The Effect of Some Polyethyleneglycol Derivatives On the Toxicity of Nicotine to Insects

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THE CONNECTICUT AGRICULTURAL
EXPERIMENT STATION
NEW HAVEN

Bulletin 543 - January, 1951

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Some brief experiments by Wigglesworth (25) on the effect of polyethyleneglycol derivatives on the penetration of nicotine through the insect cuticle attracted the attention of the senior author. In these experiments, fifth stage nymphs of Rhodnius were treated with either a 2 per cent solution of nicotine in refined paraffin oil or a similar nicotine solution in the monocetyl ether of tetraethyleneglycol. The nymphs treated with the latter solution were all dead in 24 hours while the 2 per cent nicotine solution in paraffin oil had practically no effect after two days. Suspensions of rotenone in these two solvents affected the nymphs similarly. These rather striking results with a non-ionic wetting agent were thought worthy of further investigation.

The effect of wetting agents on the toxicity of nicotine used in sprays has been investigated for many years. In 1916, Smith (18) studied the relation between wetting power of fish-oil soap and the toxicity of nicotine-soap sprays to pea aphids (*Macrosiphum pisi* Kalt.), spinach aphids (*Myzus persicae* Sulz.) and red spiders (*Tetranychus* sp.) on strawberries. The wetting power of the spray was increased by each additional amount of soap to the spray mixture. Toxicity to the insects increased as soap was added up to 4 pounds in 50 gallons of spray, and then declined as more soap was added. Obviously then, wetting power was not the only factor involved.

McIndoo in the same year (10) studied the way in which nicotine entered the bodies of insects, using a variety of species. He concluded that nicotine entered as a vapor, and found no chemical evidence that sprays passed into the spiracles or penetrated the cuticle. Moore and Graham (11) measured the penetration of a variety of materials into the spiracular system of roaches (*Blattella germanica* Linn.), wax moth larvae (*Galleria mellonella* Linn.) and larvae of the Indian meal moth (*Plodia interpunctella* Hubn.). They concluded a contact insecticide must be able to penetrate by vaporization or in the liquid form to be effective. Those materials which were either soluble in chloroform or ether or were fat solvents were able to enter the trachea.

In 1929 McGovran (9) investigated the penetration of nicotine into the tracheal system of the honeybee (*Apis mellifera* Linn.). He found that soap carried the spray into the spiracles. Sodium oleate was more effective than fish-oil soap. Nonylic acid reduced surface tension but

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did not increase spreading power of the spray. He demonstrated the practical value of sodium oleate with nicotine by spray tests on aphids. O'Kane et al. (13) studied the relation between physical factors and penetration of solutions into tracheae of larvae of the yellow mealworm (*Tenebrio molitor* Linn.). Surface tension did not provide an index of penetration. However, surface tension and angle of contact could be used to calculate adhesion tension which was related to degree of penetration into the tracheae.

Wilcoxon and Hartzell (26) calculated the spreading coefficient of solutions of nicotine and calcium caseinate, a sulfonated spreader (*Penetrol*) and sodium oleate on the wing covers of the Colorado potato beetle (*Leptinotarsa decemlineata* Say.). They determined penetration into the tracheae of larvae of the tomato worm (*Phlegethontius quinquemaculatus* Haw.). Toxicity of the materials was measured by spraying *Aphis rumicis* Linn. They found that the toxicity and spreading coefficient were related. O'Kane et al. (12) applied 95 per cent nicotine to several areas on the body of the mealworm larva (*Tenebrio molitor* Linn.). The antennae and the membranes between thoracic segments were particularly vulnerable to penetration by nicotine.

Hurst in 1943 (6) studied penetration of chemicals through the integument of blow-fly larvae. He concluded that a drug may induce its own penetration or exert a carrier action when forming part of a mixed drug system. Penetration was facilitated most by non-polar fat solvents and cyclic aliphatic hydrocarbons, less by aromatic hydrocarbons, and least by more complex aromatic compounds such as coumarin and indene. Wigglesworth (25) used solvents and detergents to remove the wax from the insect integument and measured the loss of water through the treated cuticle. Such agents as turkey red oil, sulfonated naphthalenes and other hydrocarbons and sulfated alcohol had little effect. The monocetyl ethers of polyethyleneglycols were the most effective, and the octaethyleneglycol ether was more effective than the tetraethylene derivative. As previously mentioned, Wigglesworth also demonstrated increased toxicity of insecticides when used with these cetyl ethers.

