METHYLENEDIOXYPHENYL SYNERGISTS for INSECTICIDES

Neely Turner

THE CONNECTICUT AGRICULTURAL
EXPERIMENT STATION
NEW HAVEN

FOUNDED 1875

BULLETIN 570, APRIL, 1953

CONTENTS

Methylenedioxyphenyl Synergists For Insecticides[1]

Neely Turner[2]

The synergistic effect on pyrethrum of N-isobutylundecylenamide, a compound having relatively little toxicity to insects, was discovered by Weed (1938). The compound was used in commercial formulations with success. Eagelson (1942) found a similar type of activity in sesame oil, and Haller, La Forge and Sullivan (1942) showed that sesamin was responsible for its effectiveness. They extended their study to related compounds and found some of them to be effective synergists. Continued investigations by Gersdorff and Gertler (1944) showed that the compounds containing the methylenedioxyphenyl structure were more toxic than the analogous benzamides. Several effective materials of this type have been synthesized and introduced, including piperonyl cyclonene, piperonyl butoxide, "N-propyl isome", "sulfoxide", and N-2-ethylhexylimide of endomethylenetetrahydrophthalic acid ("264").

In addition to their effect on pyrethrum, methylenedioxyphenyl compounds have been found synergistic with rotenone by Brannon (1947), with ryania by Reed and Filmer (1950), and with allethrin and benzene hexachloride by Hewlett (1952). Perry and Hoskins (1950) reported that piperonyl cyclonene reduced toxicity of DDT to normal houseflies, but definitely increased its toxicity to DDT-resistant strains. This was at least in part a result of interference in the degradation of DDT by resistant flies. Kerr (1951) found that materials synergistic with pyrethrum had no effect on the toxicity of "Thanite".

At least three names have been applied to this class of compounds: (1) synergists, (2) activators and (3) adjuvants. Bliss (1939) has defined synergism as toxicity of joint application exceeding that predicted from the toxicity of the two materials applied alone. Inman (1929) introduced the term activator to define a material of low toxicity which "activated" insecticides. Cox (1943) proposed the general term "adjuvant" to mean "any material added to a primary pest control chemical or product when a function is not being particularly emphasized for it". He used "activator" as a special class of adjuvant, which had little or no toxicity of its own, but increased the effectiveness of another compound. He reduced synergism to a special case of activation, in which the substances involved are active ingredients. The action of these compounds obviously fits all three definitions. The toxicity of a mixture with pyrethrins is greater than predicted by a study of the materials alone. They are materials of low toxicity which "activate" pyrethrum. They are "adjuvants" because they are "activators". Since they appear to have an effect on the physiological processes of the insect (as will be discussed later), the term "synergist" seems to be entirely proper. Synergism is far too important a concept in insecticide toxicology to be relegated to a special case of activation.

The purposes of the work described here were (1) to verify the synergism between these compounds and pyrethrum and rotenone by use of

---

[1] The assistance of Mrs. Nancy W. Wheeler and Mrs. Joan T. Curtiss is gratefully acknowledged.
[2] Entomologist, The Connecticut Agricultural Experiment Station.

the injection technique, (2) to determine the relation between dosage of synergist and its effectiveness and (3) to study the effect of methylenedioxyphenyl synergists on insecticides other than pyrethrum and rotenone.

MODE OF ACTION OF SYNERGISTS

Hartzell and Scudder (1942) have studied the histological effects of pyrethrum and N-isobutylundecylenamide on the central nervous system of the housefly. They found that pyrethrum clumped the chromatin of nuclei and the synergist caused chromatolysis or dissolution of the chromatin. David (1946) reported that non-volatile oils increased droplet size and reduced loss by evaporation, thereby increasing toxicity. Sesame oil and N-isobutylundecylenamide increased toxicity more than mineral oils. David and Bracey (1947) studied the effects of synergists, and found that the effect was not entirely one of droplet size. When the synergists were applied first, and pyrethrum 15 minutes later, the increase in mortality was not lost. Furthermore, the synergism was not the result of a slower knockdown. Lindquist *et al.* (1947) also pretreated houseflies with synergists before applying pyrethrum. When pyrethrum followed the synergist in 30 seconds, toxic effect was greatest, but synergism was still noticeable when the interval was increased to four hours. When pyrethrum was applied first and the synergist later, there was some effect at an interval of 30 seconds, but none at all at a greater interval. The results implied that the synergists affected the physiology of the flies in such a way as to increase the toxicity of pyrethrum. It was also concluded that the effect of pyrethrum was slight and of short duration.

Wilson (1949) applied droplets of synergist and pyrethrum on different parts of the bodies of flies. The toxicity was almost as great when the two materials were applied to different parts of the body as when a mixture of the two was applied in one spot. Obviously, then, the synergist was not affecting penetration of the pyrethrum, nor preventing its oxidation.

Chamberlain (1950) sprayed wooden panels with piperonyl butoxide, pyrethrum, and a mixture of the two. Flies exposed to piperonyl butoxide for one hour and transferred to a pyrethrum panel were "knocked down" in 30 minutes, and there was no difference in transferal immediately or after a "rest" of three hours after exposure to butoxide. Flies exposed to pyrethrum first and transferred immediately to butoxide showed some effect of synergism, but a delay of one hour after pyrethrum exposure destroyed it. Chamberlain repeated the type of experiment of Wilson (1949), putting droplets of synergist and pyrethrum on different parts of the body. The results showed that butoxide applied to the head and pyrethrum to the abdomen were as effective as the two applied in the same spot.

Chamberlain (1950) selected lipase for studies of the effect of pyrethrum, because of the labile ester linkage in the pyrethrin molecule. He prepared lipase from the roach and tested it on mosquito larvae in combination with pyrethrum. There was little reduction in pyrethrum toxicity caused by an active preparation of roach lipase. Tests of series of synergists showed no correlation between inhibition of enzymatic hydrolysis and effectiveness as synergists. Although the results were not conclusive, Chamberlain believed that butoxide did inhibit lipase which may detoxify pyrethrum, and felt that the hypothesis warranted further investigation.

Page and Blackith (1949) concluded that the synergists formed a loose molecular complex with pyrethrum, because the synergist was most effective (in terms of knock-down threshold) in equivalent molecular concentrations with pyrethrum. On the other hand, Miller *et al.* (1952) found little evidence of formation of complexes by use of freezing point determinations and infrared spectrometer readings.

