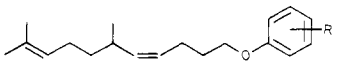
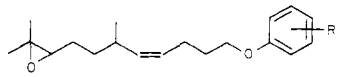
Table III. Juvenile Hormone Activity of 6,10-Dimethyl-4,9-undecadienyl and 6,10-Dimethyl-9,10-epoxy-4-undecenyl Aryl Ethers

		dosage (μg) causing juvenilization ratings of[a]			
		O. fasciatus		*T. molitor*	
no.	R	3.0	≥1	4.0	≥1
					
89	3,4-OCH_2O	0.1	0.1		1
90	3-C_2H_5	1	0.1		10
91	3-OCH_3	10	1		10
92	3-CH_3	10	10		10
93	3-Cl		10		10
94	4-CH_3		10		10
95	4-C_2H_5		*b*	10	1
96	4-Cl		*b*		10
					
97	3-C_2H_5	0.1	0.01		1
98	3,4-OCH_2O	0.1	0.1		1
99	3-OCH_3	1	1		10
100	3-CH_3	10	1		10
101	3-Cl	10	1		10
102	4-CH_3	10	10		1
103	4-C_2H_5		1	10	1
104	4-Cl		1		10

[a] See Procedure section for description of rating system. [b] Rating <1.0 at 10-μg level.

(series IV) are the most active group in Table I against *Tenebrio* and show the enhanced activity of the para-substituted ethers relative to the meta-substituted ethers.

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Insecticide Toxicity and Degradation in Houseflies as Affected by Naturally Occurring Food Plant Components

Tom W. Fuhremann and E. Paul Lichtenstein*

Studies were conducted to investigate the effects of the naturally occurring food plant components myristicin and *d*-carvone on the toxicity and in vivo degradation of parathion and paraoxon in houseflies. Simultaneous topical application of myristicin and paraoxon, or feeding diets containing myristicin followed by paraoxon application, caused substantial increases in the toxicity of paraoxon. LD_{50} values indicated a tenfold increase in paraoxon toxicity due to a topical application of myristicin at a sublethal dosage of 2 μg/fly. Degradation of [^{14}C]paraoxon was inhibited in flies fed myristicin, resulting in its increased toxicity. With parathion, simultaneous application of myristicin increased housefly mortalities, while feeding this natural compound to flies followed by application of the insecticide resulted in decreased mortalities. Feeding myristicin inhibited the metabolism of [^{14}C]parathion and presumably the production of its toxic analogue, paraoxon. Contrary to results obtained with myristicin, feeding *d*-carvone increased the toxicity and metabolism of parathion, but had no apparent effect on paraoxon toxicity or its degradation. Data reported indicate that some compounds occurring naturally in food plants have the ability to alter the toxicity of insecticides as a result of their effects on insecticide degrading systems.

The presence of naturally occurring, insecticidal plant components has been recognized for centuries (Jacobson and Crosby, 1971). Some of these compounds such as nicotine, rotenone, and the pyrethrums have been commercialized for insect control. In more recent years, compounds having insecticidal activity were isolated in this laboratory from edible portions of some food plants, belonging to the Cruciferae and Umbelliferae plant families.

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Some of these compounds also synergized the toxicity of commercially available synthetic insecticides. Thus, myristicin (5-allyl-1-methoxy-2,3-methylenedioxybenzene) was isolated from parsnip roots (*Pastinaca sativa* L.) and found to exhibit both insecticidal and insecticide synergistic activity (Lichtenstein and Casida, 1963). Myristicin was superior to the commercial insecticide synergist piperonyl butoxide when applied topically with the carbamate insecticides carbaryl and dimetilan to CSMA strain houseflies.

A number of insecticidal and synergistic compounds were also isolated from dill plants, *Anethum graveolus* L. (Lichtenstein et al., 1974). These compounds were *d*-carvone (*p*-mentha-6,8-dien-2-one) and the methylenedioxybenzene compounds, apiol, dill apiol, and myristicin. The toxicity of *d*-carvone and myristicin to houseflies (*Musca domestica* L.) was found to be rather low, but sublethal dosages of *d*-carvone increased the contact toxicity of carbaryl, carbofuran, and parathion to *Drosophila melanogaster* (Meigen), and myristicin, which was more toxic than *d*-carvone, also synergized the contact toxicity of carbaryl and parathion at considerably lower dosages.