Webb and Green (24) approached the problem in a somewhat different manner. They tested the effect of solvents on the insecticide. Cresols, xylenol and benzyl alcohol increased the speed of action of diphenylamine. Later Webb (23) found that insecticides differed in their reaction with certain solvents, because benzyl alcohol had no effect on a pyrethrum spray. For high carrier efficiency, Webb postulated the following requirements: (1) the insecticide must be soluble in the carrier; (2) the carrier must penetrate the cuticle rapidly; (3) the carrier must have a relatively high partition coefficient between water and wax; (4) the insecticide must be more soluble in a solution of the carrier in water than in water alone, and (5) the carrier must be comparatively non-volatile.

It is obvious from this discussion that the use of a wetting agent with nicotine is not a simple matter of spreading the spray over the body of the insect. Both the alkaloid and the sulfate forms of nicotine are water-soluble. Both forms do penetrate the integument of insects.

However, they do not represent the types of materials found most penetrating by Hurst (6), Wigglesworth (25) and Webb and Green (24). Certain modifications of nicotine that we have tried previously (20) seemed even less favorable. Alkyl and aralkyl nicotinium salts generally compared more favorably with nicotine when injected into the larger milkweed bug *Oncopeltus fasciatus* Dal. than they did when sprayed on *Aphis rumicis* Linn. This might mean that this type of modification in the nicotine molecule results in reduced penetrating power. Therefore, it seems of some importance to study the ability of compounds similar to the monocetyl ethers of polyethyleneglycols to induce penetration of nicotine into the integument of insects. In the present study, chief emphasis was made on comparative effectiveness of a series of long chain alkyl ethers and analogous fatty acid esters of polyethyleneglycols as penetrating agents for nicotine.

The action of soap as a wetting agent deserves some review. deOng (3) found a close correlation between volatility of nicotine and toxicity. Addition of alkaline materials such as sodium hydroxide and soap as a wetting agent increased toxicity. However, Worthley (27) reported that alkalinity as such was not the only governing factor in liberating nicotine. Sodium carbonate freed nicotine very rapidly and was highly toxic; soap freed nicotine much more slowly and was equally toxic. deOng (3) found that the toxicity of nicotine sulfate to aphids increased as the pH of the solution was increased from 6.5 to 8.2. Richardson and Shepard (15) immersed larvae of the house mosquito (*Culex pipiens* Linn.) in solutions of nicotine and nicotine sulfate at adjusted pH values. Sulfuric and hydrochloric acids both decreased toxicity of the free base; in other words, toxicity increased as the pH increased from 2.4 to 9.7. The free base (pH 8.5 to 10.0) was five to seven times more toxic than nicotine sulfate at a pH of 5.0. Addition of sodium hydroxide to the free base did not alter toxicity. Later Richardson (14) found a similar relation of toxicity of nicotine to the cockroach *Periplaneta americana* Linn. by immersion. It was concluded that nicotine molecules had greater penetrating ability and greater toxicity than nicotine ions.

Martin (8) has shown that spray deposit (maximum initial retention) of simple homogenous solutions decreased as wetting and spreading properties were improved. This would explain the loss of toxicity which Smith (18) reported as increasing amounts of soap were added to nicotine solutions. Richardson and Smith (16) found that fish oil soap had some toxicity to insects, and O'Kane et al. (13) published toxicity data for sodium oleate. Soap was therefore involved in four ways: (1) the alkaline ion acted to liberate nicotine from the sulfate; (2) penetration of the cuticle may have been increased; (3) the amount of the spray reaching the insect was reduced because of the smaller droplet size of the spray, and (4) the soap itself was somewhat toxic.