Hewlett (1951) showed that piperonyl butoxide did not make films of pyrethrins more persistent. He also found that the pyrethrin equivalent of a given pyrethrin-piperonyl butoxide mixture could differ according to the method of application or the species of test insect.

Kerr (1951) studied the relation between chemical structure and toxicity and the mode of action of synergists for pyrethrum. Topical application of measured drops showed that all tested had a primary effect in addition to their influence on dosage. Most of the synergists actually delayed the paralysis in topical tests. In tests on flies in motion, many synergists increased activity. Kerr (1951) also added materially to the knowledge of chemistry and effectiveness of the methylenedioxyphenyl compounds.

Mallis *et al.* (1952) added other synergists to a mixture of pyrethrum and piperonyl butoxide in an effort to produce double synergism. There was no evidence of any such phenomenon.

It is obvious that the mode of action of these synergists is still unknown. Their effect on droplet size and rate of evaporation of droplets, while marked, does not account for their synergism. Likewise, they do not enter into any physical or chemical combination with pyrethrum on the surface of the insect. In many respects their behavior is like that of nicotine combined with pyrethrum (Turner and Bliss, 1953). It would seem logical to investigate their mode of action in exactly the same way in which the mode of action of an insecticide is studied.

EXPERIMENTAL

At the time these studies were started there was some conjecture as to the way in which the synergists acted. The technique chosen for application was injection, in order to avoid any effect of the synergist on penetration of the cuticle. The insects were adult *Oncopeltus fasciatus* used seven to ten days after molting. The amount injected was standardized at three microliters. The water-soluble materials nicotine, arsenic and sodium azide were used without additions. DDT and rotenone were dissolved in xylene and emulsified with *Triton X-100*. The pyrethrum was in petroleum oil solution and was emulsified with *Triton X-100*. The synergists were emulsified with the same agent. Unless stated otherwise in the text and tables, the result of each test includes four lots of 12 insects each, or a total of 48 insects for each dosage used. Results were obtained in 48 hours, and the percentage dead included those individuals so badly paralyzed that their recovery was improbable.

Tests for Synergism with Pyrethrum, Rotenone and Allethrin

There is general agreement in the literature that the methylenedioxyphenyl compounds act as synergists with pyrethrum. The tests on which this conclusion was based were by external application. Four of the materials commercially available were tested for synergism by injection.

TABLE 1. TEST FOR SYNERGISM BETWEEN PYRETHRUM, AND PIPERONYL BUTOXIDE AND PIPERONYL CYCLONENE. INJECTION OF MILKWEED BUGS, EACH PERCENTAGE THE RESULT OF 4 REPLICATES TOTALLING 48 INSECTS.

Per cent pyrethrum	Per cent synergist	Per cent mortality
.014	....	88
.01	....	69
.007	....	56
.005	....	23
	Piperonyl butoxide	
....	4.0	10
....	2.8	17
....	2.0	0
....	1.4	12
	Piperonyl cyclonene	
....	4.0	31
....	2.8	12
....	2.0	15
....	1.4	8
	Piperonyl butoxide	
.005	1.0	100
.0035	1.0	83
.0025	1.0	83
.00175	1.0	60
	Piperonyl cyclonene	
.005	1.0	100
.0035	1.0	100
.0025	1.0	98
.00175	1.0	96

EXPECTED TOXICITY ON THE BASIS OF SIMILAR ACTION

1 per cent butoxide = .0021 per cent pyrethrum

1 per cent cyclonene = .0015 per cent pyrethrum

	Expected	Observed	Synergism
Butoxide			
.005 + .0021 = .0071	61	100	+++
.0035 + .0021 = .0056	36	83	+++
.0025 + .0021 = .0046	24	83	+++
.00175 + .0021 = .00385	16	60	+++
Cyclonene			
.005 + .0015 = .0065	46	100	+++
.0035 + .0015 = .005	30	100	+++
.0025 + .0015 = .004	17	98	+++
.00175 + .0015 = .00325	10	96	+++

The results, given in Tables 1, 2 and 3, show that there was good evidence of synergism. Piperonyl butoxide and piperonyl cyclonene were not very toxic to milkweed bugs by injection. "Sulfoxide" and "264" had substantial toxicity. However, all were more effective when used with pyrethrum than would be expected on the basis of similar action. It is evident that their effectiveness is not limited to external application.

TABLE 2. TEST FOR SYNERGISM BETWEEN PYRETHRUM AND SULFOXIDE. EACH PERCENTAGE MORTALITY REPRESENTS 4 REPLICATES TOTALLING 48 INSECTS.

Per cent pyrethrum	Per cent sulfoxide	Per cent mortality
.025	....	92
.0175	....	83
.0125	....	63
.0087	....	52
.0062	....	40
....	4.0	88 (73)[1]
....	2.8	27 (44)
....	2.0	27 (38)
....	1.4	25 (21)
.0062	1.0	96
.0044	1.0	94
.0031	1.0	92
.0022	1.0	54

EXPECTED TOXICITY ON THE BASIS OF SIMILAR ACTION
1 per cent sulfoxide = .0025 pyrethrum

	Expected	Observed	Synergism
.0062 + .0025 = .0087	53	96	+++
.0044 + .0025 = .0069	43	94	+++
.0031 + .0025 = .0056	34	92	+++
.0022 + .0025 = .0047	26	54	+++

[1] Data from a similar test made 3 days earlier.

TABLE 3. TEST FOR SYNERGISM BETWEEN PYRETHRUM AND 264. EACH PERCENTAGE MORTALITY REPRESENTS 4 REPLICATES TOTALLING 48 INSECTS.

Per cent pyrethrum	Per cent 264	Per cent mortality
.02	....	98
.014	....	85
.01	....	85
.007	....	56
....	4.0	71
....	2.8	56
....	2.0	28
....	1.4	10
.01	1.0	100
.007	1.0	100
.005	1.0	100
.0035	1.0	96

EXPECTED TOXICITY ON THE BASIS OF SIMILAR ACTION
1 per cent 264 = .0024 pyrethrum

	Expected	Observed	Synergism
.01 + .0024 = .0124	87	100	+++
.007 + .0024 = .0094	74	100	+++
.005 + .0024 = .0074	58	100	+++
.0035 + .0024 = .0059	41	96	+++

The results, given in Tables 1, 2 and 3, show that there was good evidence of synergism. Piperonyl butoxide and piperonyl cyclonene were not very toxic to milkweed bugs by injection. "Sulfoxide" and "264" had substantial toxicity. However, all were more effective when used with pyrethrum than would be expected on the basis of similar action. It is evident that their effectiveness is not limited to external application.

