples, but reduction below the raw tissue was not significant in most cases. Lindane was lowered considerably in the steamed tissues, but reductions were minor when the tissues were either baked or fried. The amounts of endrin in the cooked samples were below the raw controls, but there were no differences between methods of cooking. Heptachlor, determined as heptachlor epoxide, was reduced slightly in the baked and steamed samples, but the concentration in the fried samples was similar to the control. The amounts of dieldrin and aldrin were reduced by cooking, but there were no differences in the cooking methods.

When samples of chicken containing the residue were heated for 30, 60, and 90 min in closed containers, residues of lindane and heptachlor epoxide were reduced. Lindane was reduced from 7.3 to 1.5 ppm in the tissue heated for 90 min. Heptachlor epoxide was reduced from 28.1 to 18.2 ppm at 90 min, but this level was the same at 30 and 60 min. Any reduction of heptachlor epoxide occurred in the initial 30 min with no further loss of the residue. However, endrin, dieldrin, and aldrin were not reduced significantly below the raw control. Heating had no effect on these latter three pesticides and any loss which occurred in the cooked samples was apparently through leaching of fat and water. Losses of lindane and heptachlor occurred by both leaching and destruction by heat.

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Influence of Sequential Herbicide Applications on Cottonseed Oil Composition

Robert E. Wilkinson* and Willis S. Hardcastle

Influence on cottonseed oil quality of four sequential herbicide applications selected from the compounds trifluralin, chlorpropham, fluometuron, DCPA, norea, diuron, MSMA, linuron, and prometryne was measured by gas–liquid chromatography. The sequential herbicide treatments did not greatly influence the fatty acid composition of cottonseed oil which consisted of myristic (1.0%), palmitic (23.1%), palmitoleic (0.6%), stearic (2.6%), oleic (18.5%), linoleic (53.7%), linolenic (0.2%), and arachidic (0.3%) acids.

Herbicides are requisite tools in current cotton (*Gossypium hirsutum* L.) production practices, and since herbicides alter plant metabolic processes, quality of food produced from herbicide-treated cotton has been an area of increasing concern. Approximately 568 million kg of cottonseed oil was used in the United States for human food in 1966 (Fats and Oils Situation, 1967). In that year herbicides were applied to 3,017,000 ha of cotton (Quantities of Pesticides Used by Farmers in 1966, 1970) from a total 3,867,000 ha of cotton harvested (Supplement to Statistical Bulletin, 1969). Thus, 78% of the shortening, margarine, or salad and cooking oils prepared in the United States from cottonseed oil grown in 1966 came from herbicide-treated plants.

Numerous studies have been reported on the influence of herbicides on seed germination, plant emergence, growth, and development, photosynthesis, respiration, and yield of various crops. Wilkinson and Hardcastle (1971) demonstrated that individual applications of several herbicides did influence the fatty acid content of cottonseed oil less than seasonal fluctuation, soil fertility, or soil type. However, current agronomic practices utilize several sequential herbicide applications at various growth stages of the cotton crop. The possibility of synergistic reactions from sequential herbicide applications presently needed for effective cropping remains a problem of concern for which data have not been reported. Therefore, samples of seed from field grown cotton treated with sequential herbicide applications were collected. Gas–liquid chromatographic analyses of the cottonseed oil quality are reported herein.

METHODS AND MATERIALS

Cotton (cv. Atlas 67) was grown in 1969 according to accepted agronomic practices with uniform fertilization of the Cecil fine sandy loam in the entire field area as recommended by soil testing. Insecticides [*i.e.*, dichlorodi-

Table I. Fatty Acid Content of Seed Oil from Cotton Treated with Various Sequential Herbicide Applications. Each Value is the Average of Five Determinations

