until reaction is complete. Ash overnight at 450° C. in a muffle furnace. Dissolve the ash in 6 ml. of concentrated hydrochloric acid and sufficient water to give a final volume of 25 ml. Filter.

Analytical Procedure. Make analyses in duplicate.

Place a 3-ml. aliquot of sample or standard solution in each test tube, add 2 ml. of dipropylene glycol reagent, and mix thoroughly, then, at once, add 5 ml. of barium chloride reagent and again mix thoroughly.

Let mixture stand for 15 minutes to 1 hour and read transmittance at 720 mμ, using reagent blank as reference sample. Determine concentration of sulfur from standard curve.

If a smaller aliquot is used, enough zero sulfur standard should be added to make a volume with the aliquot of 3 ml.

Reading the transmittance at about 410 mμ increases sensitivity about two- or threefold.

Discussion

Comparison with Gravimetric Method. Results obtained by the proposed method are in good agreement with those obtained by the official AOAC method (*3*), as shown in Table I.

Analysis of variance showed no significant differences due to method of chemical analysis. There was an interaction, barely significant at the 5% level, between sulfur level in the sample and the method of analysis. The proposed method gave slightly higher values than the official method on samples containing about 0.2% sulfur and slightly lower values on samples containing 0.4% sulfur. The method gave equally good results with oily material of the seed and the nonoily root material.

Use of Alcoholic Magnesium Nitrate Solution. Alcoholic magnesium nitrate was used instead of the aqueous solution described in the official AOAC method to give better penetration of oily materials such as soybean meal.

Effect of Added Salts. The presence of magnesium and chloride ions at the high levels contained in the ash solution increases the absorbance by a factor of about 1.8 following addition of barium to a sulfate solution. It was therefore necessary to include magnesium chloride reagent in the standard solutions.

Table I. Results Obtained with Proposed Method and with AOAC Method

Sample No.	Material	Sulfur, % Proposed Method		Official Method		Mean Difference
1	Seed	0.20	0.20	0.21	0.22	−0.015
2	Seed	0.21	0.22	0.20	0.19	+0.02
3	Seed	0.23	0.23	0.21	0.22	+0.015
4	Seed	0.28	0.27	0.28	0.28	−0.005
5	Seed	0.27	0.28	0.28	0.28	−0.005
6	Roots	0.30	0.30	0.30	0.32	−0.01
7	Roots	0.39	0.38	0.40	0.41	−0.02
8	Roots	0.39	0.38	0.38	0.38	+0.005

The increase in turbidity with the addition of magnesium chloride reagent was not due to traces of sulfate, as the same amount of reagent was added to each standard, nor was it due specifically to either magnesium ions, chloride ions, or pH as the addition of sodium chloride, hydrochloric acid, or nitric acid had similar effects. In agreement with Toennies and Bakay (*4*), the effect of added salts was found to be small at low sulfur concentrations.

The amount of salts in the ash solution originating from the sample itself was small compared to that which was added. Variations of salt concentration of the magnitude owing to differences in ash content of the samples were found to have no effect on turbidity. The addition of phosphate to the standards to give concentrations higher than would be found in the ash solutions had no effect.

In the presence of only moderate concentrations of salts it is possible to determine sulfate volumetrically. Asghar, Quayyum, and Rana (*1*) have recently published a rapid method in which sulfate is precipitated as barium sulfate and the excess barium is titrated with the disodium salt of (ethylenedinitrilo)-tetraacetic acid. However, the high concentration of magnesium necessary to prevent the loss of sulfur during ashing of plant materials would result in too high a blank for good accuracy with this method.

Spectrophotometry. Reading transmittance of 720 mμ gave satisfactory sensitivity over a wide range of sulfur concentrations, so that it was seldom necessary to change aliquot size. Greater sensitivity is possible at 410 mμ, but aliquot size must then be varied according to the expected percentage of sulfur. However, if the sample contains less than about 200 γ of sulfur, the greater sensitivity at the lower wave length is worth while.

Commercially available reagents were used except in the case of magnesium nitrate and no difficulty was experienced due to the trace of sulfur which the reagents may have contained. Deionized water prepared by passing distilled water through a column of Amberlite Monobed No. 3 resin proved very satisfactory.