TABLE 2. TEST FOR SYNERGISM BETWEEN PYRETHRUM AND SULFOXIDE. EACH PERCENTAGE MORTALITY REPRESENTS 4 REPLICATES TOTALLING 48 INSECTS.

Per cent pyrethrum	Per cent sulfoxide	Per cent mortality
.025	....	92
.0175	....	83
.0125	....	63
.0087	....	52
.0062	....	40
....	4.0	88 (73)[1]
....	2.8	27 (44)
....	2.0	27 (38)
....	1.4	25 (21)
.0062	1.0	96
.0044	1.0	94
.0031	1.0	92
.0022	1.0	54

EXPECTED TOXICITY ON THE BASIS OF SIMILAR ACTION
1 per cent sulfoxide = .0025 pyrethrum

	Expected	Observed	Synergism
.0062 + .0025 = .0087	53	96	+++
.0044 + .0025 = .0069	43	94	+++
.0031 + .0025 = .0056	34	92	+++
.0022 + .0025 = .0047	26	54	+++

[1] Data from a similar test made 3 days earlier.

TABLE 3. TEST FOR SYNERGISM BETWEEN PYRETHRUM AND 264. EACH PERCENTAGE MORTALITY REPRESENTS 4 REPLICATES TOTALLING 48 INSECTS.

Per cent pyrethrum	Per cent 264	Per cent mortality
.02	....	98
.014	....	85
.01	....	85
.007	....	56
....	4.0	71
....	2.8	56
....	2.0	28
....	1.4	10
.01	1.0	100
.007	1.0	100
.005	1.0	100
.0035	1.0	96

EXPECTED TOXICITY ON THE BASIS OF SIMILAR ACTION
1 per cent 264 = .0024 pyrethrum

	Expected	Observed	Synergism
.01 + .0024 = .0124	87	100	+++
.007 + .0024 = .0094	74	100	+++
.005 + .0024 = .0074	58	100	+++
.0035 + .0024 = .0059	41	96	+++

TABLE 4. EFFECT OF PIPERONYL CYCLONENE AND BUTOXIDE ON THE TOXICITY OF ROTENONE. MEAN OF 4 REPLICATES, ADULT MILKWEED BUGS INJECTED.

Per cent rotenone	Synergist	Per cent dead and paralyzed
.0043	....	89
.0031	....	77
.0022	....	39
.0015	....	29
.0031	P. cyclonene 1%	96
.0022	" " "	75
.0015	" " "	50
.0011	" " "	27
.0031	P. butoxide 1%	96
.0022	" " "	85
.0015	" " "	43
.0011	" " "	33
....	P. cyclonene 2.0%	31
....	" " 1.4	25
....	" " 1.0	10
....	" " .7	4
....	P. butoxide 2.0%	25
....	" " 1.4	15
....	" " 1.0	10
....	" " .7	12

EXPECTED TOXICITY ON THE BASIS OF SIMILAR ACTION
1 per cent cyclonene or butoxide = .001 per cent rotenone

	Expected	Observed		Synergism
		cyclonene	butoxide	
.0031 + .001 = .0041	85	96	96	+
.0022 + .001 = .0032	72	75	85	+
.0015 + .001 = .0025	58	50	43	−
.0011 + .001 = .0021	45	27	33	−

TABLE 5. TEST FOR SYNERGISM BETWEEN ROTENONE AND SULFOXIDE. EACH PERCENTAGE MORTALITY REPRESENTS 4 REPLICATES TOTALLING 48 INSECTS.

Per cent rotenone	Per cent sulfoxide	Per cent mortality
.016	....	89
.0112	....	79
.008	....	54
.0056	....	31
....	4.0	89
....	2.8	77
....	2.0	50
....	1.4	31
.0112	1.0	92
.008	1.0	92
.0056	1.0	77
.004	1.0	73

EXPECTED TOXICITY ON THE BASIS OF SIMILAR ACTION
1 per cent sulfoxide = .0035 per cent rotenone

	Expected	Observed	Synergism
.0112 + .0035 = .0147	86	92	+
.008 + .0035 = .0115	76	92	+
.0056 + .0035 = .0091	65	77	+
.004 + .0035 = .0075	56	73	++

A similar test of synergism between three of the four synergists and rotenone has been summarized in Tables 4 and 5. Piperonyl butoxide and cyclonene were apparently somewhat synergistic with the two higher dosages of rotenone, but were not effective at the two lower dosages. Furthermore, the degree of synergism was far less than in the tests with pyrethrum. "Sulfoxide" showed synergism with all four dosages of rotenone, but again was less synergistic than with pyrethrum.

The four synergists were tested with allethrin. The results summarized in Table 6 show that piperonyl butoxide, piperonyl cyclonene and "sulfoxide" showed no evidence of synergism with allethrin. Toxicity of allethrin with these synergists was about equal to toxicity of allethrin alone. There was some increased toxicity when allethrin was injected with "264", but far less than the same synergist produced with pyrethrum (see Table 3). The only conclusion possible is that there is enough difference in the mode of action of pyrethrum and allethrin to make these synergists ineffective on the milkweed bug.

Table 6. Effect of Four Synergists On the Toxicity of Allethrin Injected Into Milkwood Bugs. Each Figure Is the Result of 4 Replicates Totalling 48 Insects.

Per cent allethrin	Per cent synergist	Per cent dead
.07	....	94
.05	....	75
.035	....	40
.025	....	23
	Piperonyl butoxide	
.035	.5	40
.025	.35	27
.0175	.25	6
.0125	.175	10
	Piperonyl cyclonene	
.035	.5	6
.025	.35	4
.0175	.25	10
.0125	.175	4
.07	....	96
.05	....	54
.035	....	35
.025	....	10
	"264"	
.035	.5	50
.025	.35	23
.0175	.25	12
.0125	.175	4
	Sulfoxide	
.035	.5	37
.025	.35	15
.0175	.25	6
.0125	.175	6

Relation Between Amount of Synergist and Effectiveness

There has been some disagreement as to the relation between amount of synergist and its effectiveness. Page and Blackith (1949) found that a ratio of one part pyrethrins and five parts piperonyl butoxide provided the maximum degree of synergism. Their criterion was the amount of material required to knock down *Calandra granaria* adults. On the other hand, Goodwin-Bailey and Holborn (1952) reported that the degree of synergism increased as the ratio of piperonyl butoxide was increased. The degree of synergism was calculated by dividing the concentration of pyrethrins required to produce 50 per cent mortality by the amount required for equal mortality when used with piperonyl butoxide. Their degree of synergism plots as a straight line in relation to the logarithm of the ratio of piperonyl butoxide. However, if the concentration of pyrethrins required to produce 50 per cent mortality is plotted arithmetically against the ratio of piperonyl butoxide, the result is a curve not unlike the one drawn by Page and Blackith (1949). This appears to be one of the many instances in which the way the data are plotted may determine the interpretation of the results.