The effects of myristicin and *d*-carvone on the degradation of the organophosphorus insecticides parathion, paraoxon, and fonofos in rats was recently reported by Fuhremann et al. (1978).

The present study was conducted to investigate the effects of myristicin and *d*-carvone on the toxic effects of the insecticide parathion and its activation product, paraoxon, on houseflies and to determine the effects of these naturally occurring compounds on the in vivo degradation of these insecticides.

MATERIALS AND METHODS

Chemicals. Parathion and paraoxon were obtained through the courtesy of Farbenfabriken-Bayer, Leverkusen, West Germany. [^{14}C]Parathion (97% radiopurity) labeled in the 2,6-phenyl positions (ICN Corporation, Irvine, Calif.) was diluted with nonradioactive parathion to a specific activity of 1.44 mCi/mmol. [^{14}C]Paraoxon was prepared by oxidation of [^{14}C]parathion with bromine as previously described (Fuhremann and Lichtenstein, 1972). Myristicin (98%) was isolated from nutmeg spice powder as previously described (Fuhremann et al., 1978) and *d*-carvone (96%) was purchased from Aldrich Chemical Co., Milwaukee, Wis.

Solvents used were redistilled acetone, chloroform, hexane, and absolute AR grade diethyl ether.

Insects and Application of Chemicals. The houseflies (*Musca domestica* L.) used in this study were a DDT-susceptible strain (CSMA-1948), maintained in this laboratory without insecticide selection. Only female flies were used to eliminate variation in mortalities or in vivo insecticide degradation.

Insecticides or combinations of insecticides and the naturally occurring compounds were topically applied in 1 μL of acetone with an Isco M-420 microapplicator onto the abdominal sternites of 3–4-day-old houseflies previously anesthetized with CO_2.

Extraction and Analysis. In the in vivo degradation studies, ^{14}C insecticides and their degradation products were extracted from the insects and their excrements. For this purpose, flies previously treated with ^{14}C insecticides were anesthetized with CO_2, placed in a 250-mL Erlenmeyer flask containing 100 mL of hexane/acetone (1:1), and blended for 1 min with a Polytron homogenizer (Kinematica GmbH). This macerate was filtered under vacuum. The extracted fly pulp was rinsed successively with 25-mL aliquots of water, acetone, and hexane. It was then air-dried and combusted to release and trap $^{14}CO_2$ for the determination of unextracted (bound) ^{14}C insecticide residues as described by Fuhremann and Lichtenstein (1978).

The fly extract was diluted with 100 mL of water and partitioned with 3 × 50 mL of hexane which quantitatively extracts parathion. When flies had been incubated with [^{14}C]paraoxon, the fly extracts were partitioned with 3 × 50 mL of hexane. The resulting water extraction phases were acidified to pH 1.5 with concentrated HCl and then reextracted with 3 × 50 mL of chloroform/ether (2:1). This procedure quantitatively extracts paraoxon and *p*-nitrophenol and minimizes troublesome chloroform emulsions (Fuhremann et al., 1978).

The walls of the 2-L glass containers in which the ^{14}C insecticide treated houseflies had been incubated contained fly excreta. These residues were removed by successively scrubbing the jar walls with 50-mL aliquots each of water and acetone. The acetone was removed by flash evaporation at 40 °C and the resulting water was partitioned with 3 × 50 mL of hexane. All extraction phases were adjusted to volume and analyzed for radiocarbon content by liquid scintillation counting (LSC) techniques as described by Fuhremann and Lichtenstein (1978).

Gas–liquid chromatography (GLC) was used to analyze the organic extraction phases for the presence of undegraded [^{14}C]parathion or [^{14}C]paraoxon. Hexane extracts containing parathion were analyzed as previously described (Lichtenstein et al., 1973). Pooled hexane and chloroform–ether extracts containing [^{14}C]paraoxon were analyzed using a flameless, alkali sensitized, thermionic nitrogen–phosphorus detector, which was obtained during the course of these studies. A Tracor Model 560 gas chromatograph equipped with a Tracor Model 702 N–P detector and a 122 cm × 2 mm i.d. Pyrex column containing 10% OV-3 silicone grease on 80–100 mesh Chromosorb W-HP was used. The column was held at 185 °C and a helium carrier flow rate of 45 cm^3/min. The N–P detector was operated with 3 cm^3/min of hydrogen and 125 cm^3/min of air at a temperature of 250 °C. The temperature of a newly installed alkali source was adjusted to produce a background current of 1.5×10^{-11} amp. Under these conditions, paraoxon had a retention time of 5 min and a detection limit of 50 pg.