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Endrin Content of Milk and Body Tissues of Dairy Cows Receiving Endrin Daily in Their Diet—Correction

On page 520 [Kiigemagi, Ulo, Sprowls, R. G., Terriere, L. C., J. AGR. FOOD CHEM. **6,** 518 (1958)], in Table V, the heading of the fourth column should read "Ratio, Output/Intake."

Enzymic Hydrolysis of Naringin in Grapefruit—Correction

On page 548 [J. AGR. FOOD CHEM. **6,** 546 (1958)], in the legend of Figure 5, the broken line should be the hydrolyzed sample and the solid line the original sample.

S. V. TING

INSECT ATTRACTANTS

Esters of 6-Methyl-3-cyclohexene-1-carboxylic Acid as Attractants for the Mediterranean Fruit Fly

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ATTRACTANTS are useful in the control and eradication of insect pests. In combination with a toxicant, they may lure insects to their doom, or traps may be baited with them to determine the location and extent of insect infestation (*1*), so that control measures may be applied only when and where needed. Attractants are particularly helpful in detecting

Thirty-one esters of 6-methyl-3-cyclohexene-1-carboxylic acid have been synthesized and tested as attractants for the Mediterranean fruit fly [*Ceratitis capitata* (Wied.)]. Approximately one fourth of the esters showed considerable attractiveness to the male of this species when tested by the olfactometer method. The most effective compounds in the field tests were esters of secondary alcohols with short chains, not exceeding five carbons.

the presence of incipient infestations of foreign species. Research to find lures for certain tropical fruit flies was intensified in 1949, when the oriental fruit fly infestation reached alarming proportions in Hawaii, and later when the Mexican fruit fly was first found in the Tijuana area of Mexico and southern California.

In 1955, shortly after the discovery of the Mediterranean fruit fly [*Ceratitis capitata* (Wied.)] in Costa Rica, the Entomology Research Branch initiated a greatly accelerated attractants program in which many chemicals were synthesized by the chemists at Beltsville, Md., for laboratory screening, and field evaluation by entomologists in Hawaii. No outstanding lure for the Mediterranean fruit fly (Medfly) was known at this time.

In March 1956, a few weeks before the Medfly appeared in Florida, angelica seed oil was found by entomologists in Hawaii to be an excellent attractant (*6*). A vigorous eradication program was undertaken immediately by federal and state agricultural agencies. Approximately 50,000 traps containing the oil were set out in Florida to determine the extent of the infestation, to guide the spraying program, and to measure eradication progress. Results of the survey indicated that the infestation was spread over about 700,000 acres.

Only about 600 pounds of this oil are produced annually from angelica seed—a biennial crop grown in Belgium and in other countries of eastern Europe (*3*). By the latter part of 1956, the eradication program had consumed practically the entire world supply of this oil—including accumulated stocks from previous crops—and the price had risen from about $56 to $220 or more per pound. Even at the latter price supplies were unavailable, and it became imperative to find a substitute attractant to prevent disruption of the eradication program.

Among the compounds selected at Beltsville for screening was the *N,N*-diethylamide of 6-methyl-3-cyclohexene-1-carboxylic acid. This compound had been synthesized 5 years previously, and it is a fairly effective mosquito repellent (*4*). It showed no attraction for the Medfly. However, several esters of this acid were synthesized, and some of the first of those tested proved to be among the most effective attractants tried up to that time. This led to the preparation of a series of esters of 6-methyl-3-cyclohexene-1-carboxylic acid. The first field tests indicated that the isopropyl ester was the most attractive, and arrangements were promptly made for the manufacture of several thousand pounds of this compound by a commercial company. This material was immediately put to use in the Florida eradication program.

Subsequently the *sec*-butyl ester was found to be about twice as attractive as the isopropyl ester when evaporation of the latter was not controlled, even though somewhat inferior to the average angelica seed oil sample in this respect. Production was therefore shifted to the *sec*-butyl ester, and the first shipments of this material were made available to the eradication agencies in April 1957.

