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Effect of the Ratios of Nicotine to Bis(p-Chlorophenyl) Sulfide and Bis(p-Chlorophenyl) Disulfide on Synergism

E. L. Mayer and R. H. Nelson, *Bureau of Entomology and Plant Quarantine*, and C. F. Woodward and J. J. Willaman, *Bureau of Agricultural and Industrial Chemistry, Agr. Res. Adm., U.S.D.A.*

Many materials have been tested in our attempt to find synergists for nicotine. In preliminary screening tests bis(*p*-chlorophenyl) sulfide showed promise (Mayer *et al.* 1949), and later was tested in mixtures with nicotine sulfate against several species of insects (unpublished). In preliminary tests bis(*p*-chlorophenyl) disulfide also showed synergistic properties (unpublished). The results of studies to determine the effect of ratios of nicotine to both these materials on synergism are reported in this paper.

Methods.—Nicotine sulfate and nicotine alkaloid were made into dusts with attapulgite and bentonite, respectively, as the diluents by the solvent deposition method. The two nicotine materials were also used in sprays.

The armyworm, *Cirphis unipuncta* (Haw.) in the third instar was the principal insect in this study, and it was fed on the foliage of barley and corn. The other insects included all stages of the bean aphid, *Aphis fabae* Scop., on rhubarb and nasturtium; third instars of the diamondback moth, *Plutella maculipennis* (Curt.), on crucifers; fourth instars of the California oakworm, *Phryganidia californica* Pack., on live oak; third instars of the green dock beetle, *Gastrophysa cyanea* Melsh., on dock; and the second nymphal stage of the pea aphid, *Macrosiphum pisi* (Kltb.), on Windsor bean.

The materials were tested against lepidopterous and beetle larvae by placing them with the dusted or sprayed foliage in 9-cm. petri dishes. The leaf sections were dipped into a 1-per cent saponin solution just before the dust was applied so that the particles would adhere to them. Check tests were run, and this concentration of saponin had no apparent effect on the insects.

The pea aphids were reared and tested on Windsor bean plants. To obtain nymphs of the same age a young plant was stripped of all its leaves but one, and about eight apterous adults were placed on it, and 24 hours later they were removed, and the young were left. The following day the nymphs, which were on the undersides of the leaves, were dusted by inverting the plant in the settling dust chamber. The test plant was then confined in a battery jar 16.5 cm. in diameter.

The bean aphids were dusted while they were feeding on the leaf as brought in from the field, and were then also held in battery jars.

The number of replications ranged from 1 to 12. Approximately 50 aphids and 30 leaf-feeding larvae were used per test. Final mortality counts were made at the end of 24 hours for the aphids and at the end of 2 days for all other insects.

The occurrence of synergism or antagonism was determined by the short-cut method of Wadley (1949) as described in a previous paper (Mayer *et al.* 1950). The term "log ratio" is used as the difference between the log of the dosage for nicotine alone and the log of the dosage of nicotine equivalent required for the same mortality, divided by its estimated standard error. The log ratio is nearly the same as the conventional "t" value. A log ratio of 2.0 is the minimum considered to be significant.

Effect of Ratio of Nicotine to Adjunct.—Table 1 presents the results of tests arranged in order of increasing ratio of nicotine to adjunct. It also gives the percentages of the materials used in the dusts. All replications were combined for a single calculation when possible. However, in four cases the points on the semilogarithmic paper were too scattered to permit a combination; each set of data was therefore calculated separately and the average presented.

Figure 1 presents on semilogarithmic paper the effect of the ratio of nicotine sulfate to each adjunct against the army worm in dust tests. Highly significant log ratios are shown for the mixtures of nicotine and the sulfide in the ratios 0.05 to 1.5. The most effective mixtures of nico-

Table 1.—Synergistic effect of bis(*p*-chlorophenyl) sulfide and bis(*p*-chlorophenyl) disulfide in various dust mixtures with nicotine, as indicated by the log ratio, in tests against different insects.