METHODS

All spray tests were made in the laboratory using *Aphis rumicis* Linn. reared on nasturtiums (19). The preliminary screening test was made with 0.5 per cent of the test materials applied with 0.04 per cent nicotine as sulfate with four replicates. Those materials which increased the toxicity of the spray were used next in a dosage series in order to test the relation between amount used and toxicity. Toxicity of a few of the penetrating agents was determined by spraying aphids.

Because of a shortage of aphids, toxicity of the penetrating agents was determined by injection into milkweed bugs (*Oncopeltus fasciatus* Dal.) (20). The penetrating agents were also injected together with alkaloid nicotine.

The tests were made in 1948 and 1949. In some cases, results for the two years have been combined in a single table, with the year the tests were made identified.

MATERIALS USED

Two forms of nicotine were used: alkaloid nicotine (95 per cent nicotine) and nicotine sulfate (40 per cent nicotine). In all cases, however, dilution was made on the basis of nicotine content.

In preliminary tests, sodium oleate, ammonium linoleate, modified ammonium fatty acid compounds (*Blendene*), a quaternary ammonium (*Ammonyx Q*) and a non-ionic wetting agent (*Igepal 300*) were used. Most of the tests were made with the polyethyleneglycol compounds provided by the Eastern Regional Research Laboratory as follows:

ERL No.	*Chemical Designation*	*Molecular Weight*
169	Polyethyleneglycol-300 dilaurate	664[1]
170	Polyethyleneglycol-300 ditallate	
171	Polyethyleneglycol-1500 monolaurate	1102[1]
172 and 172A	Polyethyleneglycol-400 monolaurate	582[1]
173, 173A and 173B	Polyethyleneglycol-400 monooleate	664[1]
174	Polyethyleneglycol-400 ditriricinoleate	2080[1]
176	Polyethyleneglycol-400 dioleate	928[1]
177	Polyethyleneglycol-600 distearate	1132[1]
178 and 178A	Polyethyleneglycol-600 monooleate	864[1]
179 and 179A	Polyethyleneglycol-600 dioleate	1128[1]
180	Polyethyleneglycol-600 ditallate	
181 and 181A	Polyethyleneglycol-1000 monooleate	1264[1]
182	Decaethyleneglycol mono-*p*-(1,1;3,3-tetramethyl-butyl)-phenyl ether (*Triton X-100*)	647[1]
183	Tetraethyleneglycol monooctyl ether	306.4
184	Tetraethyleneglycol dioctyl ether	418.6
185	Tetraethyleneglycol monododecyl ether	362.5
186	Tetraethyleneglycol didodecyl ether	530.8
187	Tetraethyleneglycol monocetyl ether	418.6

[1]Estimated.

ERL No.	*Chemical Designation*	*Molecular Weight*
188	Tetraethyleneglycol dicetyl ether	643.1
189	Tetraethyleneglycol monooctadecyl ether	446.7
190	Tetraethyleneglycol dioctadecyl ether	699.2
193	Polyethyleneglycol-200 monolaurate	382[1]
194	Polyethyleneglycol-300 monolaurate	482[1]
196	Polyethyleneglycol-600 monolaurate	782[1]
198	Polyethyleneglycol-1000 monolaurate	1182[1]
200	Polyethyleneglycol-200 monooleate	464[1]
201	Polyethyleneglycol-300 monooleate	564[1]
202	Polyethyleneglycol-1540 monooleate	1804[1]
203	Octaethyleneglycol monododecyl ether	538.7
204	Octaethyleneglycol monocetyl ether	594.8

Polyethyleneglycol Esters

The polyethyleneglycol fatty acid esters which comprise ERL numbers 169-181 and 193-202 inclusive were obtained commercially. Samples 173, 173A and 173B were obtained from three different manufacturers; 172A and 179A differed from 172 and 179 in the same way. Samples 178A and 181A were obtained from the same supplier as 178 and 181 but in different years. No sample of this type is a pure chemical compound but consists of a mixture of closely related compounds of which the most abundant member or the member that most nearly approximates the average molecular weight gives the name to the material. In general, the monoesters are prepared either by treating a fatty acid with ethylene oxide under pressure or by esterification of a fatty acid with a commercially available polyethyleneglycol (17). The latter method is probably used to prepare most of the diesters (4). These esters vary in physical form from mobile liquids to fairly hard wax-like solids. The liquids were variously soluble, miscible, or dispersible in water; the solid materials could be dispersed with water by heating.