EXPERIMENTAL SECTION

Basically two experimental approaches were utilized with houseflies to investigate the interaction of the naturally occurring food plant components *d*-carvone and myristicin with the insecticide parathion and its activation product, paraoxon. In the first series of experiments bioassays were conducted to determine the effects of the naturally occurring compounds on insecticide toxicity after simultaneous topical application of one of these compounds with one of the insecticides or after feeding one of the naturally occurring compounds to flies for 3 days prior to the topical application of one of the insecticides. In the second series, the in vivo degradation of the insecticides in houseflies was examined after topical insecticide application to insects, which had been fed for 3 days with diets containing one of the naturally occurring compounds.

Effects of Simultaneous Application of Myristicin or *d*-Carvone with Parathion or Paraoxon on Housefly Mortalities. To determine the effects of the naturally occurring compounds on the toxicity of the insecticides, combinations of myristicin or *d*-carvone, and of parathion or paraoxon, were simultaneously applied to houseflies.

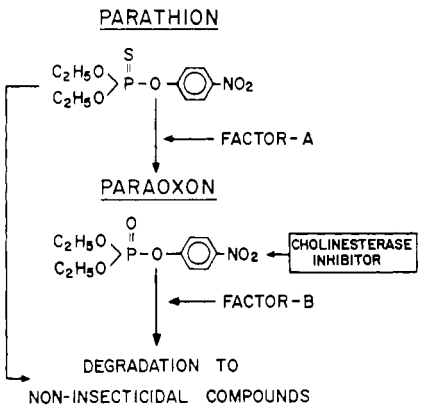

Figure 1. Activation and degradation of parathion.

For this purpose, 1 μL of acetone containing 0.06 μg of parathion plus a sublethal dose (2.0 μg) of either myristicin or *d*-carvone, or 0.06 μg of paraoxon plus 2.0 μg of either myristicin or *d*-carvone, was topically applied to the abdominal sternites of each 3–4-day-old fly. Flies treated with the insecticides alone or the natural products alone served as controls. For each combination of compounds, 60–100 flies were treated. They were then distributed into 4-oz glass jars (10 flies/jar) and covered with screens, and applesauce was provided as food. Mortality of the flies was recorded during a 24-h holding period. Each experiment was repeated twice on different dates with different CSMA fly populations.

Because a large synergistic effect of myristicin in combination with paraoxon was detected in these experiments, dose mortality curves with paraoxon by itself and in combination with myristicin (2 μg/fly) were also determined. For this purpose 30 houseflies were each treated with 1 μL of acetone containing various dilutions of paraoxon (0.1 to 0.22 μg/fly) or 0.01 to 0.024 μg of paraoxon plus 2 μg of myristicin per fly (30 flies/dose). Mortalities were recorded after a 24-h holding period and used to prepare dose–mortality curves.

Effects of Myristicin or *d*-Carvone in Housefly Diets on the Toxicity of Parathion and Paraoxon. In this series of experiments the naturally occurring compounds were fed to the houseflies for 3 days prior to topical insecticide application. This was done to utilize another route and a longer exposure of the insects to the natural compounds. In addition, feeding myristicin or *d*-carvone could eliminate any possible effects they might have on the penetration of the insecticides after simultaneous topical application. For this purpose, three groups of ca. 600 one-day-old male and female flies, contained in 30 × 30 × 30 cm screen cages, were fed for 3 days with milk only (controls) or milk containing 0.5% v/v myristicin or 0.5% v/v *d*-carvone. On the fourth day, dead flies were discarded while the living flies were anesthetized with CO_2. The female flies from each feeding group were topically treated with 1 μL of acetone/fly containing 0.06 μg of parathion or 0.06 μg of paraoxon. Each treatment group consisted of 80–100 flies which were held for 24 h when mortalities were recorded. Each experiment was repeated twice on different dates with different CSMA fly populations.