Program no.	Treatment PP[a]	Pr	Po	L	Percentage Composition: Myristic S[b]	Palmitic S	Palmitoleic U	Stearic S	Oleic U	Linoleic U	Linolenic U	Arachidic S	Total S	Total U
1	T[c]	C	FA	P	0.92ab[d]	23.08cd	0.60ab	2.20b	16.15bc	56.80bc	0.12bc	0.12bc	26.32cd	73.67bc
2	T	F	M	Di	0.85abc	23.78ab	0.48b	2.20b	15.32d	57.17b	0.10c	0.10c	26.92b	73.07d
3	T	D	FA	L	0.85abc	23.62abc	0.55b	2.18b	15.52cd	56.97b	0.12bc	0.18abc	26.82b	73.17d
4	T	N	M	FA	0.82bc	22.95d	0.60ab	2.10b	15.30d	57.75a	0.15abc	0.32a	26.20d	73.80b
5	T	DC	FA	Di	0.80c	23.80ab	0.55b	2.12b	15.25d	57.07b	0.17abc	0.22abc	26.95b	73.05d
6	T	Di	M	FA	0.80c	22.38e	0.70a	2.20b	15.35d	58.07a	0.20ab	0.30ab	25.68e	74.32a
7	TC		N	N	0.80c	23.28bcd	0.60ab	2.20b	16.24b	56.40c	0.18abc	0.30ab	26.58bcd	73.42bcd
8	T	C	FA	FA	0.85abc	24.03a	0.60ab	2.40a	16.12bc	55.47d	0.20ab	0.32a	27.60a	72.40ed
9					0.95a	22.97d	0.65ab	2.55a	18.45a	53.90e	0.22a	0.30ab	26.77bc	73.22c
Avg					0.85	23.32	0.59	2.24	15.97	56.62	0.16	0.24	26.65	73.35
Retention time (min)					1.4	2.3	2.8	4.1	4.7	6.0	8.1	7.7		

[a] PP = preplant incorporated; Pr = preemergence; Po = postemergence, and L = Lay-by applications. [b] S = saturated; U = unsaturated. [c] T = trifluralin; TC = trifluralin + chlorpropham; F = fluometuron; D = DCPA; N = norea; C = chlorpropham; DC = DCPA + chlorpropham; Di = diuron; FA = fluometuron + Adj T; M = MSMA; P = prometryne; L = linuron. [d] Values in a column followed by the same letter or letters are not significantly different at the 1% level.

Table II. Common Name, Chemical Name, Formulations, and Application Rates of Herbicides Applied Sequentially to Cotton for Season-Long Weed Control

Application time[a]	Common name	Chemical name	Formulation[b]	Rate, kg/ha	Program 1	2	3	4	5	6	7	8
PPI	Trifluralin	α,α,α-Trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine	EC	1.12	1[c]	1	1	1	1	1		1
	Trifluralin + Chlorpropham	Isopropyl *m*-chlorocarbanilate	EC + EC	1.12 + 5.04							1	
Pre	Chloropropham		EC	10.08	2							2
	Fluometuron	1,1-Dimethyl-3-(α,α,α-trifluoro-*m*-tolyl)urea	WP	2.24		2						
	DCPA	Dimethyl tetrachloroterephthalate	WP	11.12			2					
	Norea	3-(Hexahydro-4,7-methanoindan-5-yl)-1,1-dimethylurea	WP	1.79				2				
	Chlorpropham + DCPA		EC + WP	3.36 + 5.60					2			
	Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea	WP	1.59						2		
Post	Fluometuron + Adj T		WP + L	2.24 + 0.5% v/v	3		3		3			3
	MSMA	Monosodium methanearsonate	L	2.24		3		3		3		
	Norea		WP	1.12								3
Lay-by	Prometryne	2,4-Bis(isopropylamino-6-methylthio)-*s*-triazine	WP	1.12	4							
	Diuron		WP	1.59		4			4			
	Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea	WP	1.68			4					
	Norea		WP	1.12							4	
	Fluometuron + Adj T		WP + L	2.24 + 0.5% v/v				4		4		4

[a] PPI = preplant incorporated; Pre = preemergence; Post = postemergence; Lay-by = late postemergence when crop cannot be cultivated. [b] EC = emulsifiable concentrate; WP = wettable powder; L = liquid. [c] Numbers refer to order in which herbicides were applied.

phenyltrichloroethane (DDT), chlorinated camphenes (toxaphene), and *O*,*O*-dimethyl-*S*-1,2-di(ethoxycarbamyl) ethylphosphorodithioate (malathion)] were uniformly applied when required during the growth season. Seed cotton was hand picked, ginned, and the oil extracted from 100-g seed samples by a Carver press at 6.9×10^8 dynes/cm^2 for 5 min. Oil samples were methylated and quantitated by methods previously described (Wilkinson and Hardcastle, 1971) on a gas–liquid chromatograph (Hewlett-Packard Model 5751A) equipped with dual flame ionization detectors and a digital integrator (Infotronics CRS-100). Chromatographic contions were: 2.43-m × 4.76-mm i.d. copper columns filled with 70/80 mesh Chromosorb W (AW) (DMCS) carrying 10% w/w stabilized diethyleneglycolsuccinate (DEGS). Carrier gas flow was helium at 75 ml/min. Temperatures utilized were: oven, 200°C isothermal; detector, 280°C; and injector port, 250°C. Retention times of the fatty acid methyl esters are shown in Table I. Quantitization was by normalization.