Polyethyleneglycol Ethers

Decaethyleneglycol mono-*p*-(1,1,3,3-tetramethylbutyl)-phenyl ether (ERL-182) is available commercially as *Triton X-100*. This consists of the mono-*p*-(1,1,3,3-tetramethylbutyl)-phenyl ethers of a short series of polyethyleneglycols, the intermediate member of which is decaethyleneglycol.

The tetraethyleneglycol and octaethyleneglycol alkyl ethers which comprised ERL numbers 183-190, 203 and 204 are chemically pure compounds. The tetraethyleneglycol derivatives were prepared by treating one mol of an alkyl halide with one mol of sodium metal dissolved in 1.1 mol of tetraethyleneglycol and dry dioxane. Both the mono and diethers were obtained and could be separated either by low pressure fractional distillation or by fractional crystallization. The

[1]Estimated.

octaethylene derivatives were obtained by converting a tetraethyleneglycol monoalkyl ether to a corresponding halide and using this compound in the same reaction as was used for the alkyl halide above. The final product was purified by fractional crystallization.

The tetraethyleneglycol monooctyl and monododecyl ethers are mobile, colorless liquids which are readily miscible with water. The monocetyl and monooctadecyl ethers of tetraethyleneglycol and the monododecyl and monocetyl ethers of octaethyleneglycol are low-melting crystalline solids which are dispersible in water on warming. The dioctyl ether of tetraethyleneglycol is a mobile, colorless oil while the remaining dialkyl ethers are low melting crystalline solids. All these dialkyl ethers are difficultly dispersible in water.

RESULTS

Preliminary Tests

The effect of sodium oleate on the toxicity of alkaloid nicotine and nicotine sulfate was determined (Table 1). Dosages were varied by altering the length of time the aphids were sprayed, and there were two replicates of each test. It must be assumed that the sodium oleate was equally toxic to the aphids in both cases, and that any differences in results have been caused by the differential effect of the sodium oleate on alkaloid nicotine and nicotine sulfate. Dosage-response curves (Figure 1) show that sodium oleate increased the toxicity of nicotine as sulfate much more than it affected alkaloid nicotine. In terms of dosage for 70 per cent control, one unit of alkaloid nicotine equalled in toxicity about 0.6 unit of alkaloid nicotine plus 0.1 per cent sodium oleate. One unit of nicotine as sulfate was equal in effectiveness to 0.3 unit of nicotine as sulfate with 0.1 per cent sodium oleate.

TABLE 1. EFFECT OF SODIUM OLEATE ON THE TOXICITY OF TWO FORMS OF NICOTINE AS SPRAYS ON APHIDS

Nicotine				Mortality, Spray Time, Secs.			
Form	Per Cent	Wetting Agent		2.5	5	10	20
Alkaloid nicotine	.04	None		36.2	35.4	44.6	57.1
				40.5	52.7	71.1	75.3
			Mean	38.3	44.0	57.8	66.2
Alkaloid nicotine	.04	Sodium oleate .1%		39.2	40.0	57.1	69.6
				56.2	62.8	71.6	79.6
			Mean	47.7	51.4	64.3	74.5
Nicotine sulfate	.04	None		33.3	39.7	61.5	63.1
				45.4	53.0	66.7	80.0
			Mean	39.3	46.3	64.0	71.5
Nocotine sulfate	.04	Sodium oleate .1%		54.2	76.0	83.7	93.9
				66.6	78.5	83.3	92.5
			Mean	60.4	77.2	83.5	93.2

Results of tests with other types of wetting agents are given in Table 2. Ammonium linoleate had little effect on the toxicity of nicotine sulfate and apparently decreased the toxicity of the alkaloid. The ammonium soap *Blendene* increased the toxicity of the alkaloid to a much greater extent than that of the sulfate. The cationic quaternary ammonium *Ammonyx Q* had little effect on either form of nicotine. Similarly the non-ionic *Igepal 300* did not change toxicity of either form markedly.