Experimental

Methods of Preparation. Thirty-one esters of 6-methyl-3-cyclohexene-1-carboxylic acid were synthesized. Most of them were prepared by azeotropic esterification of the acid with the appropriate alcohol, using benzene as a solvent and either *p*-toluenesulfonic or sulfuric acid as a catalyst. Others were made by reacting the acid chloride with the alcohol, either in the presence of an acid acceptor, such as pyridine, or by heating the components under reflux in a suitable solvent. These esters are listed in Table I with the physical constants of the purified compounds.

Olfactometer Tests. The attractiveness of the compounds was measured by the olfactometer method described by Gow (*2*). Their ratings based on the number of flies attracted in comparison with the number attracted by the isopropyl ester as a standard are shown in Table I. As the compounds were tested at different times, the comparative ratings are only approximate. The ratings represent attractiveness almost solely to the male Medfly, as only a small proportion of females were caught in the olfactometer traps or in the field tests.

Field Tests. Ultimate effectiveness as a lure is determined by field tests, where field traps are hung on the branches of trees and shrubs. Experience has shown that a compound may prove to be either more or less effective in field tests than in the laboratory olfactometer tests.

Table I. Chemical and Attractant Data on Esters of 6-Methyl-3-cyclohexene-1-carboxylic Acid

	Chemical Data			Olfactometer Data	
Ester	Yield, %	Boiling point, °C./mm. Hg	n_D^{25}	Concentration, %	Attractant rating (isopropyl 100)
Methyl	73	80–1/14	1.4533	0.02	48
Ethyl	73	91–2/14	1.4450	0.02	122
Propyl	90	106–7/14	1.4517	0.02	96
Isopropyl	58	97–8/14	1.4454	0.02	100
Butyl	74	122/14	1.4518	0.01	71
Isobutyl	84	117–8/15	1.4496	0.02	99
sec-Butyl	31	113–4/15	1.4484	0.02	87
tert-Butyl	44	133/0.05	1.4884	0.02	8
Pentyl	83	129–31/14	1.4512	0.02	68
Isopentyl	70	82/0.5	1.4528	0.02	65
1-Ethylpropyl[a]	69	79/0.7	1.4458	0.02	83
1,1-Dimethylpropyl[a]	30	133/0.7	1.4890	0.02	13
Hexyl	50	97–8/0.5	1.4536	0.02	5
2-Ethylbutyl	69	91–2/0.5	1.4553	0.02	61
1,1-Dimethylbutyl[a]	92	129/13	1.4517	0.05	45
1-Ethylpentyl[a]	90	107–8/1.4	1.4555	0.05	11
2-Ethylhexyl[a]	92	121–3/1.4	1.4585	0.05	9
1-Methylheptyl[a]	75	109/0.7	1.4551	0.05	15
1-Isobutyl-3-methylbutyl[a]	90	101–2/0.6	1.4538	0.05	5
Allyl	87	106–7/13	1.4641	0.02	107
2-Propynyl	46	117–8/15	1.4727	0.02	83
2-Chloroethyl	49	133/14	1.4750	0.02	86
2-Bromoethyl[a]	86	91–2/0.7	1.4935	0.1	115
2-Methoxyethyl	65	129–30/14	1.4566	0.1	0
2-Butoxyethyl	54	109–10/0.5	1.4523	0.02	2
Benzyl	63	119–20/0.5	1.5193	0.1	0
Phenethyl[a]	90	130/0.7	1.5142	0.05	0
Cyclopentyl[a]	73	141–3/13	1.4753	0.02	79
Cyclohexyl	23	108/1	1.4757	0.02	39
2-Methylcyclohexyl[a]	91	104–5/0.7	1.4759	0.05	67
Tetrahydro-2-furfuryl[a]	90	108–9/0.5	1.4780	0.05	13

[a] Prepared from the acid chloride.