It has been suggested by Lindquist *et al.* (1947), Wilson (1949) and Chamberlain (1950) that these synergists affect the physiology of some insects in such a way that toxicity of pyrethrum is increased. If this is true, the amount of synergist would be expected to act in a similar way as the amount of pyrethrum. In other words, the dosage would be in a logarithmic relationship. However, since the synergist must be used with an insecticide, the response as measured includes the effects of both synergist and insecticide. If, for instance, a constant concentration of insecticide is applied with a dosage series of synergist, the resulting dosage-response curve might not be a straight line on the log-probit grid. The line would be expected to show curvature as the result of addition of a constant mortality caused by the insecticide. Gersdorff and Gertler (1944) have published curves of this type.

Two series of tests were made to test this hypothesis. In the first, summarized in Table 7, piperonyl cyclonene and piperonyl butoxide were used in dosage series with a constant concentration of .0015 per cent rotenone. The length of the series made it necessary to carry it out as two different experiments, so that the results cannot be combined. Piperonyl cyclonene proved to be a relatively poor synergist for rotenone in this test. Piperonyl butoxide was far more effective. In neither case, however, was there any substantial evidence of curvature of the dosage-response curves.

In the second test "264" was used with .005 per cent pyrethrum (Table 8). Again there was no evidence of distinct curvature in the dosage-response curve (Figure 1).

TABLE 7. DOSAGE TESTS OF TWO SYNERGISTS INJECTED INTO MILKWEED BUGS WITH .0015 PER CENT ROTENONE.

Per cent synergist	Per cent mortality	
	Piperonyl cyclonene	Piperonyl butoxide
None	31[1]	31[1]
.0078	35	27
.0156	28	38
.0312	45	40
.0625	49	43
None	30[2]	30[2]
.0625	53	52
.125	36	50
.25	55	64
.5	64	84
1.0	64	88
2.0	68	93

[1] Mean of 4 replicates, a total of 48 insects.
[2] Mean of 8 replicates, a total of 96 insects.

TABLE 8. DOSAGE TEST OF "264" INJECTED INTO MILKWEED BUGS WITH .005 PER CENT PYRETHRUM. EACH FIGURE IS THE MEAN OF 4 REPLICATES TOTALLING 48 INSECTS.

Per cent "264"	Per cent mortality
None	63
.01875	46
.0375	65
.0625	85
.125	81
.25	98
.5	100

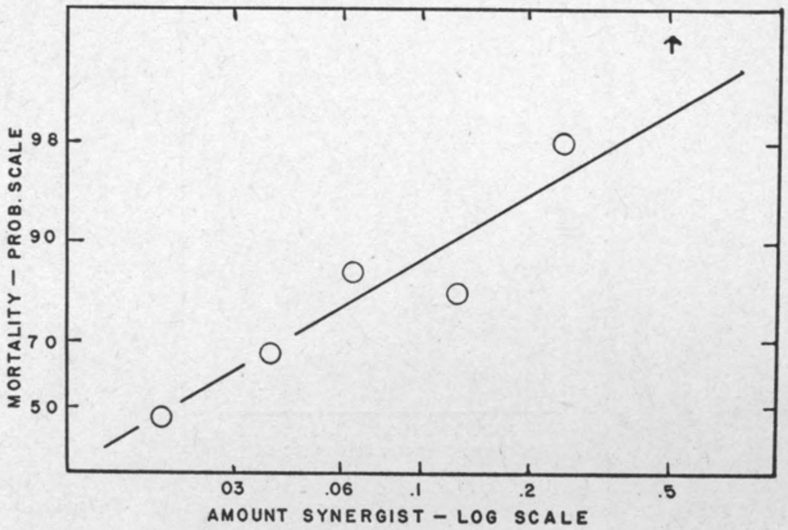

Figure 1. Relation between concentrations of "264" applied with .005 per cent pyrethrum and mortality.

TABLE 7. DOSAGE TESTS OF TWO SYNERGISTS INJECTED INTO MILKWEED BUGS WITH .0015 PER CENT ROTENONE.

	Per cent mortality	
Per cent synergist	Piperonyl cyclonene	Piperonyl butoxide
None	31[1]	31[1]
.0078	35	27
.0156	28	38
.0312	45	40
.0625	49	43
None	30[2]	30[2]
.0625	53	52
.125	36	50
.25	55	64
.5	64	84
1.0	64	88
2.0	68	93

[1] Mean of 4 replicates, a total of 48 insects.
[2] Mean of 8 replicates, a total of 96 insects.

TABLE 8. DOSAGE TEST OF "264" INJECTED INTO MILKWEED BUGS WITH .005 PER CENT PYRETHRUM. EACH FIGURE IS THE MEAN OF 4 REPLICATES TOTALLING 48 INSECTS.

Per cent "264"	Per cent mortality
None	63
.01875	46
.0375	65
.0625	85
.125	81
.25	98
.5	100

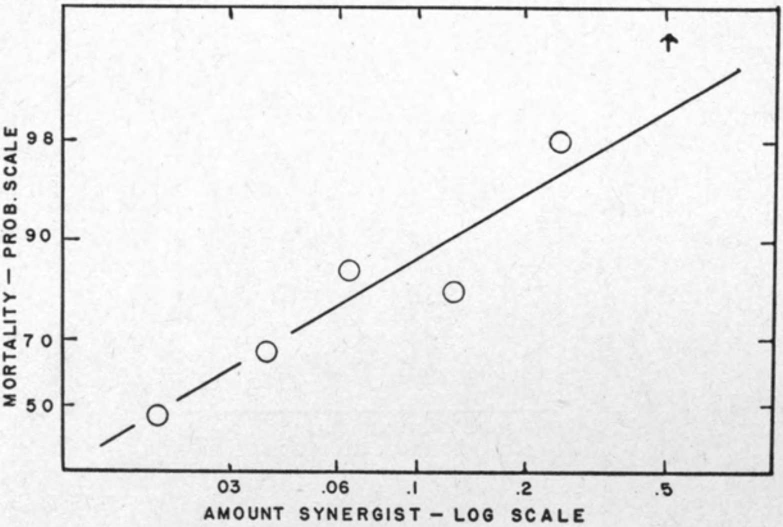

Figure 1. Relation between concentrations of "264" applied with .005 per cent pyrethrum and mortality.