TABLE 2. EFFECT OF FOUR WETTING AGENTS OF THREE TYPES ON ALKALOID NICOTINE AND NICOTINE SULFATE AS SPRAYS FOR APHIDS

Nicotine			Mortality, Spray Time, Secs.			
Form	Per Cent	Wetting Agent	2.5	5	10	20
Alkaloid nicotine	.04	None	23.5	60.0	51.7	62.2
Alkaloid nicotine	.04	*Blendene* .1%	53.5	63.6	76.0	82.8
Nicotine sulfate	.04	None	50.0	56.2	72.4	71.4
Nicotine sulfate	.04	*Blendene* .1%	55.1	59.5	79.1	90.4
Alkaloid nicotine	.04	None	59.4	68.7	77.4	82.7
Alkaloid nicotine	.04	Ammonium linoleate .1%	43.2	57.1	79.4	68.4
Nicotine sulfate	.04	None	55.8	71.4	68.9	81.0
Nicotine sulfate	.04	Ammonium linoleate .1%	58.4	64.8	76.1	86.5
Alkaloid nicotine	.04	None	42.0	54.1	58.1	74.3[1]
Alkaloid nicotine	.04	*Ammonyx Q* .1%	43.3	52.3	64.7	75.1[1]
Nicotine sulfate	.04	None	48.0	60.1	64.7	73.9[1]
Nicotine sulfate	.04	*Ammonyx Q* .1%	57.9	60.7	72.7	74.5[1]
Alkaloid nicotine	.04	None	37.8	52.7	53.2	55.9[1]
Alkaloid nicotine	.04	*Igepal 300* .1%	45.6	52.5	60.2	66.5[1]
Nicotine sulfate	.04	None	44.1	45.0	57.7	74.0[1]
Nicotine sulfate	.04	*Igepal 300* .1%	49.5	57.2	68.1	77.6[1]

[1]Mean of two replicates.

If the six series of tests in Tables 1 and 2 are averaged, it is seen that nicotine sulfate was slightly more toxic to aphids than the alkaloid. The difference was not significant statistically, however.

These tests with anionic, cationic and non-ionic wetting agents were made to study the differences in results when they were used with alkaloid and sulfate nicotine. There was no obvious pattern of difference. Sodium oleate had the greatest effect on toxicity of the sulfate, and the ammonium soap *Blendene* on the alkaloid. The one non-ionic material used had no effect on either form of nicotine. Since the study was devoted chiefly to non-ionic polyethyleneglycol compounds, no further work was done with ionic agents.

Screening Tests With Polyethyleneglycol Esters and Ethers

Results of the screening tests with nicotine sulfate are given in Table 3. For the purposes of this discussion, the compounds can be grouped in three classes: (1) those which applied with nicotine sulfate increased the toxicity as compared with nicotine sulfate alone; (2) those which had little effect and (3) those which reduced toxicity. Thirty compounds were tested in the two years. Two-thirds of these materials increased the toxicity of nicotine sulfate to aphids. These include ERL numbers 169, 171, 187, 198, 200 and 201 in addition to those listed in Table 4.

The compounds which had little effect were:

189 Tetraethyleneglycol monooctadecyl ether
186 Tetraethyleneglycol didodecyl ether
170 Polyethyleneglycol-300 ditallate
173A Polyethyleneglycol-400 monooleate
176 Polyethyleneglycol-400 dioleate
177 Polyethyleneglycol-600 distearate

The materials which reduced toxicity were:

185 Tetraethyleneglycol monododecyl ether
184 Tetraethyleneglycol dioctyl ether
188 Tetraethyleneglycol dicetyl ether
190 Tetraethyleneglycol dioctadecyl ether
182 Decaethyleneglycol mono-*p*-(1,1,3,3-tetramethylbutyl)-phenyl ether. (*Triton X-100*)

Number 178 was ineffective in the 1948 tests. An additional sample of the same material from the same source was tested in 1949 under the number 178A and was found effective. Number 179 was not very effective in 1948. A sample with the same name obtained from a different manufacturer, 179A, was very effective in 1949. Likewise 173 was effective, but 173A from a different source but having the same name was not highly effective. Some of the polyethyleneglycol compounds used here are known to be mixtures of two materials blended to have the required physical characteristics. It is probable that these blends will not perform identically unless they have been made of the same basic materials in approximately the same way.