Effects of Myristicin or *d*-Carvone in Housefly Diets on the in Vivo Metabolism of [^{14}C]Parathion or [^{14}C]Paraoxon. The previously described experiments were designed to determine the effects of the naturally occurring compounds on insecticide toxicity to houseflies. These effects could be related to the interaction of the natural compounds with enzymes responsible for the activation (factor A, Figure 1) or degradation (factor B, Figure 1) of the insecticides. Therefore, experiments were conducted to determine the effects of feeding myristicin or *d*-carvone on the in vivo degradation of [*phenyl*-^{14}C]parathion and [*phenyl*-^{14}C]paraoxon in houseflies. One-day-old houseflies were fed milk only (controls) or milk containing 0.5% v/v myristicin or 0.5% v/v *d*-carvone for 3 days prior to insecticide application. Each feeding group, consisting of 200 four-day-old flies, were then topically treated with 1 μL of acetone/fly containing 0.05 μg of [^{14}C]parathion or 0.05 μg of [^{14}C]paraoxon. Thus 200 flies received a total of 10 μg (ca. 0.05 μCi) of either of the two ^{14}C insecticides. Flies treated with [^{14}C]parathion were held for 4 h at 22 ± 2 °C in 2-L glass jars covered with cheesecloth. Flies treated with [^{14}C]paraoxon were held under identical conditions but for only 30 min since the degradation of paraoxon is more rapid than that of parathion (Heath, 1961). At the end of the incubation periods the flies and their excrements were removed, extracted, and analyzed by LSC and GLC as described above. Each experiment was repeated three times at different dates with different CSMA fly populations.

Table I. Effects of Myristicin and *d*-Carvone on the Toxicity of Parathion and Paraoxon to Houseflies

natural compds applied	insecticide applied topically to houseflies per 24 h[b]				
	none	parathion		paraoxon	
topically[a]	% mortality/24 h[b]		change		change
none	0	51		4	
myristicin	0	75	+24	87	+83
carvone	0	48	−3	13	+9
in fly diet[c]					
none	0	55		5	
myristicin	0	34	−21	97	+92
carvone	0	68	+13	1	−4

[a] Natural compounds (2 μg/fly) and insecticides (0.06 μg/fly) topically applied to houseflies simultaneously in 1 μL of acetone. [b] Results are averages of two separate experiments. [c] Milk diets containing 0.5% v/v myristicin or 0.5% v/v *d*-carvone were fed to the houseflies for 3 days prior to topical insecticide application. Control flies were fed milk only.

RESULTS AND DISCUSSION

For clarity purposes, data obtained with myristicin will be reported first, followed by a discussion of results obtained with *d*-carvone.

Effects of Simultaneous Application of Myristicin or *d*-Carvone with Parathion or Paraoxon on Housefly Mortalities. Results summarized in Table I indicate that simultaneous topical application of a sublethal dose of myristicin (2 μg/fly) with 0.06 μg of parathion/fly caused a small increase in housefly mortalities in comparison to flies treated with the insecticide only (from 51 to 75% mortality/24 h). However, with 0.06 μg of paraoxon and a simultaneous application of 2 μg of myristicin, an increase from 4% (controls) to 87% fly mortalities occurred. Results therefore indicate that myristicin was responsible for increasing the toxic effects of these insecticides, especially paraoxon. Myristicin may have caused an increase in the penetration of these insecticides into the flies or it may have reduced the rate of detoxification of the insecticides.

Because large increases in paraoxon toxicity were observed, the LD_{50} values for paraoxon alone and in combination with myristicin were determined. After topical application of various dilutions of paraoxon or simultaneous application of sublethal doses of myristicin (2 μg/fly)

Table II. Effects of Myristicin or *d*-Carvone in Housefly Diets on the in vivo Metabolism of Parathion and Paraoxon