In another type of test, pyrethrum was injected alone in a dosage series, and together with synergists, in six dosage series of different ratios of pyrethrum and synergist. The results have been summarized in Tables 9 and 10 and in Figures 2 and 3. The size of the test made it impossible to complete in one day. The three sections of each table represent the tests made on three days, each with its standard of pyrethrum alone. Variation between the tests with pyrethrum on the different days was not large.

TABLE 9. RELATION BETWEEN AMOUNT OF "264" AND TOXICITY OF MIXTURES WITH PYRETHRUM. EACH FIGURE IS THE RESULT OF 4 REPLICATES, 48 INSECTS.

Per cent pyrethrum	Per cent mortality	Per cent		Per cent mortality	Per cent		Per cent mortality
		Pyrethrum	264		Pyrethrum	264	
.014	92	.005	.5	98	.005	.25	98
.01	90	.0035	.35	96	.0035	.175	90
.007	61	.0025	.25	80	.0025	.125	77
.005	21	.00175	.175	69	.00175	.0875	45
.014	90	.005	.125	90	.007	.0625	92
.01	81	.0035	.0875	69	.005	.0438	77
.007	62	.0025	.0625	48	.0035	.0312	51
.005	27	.00175	.0438	23	.0025	.0219	29
.014	94	.01	.0375	87	.01	.0188	79
.01	77	.007	.0263	77	.007	.0131	73
.007	52	.005	.0188	56	.005	.0093	25
.005	38	.0035	.0131	16	.0035	.0066	21

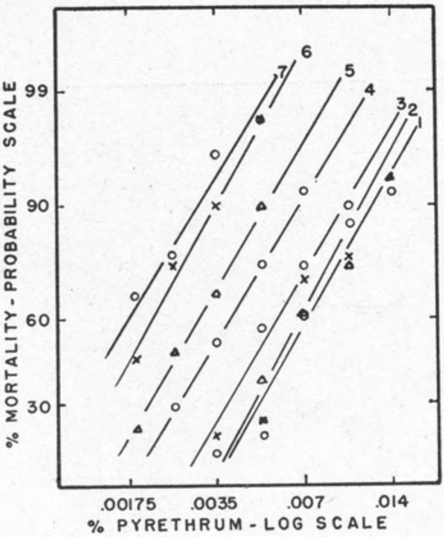

Figure 2. Dosage response curves for differing amounts of "264" applied with pyrethrum.
1 = none, 2 = .0188%, 3 = .0375%, 4 = .0625%, 5 = .125%, 6 = .25%, 7 = .5%.

The results for "264" appear to form a family of parallel curves, not necessarily equidistant. However, the distribution of the curves does not indicate that any particular ratio was more efficient than the others. In the case of sulfoxide (Table 10, Figure 3), the highest concentrations were equally effective. The lowest concentration, .01875 per cent, produced a curve of much lower slope than the other concentrations.

TABLE 10. RELATION BETWEEN AMOUNT OF "SULFOXIDE" AND TOXICITY OF MIXTURES WITH PYRETHRUM. EACH FIGURE IS THE RESULT OF 4 REPLICATES, 48 INSECTS.

Per cent pyrethrum	Per cent mortality	Per cent		Per cent mortality	Per cent		Per cent mortality
		Pyrethrum	Sulfoxide		Pyrethrum	Sulfoxide	
.014	90	.005	.5	97	.005	.25	97
.01	66	.0035	.35	74	.0035	.175	85
.007	59	.0025	.25	55	.0025	.125	47
.005	29	.00175	.175	22	.00175	.0875	25
.014	94	.005	.125	65	.007	.0625	89
.01	70	.0035	.0875	54	.005	.04375	58
.007	40	.0025	.0625	12	.0035	.03125	21
.005	19	.00175	.04375	21	.0025	.02188	15
.014	87	.01	.0375	92	.01	.01875	78
.01	79	.007	.02625	85	.007	.013125	66
.007	48	.005	.01875	42	.005	.00937	48
.005	21	.0035	.01313	19	.0035	.00656	33

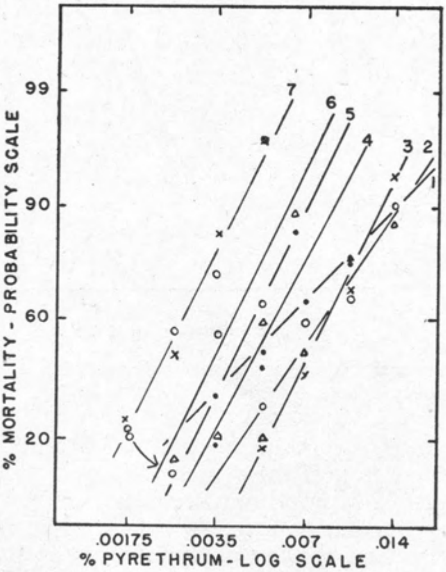

Figure 3. Dosage response curves for different amounts of sulfoxide applied with pyrethrum.

1 and 3 = none, 2 = .0188%, 3 = .0375%, 4 = .0625%, 5 = .125%, 6 = .25%, 7 = .25 and .5%.

If such a change in slope were valid, it would raise some very interesting problems. Two additional series of tests were made at this ratio. The results (Table 11, Figure 4) show that in the higher dosages the curve was apparently parallel with the curve for pyrethrum. At low dosages, the curve was less steep. This type of curve for pyrethrum has been discussed by Bliss (1939).

TABLE 11. TOXICITY OF PYRETHRUM AND OF PYRETHRUM PLUS A LOW DOSAGE OF SULFOXIDE. EACH FIGURE MEAN OF 4 REPLICATES, 48 INSECTS.