Table II. Comparison of Field and Olfactometer Ratings of Esters of 6-Methyl-3-cyclohexene-1-carboxylic Acid

Ester	Field	Olfactometer
sec-Butyl	279	87
1-Ethylpropyl	231	83
Isopropyl	100	100
Butyl	98	71
Propyl	58	96
Allyl	53	107
Isobutyl	48	99
Cyclopentyl	47	79
2-Propynyl	40	83
Ethyl	38	122
2-Chloroethyl	29	86

Eleven of the compounds that appeared most effective in the olfactometer were tested in the field. They were applied to wicks in dry traps (*5*) at 2 to 3 ml. per wick in combination with 0.5% of the toxicant 2,2-dichlorovinyl dimethyl phosphate (DDVP) and exposed in infested coffee and citrus areas. (DDVP alone in traps catches nothing. There is no definite information on its repellency, except that it gave one of the highest catches of the flies of all the insecticides tried. It, therefore, is presumed not to be appreciably repellent, if at all.) Efficiency ratings of the compounds in comparison with the isopropyl ester are shown in Table II. For the purpose of comparison, the ratings obtained in the olfactometer tests are also included.

Discussion

As a fairly large group of closely related esters of 6-methyl-3-cyclohexene-1-carboxylic acid had been tested, the results were studied to determine whether any relationship existed between the chemical structure of the alcohol component of the esters and their attractiveness to the Medfly.

The olfactometer ratings indicate that the following esters tested had attractant ratings of 85 or better at the concentrations tested: ethyl, propyl, isopropyl, isobutyl, *sec*-butyl, allyl, 2-chloroethyl, and 2-bromoethyl. The most effective compounds were among those with an alcohol moiety not exceeding four carbons. In general, the attractiveness of the compounds with larger radicals falls off sharply.

In the field, esters prepared from secondary alcohols were superior to those prepared from primary ones. Thus, the isopropyl ester proved better than the propyl, and the *sec*-butyl ester was the best of those tested in the field. The 1-ethylpropyl ester, which had only an 83 attractant rating in the olfactometer, proved effective in the field.

Performance of an attractant in the field as compared with that in the olfactometer may be affected by its volatility, and this variable, along with many other factors that affect field performance, is being investigated currently.

A large number of closely related compounds proved attractive, in some degree, to the Medfly in this study. Thus far, the action of these esters has appeared to be specific to this species, but they have not been tested extensively in areas where other species of fruit flies occur.

Acknowledgment

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INSECTICIDE RESIDUES

Demeton Residues in Collards, Lettuce, and Mustard

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Demeton residues were obtained enzymatically by using a cholinesterase inhibition technique. Differential quantities of acetic acid resulting from the enzyme hydrolysis of acetylcholine were measured by pH change in a buffered system. The usually recommended 21-day waiting period following the last application was inadequate when three or four foliar applications (4-ounce active) were applied once per week. The Miller Bill tolerance of 0.75 p.p.m. (established on lettuce) can be met on all three of these leafy vegetables if foliar applications, using recommended dosages, are spaced 2 to 3 weeks apart and a 21-day waiting period following the last application is allowed before harvest.

WITHIN THE LIMITATIONS of the law and under certain conditions, vegetable growers are resorting to the use of systemic insecticides for control of sucking types of insects such as aphids, mites, and mealybugs. As the normal weathering processes are not as effective in lowering toxic residues of certain systemics—i.e., in the case of conventional insecticides—considerable investigations are necessary to determine the quantities of this class of insecticide that persist on or in a raw agricultural commodity being prepared for interstate commerce. As the compound demeton (systox) was the first systemic insecticide to be cleared for commercial use on vegetables, it was chosen for this residue study. The active ingredient in demeton is a controlled mixture of isomeric organic phosphate esters. The two isomers are *O,O*-diethyl *O*-2-(ethylthio)ethyl phosphorothioate and *O,O*-diethyl-*S*-2-(ethylthio)ethyl phosphorothioate.

Since passage of the Miller Bill (Public Law 518), a number of official tolerances have been established recently for the use of demeton on certain commercial vegetables, fruit, alfalfa, and cotton. A tolerance of 0.75 p.p.m. has been established by the Food and Drug Administration on the following leafy vegetables: broccoli, brussel sprouts, cabbage, cauliflower, and lettuce. The U. S. Department of Agriculture, after examining pertinent residue data, specified that growers should maintain a 21-day waiting period between their last foliar application and harvesting demeton-treated leafy vegetables for which a tolerance has been