extraction phases of	^{14}C recovered in % of topically applied[a]					
	[*phenyl*-^{14}C]parathion			[*phenyl*-^{14}C]paraoxon		
	houseflies fed diets containing:					
	none	myristicin	carvone	none	myristicin	carvone
flies (F)						
hexane	20.8 ± 2.0	61.9 ± 7.6[b]	10.7 ± 1.1[b]	68.0 ± 8.9	85.6 ± 3.3[d]	69.9 ± 7.7
water	16.0 ± 1.1	11.5 ± 0.9[c]	11.8 ± 0.7[c]	17.2 ± 4.0	10.8 ± 1.8[e]	19.6 ± 6.5
bound	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.0
total (F)	37.2 ± 3.0	73.9 ± 6.9[b]	22.8 ± 1.8[b]	85.6 ± 4.9	96.6 ± 1.8[d]	89.8 ± 1.4
excreta (E)						
hexane	8.0 ± 3.3	7.6 ± 1.3	5.6 ± 2.5	3.5 ± 0.6	5.0 ± 1.6	4.3 ± 1.0
water	49.9 ± 6.1	21.5 ± 5.9[c]	70.7 ± 3.9[c]	8.2 ± 3.0	1.9 ± 0.9[d]	5.3 ± 1.3
total (E)	57.9 ± 2.9	29.1 ± 7.1[c]	76.3 ± 2.7[b]	11.7 ± 2.8	6.9 ± 0.7[d]	9.6 ± 2.1
total (F + E)	95.1 ± 3.1	102.9 ± 3.1	99.1 ± 1.3	97.5 ± 1.8	103.6 ± 1.2	99.4 ± 0.9
	Undegraded insecticide reocvered from flies in % of applied					
	11.6 ± 4.1	46.2 ± 11.3[c]	6.3 ± 2.8	65.2 ± 5.5	82.2 ± 4.2[b]	63.5 ± 5.7

[a] Two-hundred female houseflies were each topically treated with 1 μL of acetone containing 0.05 μg of (2.5×10^{-4} μCi) [*phenyl*-^{14}C]parathion and held for 4 h. With [^{14}C]paraoxon, however, flies were held for only 30 min. Data are mean ±SD for three separate experiments. [b-e] Significantly different from control values at the 0.1% (*b*), 1% (*c*), 5% (*d*), or 10% (*e*) levels as determined by Student's *t* test.

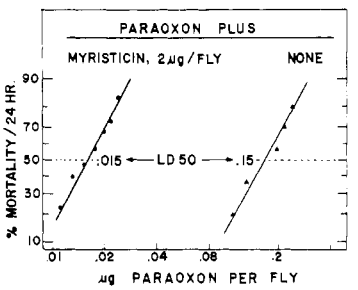

Figure 2. Effect of myristicin on the toxicity of paraoxon to houseflies after simultaneous topical application.

plus various dilutions of paraoxon, dose–mortality curves were obtained (Figure 2). Based on LD_{50} values obtained with paraoxon alone (0.15 μg/fly) or in the presence of myristicin (0.015 μg/fly) a tenfold increase in paraoxon toxicity was observed.

Effects of Myristicin or *d*-Carvone in Housefly Diets on the Toxicity of Parathion and Paraoxon. In an attempt to eliminate any possible effects of the natural compounds on insecticide penetration into the houseflies, insects were fed milk diets containing 0.5% v/v myristicin or 0.5% v/v *d*-carvone for 3 days prior to topical application of the insecticides. This feeding procedure also increased the duration of exposure to the natural compounds. Contrary to results obtained after simultaneous application of myristicin and parathion, mortalities of flies previously fed myristicin and then treated with parathion were less than those observed with control flies (Table I). Since it is unlikely that penetration of topically applied parathion could be affected by feeding myristicin, it is possible that feeding myristicin either increased the degradation of parathion to nontoxic compounds or it inhibited production of paraoxon—the activation product responsible for parathion toxicity (Metcalf and March, 1953). With paraoxon, however, feeding myristicin was responsible for a substantial increase in toxicity just as had been observed with simultaneous topical application of these compounds.

As shown in Figure 1, parathion is toxic because of its in vivo conversion to paraoxon, the actual cholinesterase inhibitor and toxicant. The dramatic increases in paraoxon toxicity observed when myristicin had been administered in fly diets or applied simultaneously with paraoxon could have been due to an inhibition of "factor β", responsible for the degradation of paraoxon to nontoxic compounds. This would result in persistence of paraoxon and higher insect mortalities over the 24-h holding period. With parathion the presence of myristicin in housefly diets could have inhibited "factor A", thus blocking the conversion (activation) to paraoxon resulting in lower fly mortalities in comparison to those observed with flies fed regular milk diets. Simultaneous topical application of myristicin and parathion probably resulted in a slower penetration of myristicin. Thus some paraoxon was produced and its degradation was subsequently blocked, thereby resulting in a small increase in housefly mortalities, but not as pronounced as was observed when paraoxon had been applied directly with myristicin.