Per cent pyrethrum	Per cent mortality	Per cent		Per cent mortality
		Pyrethrum	Sulfoxide	
.014	98	.014	.02625	100
.01	83	.01	.01875	94
.007	73	.007	.01312	83
.005	40	.005	.00937	56
		.0035	.00656	31
		.0025	.00469	21
		.00175	.00388	16

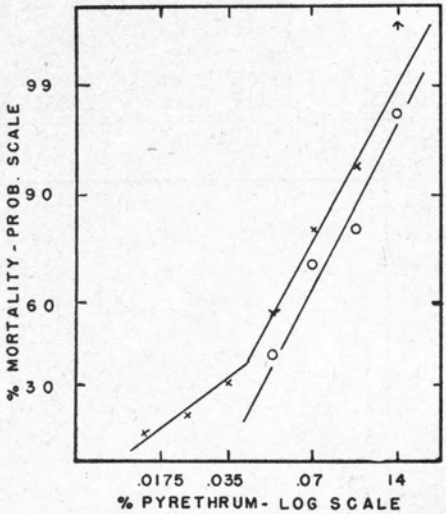

Figure 4. Dosage response curves for a low concentration of sulfoxide with pyrethrum (at left) and pyrethrum alone (at right).

If the concentration of pyrethrum required to kill 50 per cent of the insects is interpolated from Tables 9 and 10, and plotted in relation to the ratio of synergist and pyrethrum, or the amount of synergist in the mixture, curves very similar to those of Page and Blackith (1949) result (Figure 5). If, however, the log of the amount in the mixture is used, the points come much closer to forming a straight line (Figure 6). There is, therefore, a strong implication that response to addition of one of these synergists is in relation to the logarithm of the amount used, as would be expected on the basis of the theory as to their action.

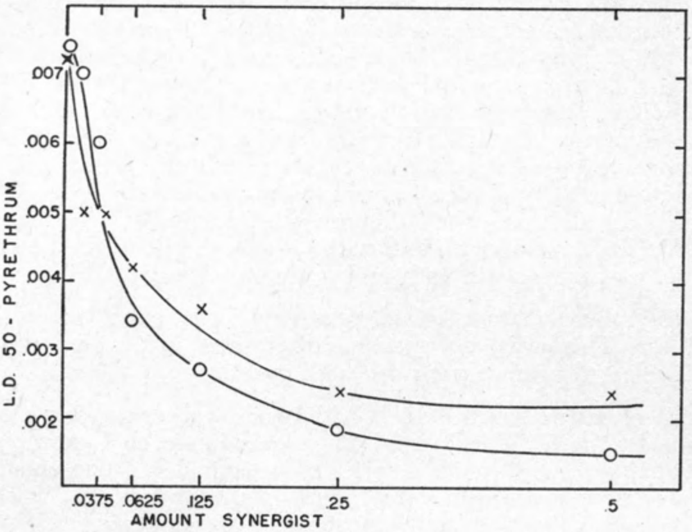

Figure 5. Effect of concentration of synergist on the amount of pyrethrum required to kill 50 per cent, arithmetic scale.

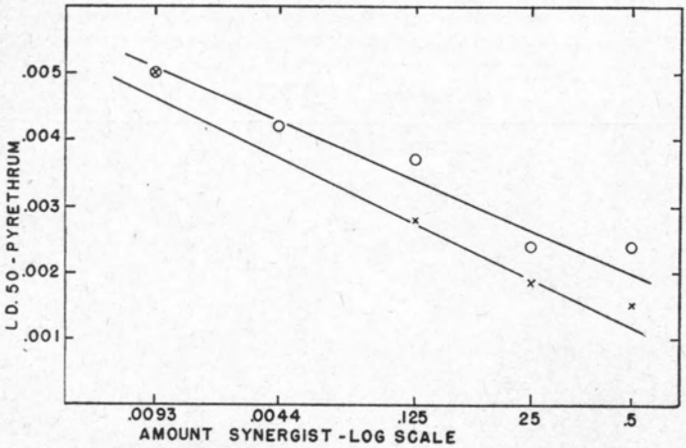

Figure 6. Effect of log of amount of synergist on amount of pyrethrum required to kill 50 per cent.

Effect of Methylenedioxyphenyl Synergists on Other Insecticides

Three synergists, N-propyl isome, piperonyl cyclonene and piperonyl butoxide, were tested with nicotine, sodium azide, DDT and arsenic in single-dosage "screening" tests. The insecticides were applied alone and with 1 per cent of the synergists. Pyrethrum was included in the tests for comparison. The dosage of insecticide was selected to kill about 50 per cent of the test insects. The results have been summarized in Table

12. Each of the synergists increased the toxicity of pyrethrum, and the one applied with rotenone was also synergistic. N-propyl isome had no effect on toxicity of sodium azide, arsenic and DDT, and reduced toxicity of nicotine. Piperonyl cyclonene apparently increased toxicity of arsenic, had little effect on nicotine and sodium azide, and reduced the toxicity of DDT. Piperonyl butoxide appeared to increase the toxicity of DDT, but a second test (not reported here) showed little effect. It had little or no effect on toxicity of nicotine and sodium azide, and reduced toxicity of arsenic. In all cases, the differences in toxicity of nicotine, sodium azide, DDT and arsenic with and without a synergist were much smaller than toxicity of pyrethrum with and without a synergist.

Obviously these materials had relatively little effect on these four insecticides. This was not particularly surprising, since they were materials selected primarily for use with pyrethrum.

A series of 10 materials which had been synthesized for tests with pyrethrum by the U. S. Industrial Chemicals Research Laboratory were made available for tests with a series of insecticides. The chemistry of these materials is given in Figure 7. All of these were used with DDT, sodium fluoride, nicotine, aldrin, rotenone and arsenic at 1 per cent concentration.

TABLE 12. RESULTS OF "SCREENING" TESTS OF INSECTICIDES WITH AND WITHOUT 3 SYNERGISTS. EACH FIGURE IS THE AVERAGE OF 4 REPLICATES AND 48 INSECTS.