Insect	Form of Nicotine	Nicotine (Per Cent)	Adjunct (Per Cent)	Ratio of Nicotine to Adjunct	Number of Replications	Log Ratio
		Bis(*p*-chlorophenyl) sulfide				
Armyworm	Sulfate–	0.1	4.9	0.02	10	4.3
		.25	4.75	.05	10	4.7
		.5	2	.25	12	12.1
		1	1.5	.66	13	8.1
		2	2	1	3	4.6
	Bentonite	2	2	1	2	2.9
Bean aphid	Sulfate–	1	1[1]	1	2	.5
		1	1[2]	1	4	2.8
	Bentonite	1	1[2]	1	4	4.4
California oakworm	Sulfate	2	2	1	1	5.5
Diamondback moth	Sulfate	2	2	1	7	13.7
	Bentonite	2	2	1	4	11.2
Green dock beetle	Sulfate	2	2	1	2	4.3
Pea aphid	Sulfate	2	2	1	3	11.7
Armyworm	Sulfate	1.5	1	1.5	11	8.1
Pea aphid	Sulfate	1.5	1	1.5	2	2.6
Armyworm	Sulfate	1.88	.63	3	8	2.7
		2	.5	4	11	1.1
Pea aphid	Sulfate	2	.5	4	2	1.8[3]
		Bis(*p*-chlorophenyl) disulfide				
Armyworm	Sulfate	0.25	4.75	0.05	3	−0.45
		.5	2	.25	4	3.7
Diamondback moth	Sulfate	.5	2	.25	2	3.1[3]
Armyworm	Sulfate	2	2	1	5	3.8
Diamondback moth	Sulfate	2	2	1	2	.5[3]
Armyworm	Sulfate	1.6	.4	4	5	1.2
Diamondback moth	Sulfate	1.6	.4	4	2	—[3]

[1] Nasturtium.
[2] Rhubarb.
[3] Not combined; average of each run separately.

tine and the disulfide are those in the ratios 0.25 to 1.0. In neither case are there any negative values of significance. The figure also shows that the sulfide is by far the better synergist.

The 0.02 end of the sulfide curve, where there is 50 times as much adjunct as nicotine, appears to level out at a high point. This mixture contains 0.1 per cent of nicotine sulfate and 4.9 per cent of the the sulfide. We wondered whether the small percentage of nicotine sulfate could have an effect on the insects. A 0.1-per cent nicotine sulfate dust used alone is not toxic to the armyworm. Therefore, we plotted curves for the sulfide alone and for the mixture, but not for nicotine sulfate, since there is no nicotine equivalent. We calculated two groups of data, consisting of five replications each, by the Wadley method of showing synergism. They gave log ratios of 4.3 and 1.9 (not shown). If the nicotine sulfate were not affecting the mortalities, these log ratios would theoretically be zero. Since they are high, it means that the small amount of nicotine sulfate is having its effect and the values at this ratio are valid.

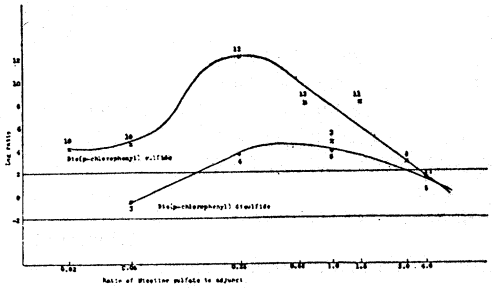

Fig. 1.—Effect of ratio of nicotine sulfate to bis(*p*-chlorophenyl) sulfide and bis(*p*-chlorophenyl) disulfide in dust tests against the armyworm. The number of replications are shown at each point.

The point at ratio 1.0 indicates the presence of a well in the curve. It must be remembered, however, that there are only three replications for this point, as compared with about 10 for all other points for this adjunct. Statistical analysis showed no significant difference between this point and the adjacent points; therefor, a smooth curve was drawn. It

Table 2.—Comparison of nicotine sulfate and the nicotine alkaloid in various mixtures with bis(*p*-chlorophenyl) sulfide when used as sprays against the armyworm.

Nicotine (Per Cent)	Adjunct (Per Cent)	Ratio of Nicotine to Adjunct	Number of Replications	Log Ratio
		Nicotine sulfate		
0.025%	0.5%	0.05	5	2.0
.2	.3	.66	2	0.7
.3	.2	1.5	2	1.9
.4	.1	4.0	2	±
		Nicotine alkaloid		
.025%	.5%	.05	5	9.6
.2	.3	.66	1	3.5
.3	.2	1.5	1	5.4
.4	.1	4.0	1	2.9

appears that any ratio of nicotine sulfate to the sulfide will produce significant synergism against the armyworm up to the point where there is three times the amount of nicotine as the adjunct. A decided peak appears at the point where there is four times as much adjunct as nicotine sulfate.

Some spray tests were run (Table 2) against the armyworm to compare the effect of nicotine sulfate and nicotine alkaloid in mixtures with bis (*p*-chlorophenyl) sulfide at the ratios of 0.05, 0.66, 1.5 and 4.0. Synergism was demonstrated with the sulfate only at the lowest ratio (0.05) where a log ratio of 2.0 was obtained, whereas, in all tests with the alkaloid the log ratios were highly significant.