"Day to day" variation in mortality in the 1949 tests with nicotine sulfate alone was relatively large. It would be prudent to place more weight on differences in mortality in those tests in which mortality of aphids sprayed with nicotine sulfate alone was relatively high.

The only numerical comparison possible between materials in Table 3 is by use of the ratio of effectiveness of nicotine sulfate with the polyethyleneglycol compounds to toxicity of nicotine alone calculated from probits. This has been done in Table 17. Such a calculation is useful in rating the screening tests, but cannot take into account differences in slope of dosage-response curves as discussed later.

TABLE 3. SCREENING TEST FOR POLYETHYLENEGLYCOL COMPOUNDS WITH NICOTINE SULFATE

ERL No.	Concentrations		Mortality of Aphids				
	Nicotine	Wetting Agent	1	2	3	4	Mean
		1948					
...	.04	...	54	55	37	35	45
183	.04	.5	58	58	67	60	61
184	.04	.5	24	26	13	14	19
185	.04	.5	46	25	33	34	35
187	.04	.5	67	57	77	60	65
188	.04	.5	47	22	37	23	38
189	.04	.5	60	57	57	38	52
...	.04	...	68	58	63	41	58
169	.04	.5	68	73	71	61	68
170	.04	.5	73	65	52	59	62
171	.04	.5	77	74	79	78	77
172	.04	.5	85	84	70	81	80
173	.04	.5	77	78	87	81	81
174	.04	.5	75	59	67	78	70
173A	.04	.5	73	60	65	64	65
...	.04	...	61	58	65	55	60
186	.04	.5	73	73	59	63	67
190	.04	.5	47	44	37	34	41
...	.04	...	65	66	67	67	66
176	.04	.5	68	66	60	69	66
177	.04	.5	59	62	64	64	62
178	.04	.5	71	68	58	65	66
179	.04	.5	69	71	75	68	71
180	.04	.5	80	77	75	73	76
181	.04	.5	92	77	90	88	87
182	.04	.5	47	56	50	42	49
		1949					
...	.04	...	45	33	38	43	40
178A	.04	.5	84	80	86	88	84
179A	.04	.5	87	78	74	83	80
193	.04	.5	69	70	69	65	68
...	.04	...	66	73	64	68	68
194	.04	.5	89	93	90	87	90
172A	.04	.5	96	86	95	95	93
196	.04	.5	89	71	94	90	91
...	.04	...	55	62	63	55	59
181A	.04	.5	86	83	86	91	86
198	.04	.5	95	90	87	75	87
200	.04	.5	83	78	86	76	81
201	.04	.5	84	80	91	88	86
...	.04	...	36	40	32	35	36
202	.04	.5	93	66	88	81	82
203	.04	.5	60	63	66	57	62
204	.04	.5	71	66	63	75	69