Insecticide	Concentration (per cent)	Per cent dead: Insecticide alone	Per cent dead: Insecticide plus 1 per cent N-propyl isome
None	....	....	2
Nicotine	.1	77	60
Sodium azide	.25	52	52
DDT	.0312	31	32
Arsenic	.01	64	60
Rotenone	.0022	54	88
Pyrethrum	.0062	25	81
			1 per cent piperonyl cyclonene
None	....	....	6
Nicotine	.1	68	60
Sodium azide	.25	68	60
DDT	.043	51	31
Arsenic	.01	64	80
Pyrethrum	.0062	53	95
			1 per cent piperonyl butoxide
None	....	....	8
Nicotine	.1	60	70
Sodium azide	.25	55	59
DDT	.043	49	66
Arsenic	.01	70	55
Pyrethrum	.0062	51	100

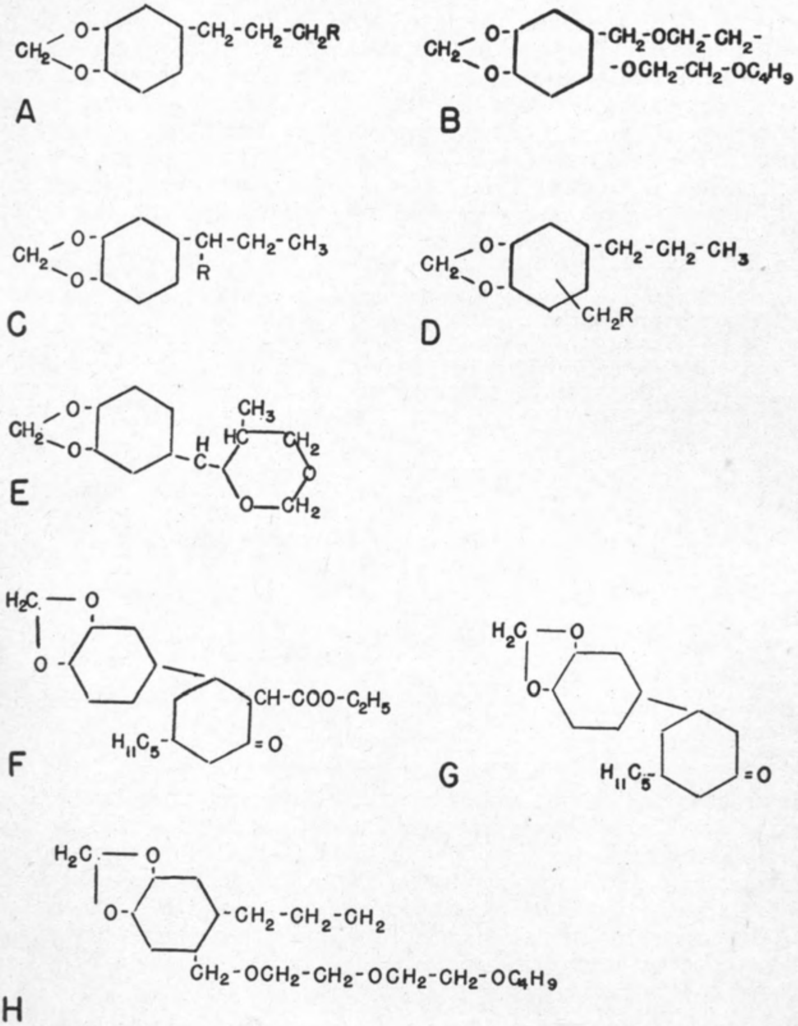

Figure 7. Structure of methylenedioxy compounds used.
A = 1 when R = $-OCH_2CH_2OC_4H_9$ and
2 when R = $-OCH_2CH_2OCH_2CH_2OC_2H_5$;
B = 3; C = 4 when R = $-OCH_2CH_2OC_4H_9$ and
5 when R = $-OCH_2CH_2OCH_2CH_2OC_4H_9$;
D = 6 when R = $-OCH_2CH_2OCH_3$, 7 when R = $-OCH_2CH_2OC_4H_9$, 8 when R = $-OCH_2CH_2OCH_2C_6H_5$ and 9 when R = $-OCH_2CH_2OCH_2CH_2OC_4H_9$;
E = 10; F and G are the two principal compounds in piperonyl cyclonene and H is piperonyl butoxide.

Results of the tests are given in Table 13, in which equal, plus and minus signs have been used to indicate the effect of the synergist on the compound in relation to toxicity of the insecticide used alone. The results show more relation between the insecticides used and all the synergists than between type of synergist and synergistic action. No compound increased the toxicity of DDT or nicotine. Only one increased the toxicity of aldrin. All materials increased the toxicity of sodium fluoride. Six increased the toxicity of arsenic. In most cases both the increases and decreases were relatively small.

Table 13. Results of Injections of Synergists With Insecticides As Compared With Toxicity of Insecticides Alone, In Terms of Less (–), Equal (=) or Greater (+) Toxicity.

	Insecticide				
Compound	DDT	Sodium fluoride	Nicotine	Aldrin	Arsenic
1	—	+	=	=	+
2	=	+	=	+	=
3	=	++	=	=	+
4	=	+	=	=	=
5	=	+	—	—	=
6	=	+	=	=	=
7	—	+	—	=	+
8	—	+	—	—	+
9	—	+	=	—	=
10	—	+	=	—	=
Piperonyl butoxide	–	+	=	=	=
Piperonyl cyclonene	=	+	–	=	+
N-propyl isome	–	++	–	=	+

In order to make a further test of some of these relations, two synergists were selected for a dosage test with each of the five insecticides (Table 14). The two selected for use with DDT and nicotine appeared to be antagonistic in the "screening" test in Table 13. In the dosage test there was little indication that either reduced toxicity of DDT in accordance with the concentration used. One of the two materials used with nicotine had no effect on its toxicity, the other (piperonyl cyclonene) apparently reduced its effectiveness.

Material 2, which appeared to increase toxicity of aldrin in the preliminary test, did not show any large effect in the dosage test. Material 8, which was antagonistic in the first test, was also antagonistic in the dosage test.

The two materials which had the largest effect on sodium fluoride, No. 2 and N-propyl isome, increased toxicity somewhat at the higher dosages used.

Material 1 was very effective in increasing toxicity of arsenic in the dosage test, but No. 7 had little effect.

TABLE 14. DOSAGE TESTS OF SOME SYNERGISTS WITH 5 INSECTICIDES

Insecticide	Concentration (per cent)	Per cent mortality	Synergist	Per cent mortality			
				Concentration of synergist with insecticide			
				.25%	.5%	1.0%	2.0%
DDT	.05	21	Piperonyl butoxide	25	23	10	25
			9	36	10	23	16
Nicotine	.1	20	7	19	13	17	25
			Piperonyl cyclonene	8	8	10	4
Aldrin	.005	25	2	8	13	21	29
			8	0	17	17	8
Sodium fluoride	.5	65	3	46	50	48	75
			N-propyl isome	54	56	71	81
Arsenic	.02	29	1	13	46	58	63
			7	25	29	38	21

These dosage tests in general confirmed the preliminary results, and showed that none of these synergists increased toxicity of DDT, nicotine and aldrin. Some were effective with arsenic and sodium fluoride. However, the increases in toxicity were relatively small in relation to the amount of synergist used. It seems obvious that none of these methylenedioxyphenyl compounds was very effective with any of these insecticides.