Effect of Season and Feed on Susceptibility of the Insect.—During the course of these tests it was thought that a variation of susceptibility of the insect might be influencing the data. All the data for bis(*p*-chlorophenyl) sulfide against the armyworm were obtained between February and July. Barley foliage was used for feed until May 1, and corn leaves thereafter. Figure 2 shows that the LD-50's for nicotine sulfate and the sulfide, when plotted against time, did not form straight lines, although the two curves did show the same general trends throughout. The nicotine equivalence was obtained by dividing the LD-50 of the nicotine by the LD-50 of the sulfide. The x-marks represent the nicotine equivalence as obtained by calculation from points on the trend curves; the circles are the actual points. The approach

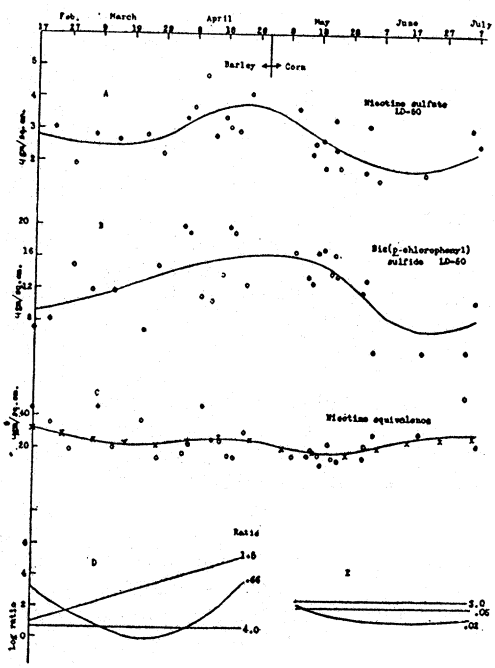

Fig. 2.—The LD-50's for materials against the armyworm as affected by season and feed: A, Nicotine sulfate and B, bis(*p*-chlorophenyl) sulfide. C, The nicotine equivalence of nicotine sulfate with bis(*p*-chlorophenyl) sulfide. Trends of the log ratio obtained when different ratios of toxicants were used: D, On barley; E, on corn.

of this curve to a straight line would indicate that there was no seasonal effect on nicotine equivalence. In the lower part of figure 2, D, when barley was used as feed, there was a definite variation in the log ratio when the ratio of nicotine to adjunct was 1.5, and 0.66, and was constant when the ratio was 4.0. In tests run from May through July, when corn was used (E), the log ratios were constant as shown by the curves.

A series of tests was run once a week for five consecutive weeks to determine the effect of corn and barley on the log ratios against the armyworm. The insects were reared on the same feed that was to be used in the test. The two ratios of nicotine to bis(*p*-chlorophenyl) sulfide employed were 0.25 and 1.5. Whether the insects were fed on corn or barley, the first mixture produced higher log ratios than the second. This was also true in previous tests shown in figure 1. The synergistic effect for each mixture was much greater in the tests in which corn was fed. The results of combining the

five replications into one calculation are as follows:

	Ratio	*Log Ratio*
Corn	0.25	21.3
	1.5	12.4
Barley	.25	13.5
	1.5	6.1

From these data it appears that if the samples tested against insects fed on corn (Fig. 1) had been tested on barley-fed insects the log ratios would have been lower, which would accent still more the peak at the middle ratios.

The effect of feed on the log ratio is also apparent when different hosts were used for the bean aphid. Table 1 shows that at a ratio of 1.0 there was a synergistic effect (2.8) when the aphids were fed on rhubarb, but a much lower log ratio (0.5) when they were fed on nasturtium.

SUMMARY.—In an attempt to find synergists for nicotine, a study was made of the effect of different ratios of nicotine to bis(*p*-chlorophenyl) sulfide and bis(*p*-chlorophenyl) disulfide on synergism. In dust form bis(*p*-chlorophenyl) sulfide strongly synergized nicotine, both as the sulfate and as the alkaloid in bentonite, in its action against the armyworm, the bean aphid, and the diamondback moth. It also synergized nicotine sulfate against the California oakworm, the green dock beetle, and the pea aphid. Against the armyworm the most effective ratios of nicotine to adjunct were in the range of 0.1 to 1.0, with a peak at 0.25. Bis(*p*-chlorophenyl) disulfide also synergized nicotine sulfate against the armyworm and the diamondback moth, but to a lesser degree.

In sprays the nicotine alkaloid and bis(*p*-chlorophenyl) disulfide mixture gave highly significant log ratios against the armyworm. Except at the low ratio of 0.05 the mixtures of nicotine sulfate and sulfide were not effective.

Synergism with bis(*p*-chlorophenyl) sulfide against the armyworm was more marked when the larvae were fed on corn leaves than when fed on barley leaves.

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