Effects of Myristicin or *d*-Carvone in Housefly Diets on the in Vivo Metabolism of [^{14}C]Parathion or [^{14}C]Paraoxon. To elucidate how the naturally occurring plant compounds myristicin and *d*-carvone were altering the toxicity of parathion and its activation product, paraoxon, to houseflies, the degradation of these ^{14}C-labeled insecticides was studied after the natural compounds had been fed to the flies. Results are summarized in Table II. They indicate that feeding myristicin blocked the degradation of parathion by living houseflies because hexane-soluble radiocarbon was three times larger in myristicin fed flies than in control flies, and a significant decrease in the production of water-soluble degradation products in flies and in their excreta was evident. While only 11.6% of the applied [^{14}C]parathion was recovered from control flies as determined by GLC, 46.2% was recovered from myristicin fed flies. The amounts of radiocarbon in the hexane extraction phases, however, were considerably greater than the amounts of parathion recovered, indicating the presence of other organic-soluble compounds. The decrease in parathion toxicity to houseflies after feeding myristicin (Table I) was therefore probably related to an overall decrease in the metabolism of parathion due to an inhibition of "factor A" (Figure 1), resulting in a decreased formation of the toxic activation product, paraoxon. Because paraoxon is rapidly degraded, it does not accumulate in flies and could not be detected by GLC.

Since in these experiments, 65% of the applied paraoxon had not been degraded in control flies (Table II), it is evident that a holding period of 30 min following paraoxon application to the flies was rather short. However, feeding

myristicin resulted in a significantly (at the 0.1% level) decreased in vivo degradation of paraoxon. This was probably due to an inhibition of "factor B" (Figure 1), as evidenced by the presence of more organic-soluble radiocarbon and more paraoxon remaining in myristicin fed flies and less water-soluble radiocarbon in flies and excreta. As shown in Table II, most of the organic-soluble radiocarbon was present as undegraded paraoxon which amounted to 82% of applied in myristicin fed flies but only 65% in control flies fed milk only. This inhibition of paraoxon degradation was reflected by a substantial increase in mortality of houseflies which had been fed diets containing myristicin (Table I).

Contrary to results obtained with myristicin, the effects of *d*-carvone in housefly diets on the persistence of [^{14}C]parathion or [^{14}C]paraoxon were either neglible or resulted in increased insecticide degradation (Table II). With [^{14}C]parathion treated flies, a decrease in hexane-soluble radiocarbon remaining in the flies and an increase in water-soluble degradation products excreted, as compared to control flies, was observed. Only 6.3% of the applied parathion was recovered from carvone fed flies while 11.6% remained in control flies. This increase in degradation of parathion due to feeding *d*-carvone was also reflected by a small increase in parathion toxicity (Table I), presumably due to an increase in paraoxon formation. Feeding *d*-carvone, however, did not significantly affect the degradation of paraoxon (Table II) or its toxicity to houseflies (Table I).

Because rather large amounts of water-soluble ^{14}C compounds derived from [*phenyl*-^{14}C]parathion were excreted by control and carvone fed flies (50 and 71% of applied), the water extraction phases from one experiment were acidified to pH 1.5 and extracted with chloroform/ether (2:1). This procedure quantitatively extracts *p*-nitrophenol. Analysis of the extraction phases by LSC indicated that 88–90% of the recovered radiocarbon remained in the water extraction phases and thus was not *p*-nitrophenol, although it could be a conjugate of this compound. Since a total of only 10 μg of either of the insecticides was originally applied to each group of 200 houseflies, not enough ^{14}C compounds were available to further identify hexane or water-soluble metabolites of [^{14}C]parathion or [^{14}C]paraoxon.

The effects of feeding myristicin or *d*-carvone to rats on the degradation of parathion and paraoxon by subcellular liver fractions has recently been reported (Fuhremann et al., 1978). In these experiments, feeding myristicin did not affect the microsomal degradation of parathion but stimulated its degradation by soluble enzyme preparations. With paraoxon, feeding myristicin was responsible for an increase in degradation by both microsomal and soluble cell fractions. With *d*-carvone, the rat microsomal degradation of parathion was stimulated but no effect on its degradation by soluble enzyme preparations was observed. With paraoxon, feeding *d*-carvone increased microsomal degradation, but inhibited degradation by soluble cell fractions.

The data reported here indicate that some naturally occurring plant components have the ability to alter the toxicity of insecticides as a result of their effects on insecticide degrading systems in insects. Biological activity of these natural compounds can also be demonstrated in mammals; however, the effects are not necessarily parallel. Thus caution must be exercised in extrapolating in vitro and in vivo data from one animal species to another.

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