SUMMARY

Several compounds of relatively low toxicity to insects have been shown to increase the toxicity of several insecticides. Such compounds meet the requirements of the definition of synergists.

The mode of action of this type of synergist has not been found. It is obviously a physiological effect of the same general type as that exerted by insecticides.

Tests by injection showed that piperonyl butoxide, piperonyl cyclonene, "sulfoxide" and N-2-ethylhexylimide of endomethylenetetrahydrophthalic acid ("264") were synergistic when applied with pyrethrum. "Sulfoxide" was also synergistic when applied with rotenone. Piperonyl butoxide and piperonyl cyclonene were synergistic at the higher dosages used. None of the four materials was synergistic when injected with allethrin at the dosages tested. These dosages were as high as effective dosages with pyrethrum.

The data obtained did not show that any particular ratio of rotenone or pyrethrum and synergist was more efficient than other ratios. In fact, the response seemed to be in relation to the logarithm of the amount of synergist used.

The methylenedioxyphenyl compounds introduced commercially were not effective as synergists for nicotine, sodium azide, DDT and arsenic.

A series of experimental materials and three commercial synergists had little effect on the toxicity of DDT, nicotine, aldrin and arsenic. All showed some synergism with sodium fluoride. Dosage tests confirmed the screening tests in general. It is obvious that there is a stronger relation between insecticide and all synergists, than between the type of synergist used and any insecticide.

LITERATURE CITED

1. Bliss, C. I. The toxicity of poisons applied jointly. Ann. Appl. Biol. **26**:585-615. 1939.
2. Brannon, L. W. Piperonyl cyclonene and Piperonyl butoxide as synergists with rotenone. Jour. Econ. Ent. **40**:933-934. 1947.
3. Chamberlain, R. W. An investigation on the action of piperonyl butoxide with pyrethrum. Am. Jour. Hyg. **52**:153-183. 1950.
4. Cox, A. J. Terminology of insecticides, fungicides and other economic poisons. Jour. Econ. Ent. **36**:813-821. 1943.
5. David, W. A. L. Factors influencing the interaction of insecticidal mists and flying insects. I. The design of a spray testing chamber and some of its properties. Bul. Ent. Res. **36**:373-393. 1946.
6. ——————— and P. Bracey. Factors influencing the interaction of insecticidal mists and flying insects. Part IV. Some experiments with adjuvants. Bul. Ent. Res. **37**:393-398. 1947.
7. Eagelson, C. Sesame in insecticides. Soap and Sanit. Chem. **18**(12):125,127. 1942.
8. Gersdorff, W. A., and S. I. Gertler. Toxicity to houseflies of certain N-substituted piperonylamides and benzamides combined with pyrethrins in oil base insect spray. Soap and Sanit. Chem. **20**(2):123,125. 1944.
9. Goodwin-Bailey, K. F., and J. M. Holborn. Laboratory and field experiments with pyrethrins/piperonyl butoxide powders for the protection of grain. Pyrethrum Post **2**(4):7-17. 1952.
10. Haller, H. L., F. B. LaForge, and W. N. Sullivan. Effect of sesamin and related compounds on the insecticidal action of pyrethrum on houseflies. Jour. Econ. Ent. **35**:247-248. 1942.
11. Hartzell, A., and H. I. Scudder. Histological effects of pyrethrum and an activator on the central nervous system of the housefly. Jour. Econ. Ent. **35**:428-433. 1942.
12. Hewlett, P. S. Piperonyl butoxide as a constituent of heavy-oil sprays for the control of stored product pests. I. Piperonyl butoxide as a synergist for pyrethrum and its effect on the persistence of pyrethrum films. Bul. Ent. Res. **42**:293-310. 1951.
13. —————. Piperonyl butoxide as a constituent of heavy-oil sprays for the control of stored product insects. II. The effect of piperonyl butoxide on the toxicities of allethrin, DDT, and BHC, and on the joint toxicity of BHC and pyrethrins, to *Tribolium castaneum*. Bul. Ent. Res. **43**:21-32. 1952.
14. Inman, M. T. Sulfonated oxidation products of petroleum as insecticide activators. Ind. Eng. Chem. **21**:542-543. 1929.
15. Jones, H. A., H. O. Schroeder and H. H. Incho. Allethrin with synergists. Soap and Sanit. Chem. **8**:109, 133, 135, 137, 139. 1950.
16. Kerr, R. W. Adjuvants for pyrethrins in fly sprays. Com. Sci. and Ind. Res. Org. (Australia) Bul. 261. 1951.
17. Lindquist, A. W., A. H. Madden and H. G. Wilson. Pre-treating houseflies with synergists before applying pyrethrum sprays. Jour. Econ. Ent. **40**:426-427. 1947.
18. Mallis, A., A. C. Miller and R. V. Sharpliss. Effectiveness against houseflies of six pyrethrum synergists alone and in combination with piperonyl butoxide. Jour. Econ. Ent. **45**:341-343. 1952.
19. Miller, A. C., J. P. Pellegrini, Jr., G. Pozifsky and J. R. Thompson. Synergistic action of piperonyl butoxide functions and observations refuting a pyrethrins-butoxide complex. Jour. Econ. Ent. **45**:94-97. 1952.
20. Moore, J. B. Relative toxicity to insects of natural pyrethrins and synthetic allyl analog of cinerin I. Jour. Econ. Ent. **43**:207-213. 1950.
21. Page, A. B. P., and R. E. Blackith. Bioassay systems for the pyrethrins. II. Mode of action of pyrethrin synergists. Ann. Appl. Biol. **36**:244-249. 1949.
22. Perry, A. S., and W. M. Hoskins. The detoxification of DDT by resistant houseflies and inhibition of this process by piperonyl cyclonene. Science **111**:600-601. 1950.
23. Reed, J. P., and R. S. Filmer. Activation of Ryania dusts by piperonyl cyclonene and N-propyl isome. Jour. Econ. Ent. **43**:161-164. 1950.
24. Turner, N., and C. I. Bliss. Tests of synergism between nicotine and the pyrethrins. Ann. Appl. Biol. **40**: (in press). 1953.
25. Weed, A. A new insecticidal compound—isobutylundecylenamide. Soap and Sanit. Chem. **14**(6):133. 1938.
26. Wilson, C. S. Piperonyl butoxide, piperonyl cyclonene, and pyrethrum applied to selected parts of individual flies. Jour Econ. Ent. **42**:423-428. 1949.