This cotton was grown for genetic seed stock increase in

plots larger than 0.5 ha. Replications of the plots were impossible as was also repetition of the treatments a second year. Therefore, from the entire seed stock from each treatment plot, quadruplicate seed samples were utilized as replications. Statistical analysis was conducted on a randomized block design for each fatty acid component, and mean differences were separated by the Duncan's multiple range test.

Common names, chemical names, formulations, application rates, and application sequences of herbicides applied to the several plots are shown in Table II.

RESULTS AND DISCUSSION

Eight sequential herbicide programs were utilized for cotton culture. None of these cultural programs resulted in a major change in the cottonseed oil quality of the treated crop. Six of the nine compounds utilized in the sequential programs in this test were reported as single herbicide applications by Wilkinson and Hardcastle (1971). Cottonseed oil quality was not greatly influenced by those six herbicides individually. Wilkinson and Hardcastle (1971) reported soil type, season, and location to have a greater influence on cottonseed oil quality than any of the six individual herbicides.

Because the cotton grown in this test was grown for genetic seed stock increase, the treatments could not be replicated in a normal agronomic test plot pattern. However, the plots were large enough to preclude major sampling errors. Comparison of the data from these analyses and those presented previously (Wilkinson and Hardcastle, 1971) indicated that the variability of cottonseed oil quality from these eight sequential herbicide programs was not greater than was found to be due to location or season. The variability of cottonseed oil quality from cotton grown on the same soil type during the same growing season was greater (Wilkinson and Hardcastle, 1971) than was found within these eight sequential herbicide programs.

Thus it must be concluded that these eight sequential herbicide programs on cotton, including the use of these nine herbicides at registered application rates, do not deleteriously influence the quality of cottonseed oil.

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Optically Pure Pyrethroids Labeled with Deuterium and Tritium in the Methylcyclopentenonyl Ring

Michael Elliott*[1] and John E. Casida

A convenient procedure is described for preparing 100-mg quantities of (+)-pyrethrolone and (+)-allethrolone labeled with ^{3}H in the methyl and methylene groups of the ring and for converting them to the corresponding esters, pyrethrins I and II, and allethrin, optically and stereochemically pure and with specific activities of 317–675 mCi per mmol. Pentadeuteroallethrolone and pentadeuteroallethrin were prepared as model compounds to establish the positions of labeling. The radiolabeled esters were sufficiently stable to be used in metabolism and mode-of-action studies, because there was no evidence of interference from radiation-induced decomposition or of isotopic exchange under biological conditions.

Research on the metabolism and mode of action of the natural pyrethrins has been hampered by lack of convenient procedures for radiosynthesis of adequate quantities of the pure isomers of these compounds with sufficient specific activity. The two most important esters in the natural material, from the flower heads of *Chrysanthemum cinerariaefolium*, are pyrethrin I and pyrethrin II (Figure 1). Of the 16 possible stereoisomers of pyrethrin I (I) and 32 of pyrethrin II (II) (Crombie and Elliott, 1961), the two natural forms probably have the highest insecticidal activity. Therefore, for metabolic and mode of action studies it is important that the products from radiosynthesis consist only of the pure, separated, optical and geometrical forms found in nature.

^{14}C-(+)-*Trans*-chrysanthemic acid (III, ^{14}C at *) was prepared and esterified with (+)-pyrethrolone (IV) and (+)-allethrolone (IV) (Nishizawa and Casida, 1965; Yamamoto and Casida, 1968) but pyrethrins I and II have not previously been available with a radioisotope in the alcohol moiety (Elliott *et al.*, 1970). Although natural and synthetic cyclopentenolones, including (±)-pyrethrolone, have been synthesized (Crombie *et al.*, 1950, 1969; Schechter *et al.*, 1949), no adequate method for optical resolution easily adapted to small quantities has yet been developed; thus only ^{14}C-(±)-allethrolone is available so far (Winteringham *et al.*, 1955; Yamamoto and Casida, 1968). We therefore examined the possibility of introducing tritium into (+)-pyrethrolone (IV) which is readily accessible from natural pyrethrum extract (Elliott, 1964; Maciver, 1968). ^{3}H-Labeling has some advantages over synthesis of compounds with ^{14}C, because products of high specific activity can often be made at reasonably low cost by exchange reactions of protons in the molecule with the tritium in tritiated water, which is available at high specific activity. If the center of optical activity in the molecule is remote from the exchanging positions, reaction conditions

Division of Entomology, University of California, Berkeley, California 94720.
[1] Present address: Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Hertfordshire, England.