TABLE 4. TOXICITY OF NICOTINE SULFATE TO APHIDS WITH INCREASING AMOUNTS OF POLYETHYLENEGLYCOL DERIVATIVES

Treatment		Concentrations: Nicotine	Concentrations: Wetting Agent	Mortality, Spray Time, Secs: 2.5	5	10	20
			1948				
Nicotine		.04	....	32	38	51	63
"	+172	.04	.0625	38	45	44	72
"	+172	.04	.125	39	54	58	73
"	+172	.04	.25	55	63	76	85
"	+172	.04	.5	58	67	81	92
Nicotine		.04	....	37	42	44	55
"	+173	.04	.0625	32	36	39	60
"	+173	.04	.125	50	60	64	80
"	+173	.04	.25	48	56	74	83
"	+173	.04	.5	60	70	78	87
Nicotine		.04	....	33	46	57	69
"	+174	.04	.0625	24	35	44	51
"	+174	.04	.125	24	38	50	61
"	+174	.04	.25	23	27	48	90
"	+174	.04	.5	29	32	68	78
Nicotine		.04	....	30	39	46	58
"	+179	.04	.0625	38	46	47	81
"	+179	.04	.125	30	52	51	90
"	+179	.04	.25	53	61	68	79
"	+179	.04	.5	26	48	81	88
Nicotine		.04	....	43	46	51	64
"	+180	.04	.0625	38	47	46	63
"	+180	.04	.125	40	55	55	69
"	+180	.04	.25	52	61	63	79
"	+180	.04	.5	52	64	67	86
Nicotine		.04	....	44	52	52	70
"	+181	.04	.0625	29	45	57	72
"	+181	.04	.125	55	61	68	87
"	+181	.04	.25	49	70	80	86
"	+181	.04	.5	77	80	84	86
Nicotine		.04	....	24	32	40	51
"	+183	.04	.0625	21	25	30	58
"	+183	.04	.125	30	38	48	65
"	+183	.04	.25	35	49	49	88
"	+183	.04	.5	55	56	74	88
			1949				
Nicotine		.04	....	25	40	54	63
"	+178A	.04	.0625	26	52	85	82
"	+178A	.04	.125	34	66	83	94
"	+178A	.04	.25	65	73	84	96
"	+178A	.04	.5	67	69	93	95
Nicotine		.04	....	24	36	48	50
"	+179A	.04	.0625	34	30	57	67
"	+179A	.04	.125	45	51	56	95
"	+179A	.04	.25	42	65	78	91
"	+179A	.04	.5	48	50	86	90

TABLE 4. (Continued) TOXICITY OF NICOTINE SULFATE TO APHIDS WITH INCREASING AMOUNTS OF POLYETHYLENEGLYCOL DERIVATIVES

Treatment		Concentrations		Mortality, Spray Time, Secs.			
		Nicotine	Wetting Agent	2.5	5	10	20
Nicotine		.04	....	53	46	..	68
"	+193	.04	.0625	40	40	43	74
"	+193	.04	.125	48	56	63	62
"	+193	.04	.25	52	62	67	87
"	+193	.04	.5	57	61	67	93
Nicotine		.04	....	29	48	48	67
"	+194	.04	.0625	59	68	69	78
"	+194	.04	.125	52	55	62	67
"	+194	.04	.25	59	71	78	82
"	+194	.04	.5	69	73	78	91
Nicotine		.04	....	33	37	38	50
"	+196	.04	.0625	44	46	67	77
"	+196	.04	.125	35	56	..	85
"	+196	.04	.25	57	56	86	88
"	+196	.04	.5	67	92	83	98
Nicotine		.04	....	24	32	39	50
"	+181A	.04	.0625	17	37	48	76
"	+181A	.04	.125	28	60	73	90
"	+181A	.04	.25	65	63	77	93
"	+181A	.04	.5	42	56	73	78
Nicotine		.04	....	42	60	64	69
"	+202	.04	.0625	30	43	72	73
"	+202	.04	.125	53	59	73	74
"	+202	.04	.25	61	63	76	89
"	+202	.04	.5	58	71	80	96
Nicotine		.04	....	22	36	56	58
"	+203	.04	.0625	28	39	61	69
"	+203	.04	.125	31	48	52	71
"	+203	.04	.25	53	64	81	89
"	+203	.04	.5	66	79	85	93
Nicotine		.04	....	32	41	52	63
"	+204	.04	.0625	31	48	44	76
"	+204	.04	.125	46	63	67	75
"	+204	.04	.25	51	63	70	77
"	+204	.04	.5	59	67	76	79
Nicotine		.04	....	37	33	35	36
"	+172A	.04	.0625	28	54	69	76
"	+172A	.04	.125	28	63	69	73
"	+172A	.04	.25	50	69	69	88
"	+172A	.04	.5	72	80	89	91

Dosage Tests

Most of the compounds which had a marked effect in the screening test were sprayed with nicotine sulfate on aphids in a dosage series of four concentrations of from 0.0625 per cent to 0.5 per cent. (A shortage of test aphids made it impossible to test all the materials in this way). The purposes of this test were: (1) to measure the relation between amount of polyethyleneglycol derivative and effectiveness and (2) to determine the effect on slope of the dosage-response curves of addition of the compounds. Results of the tests have been summarized in Table 4. Dosage-response curves have been fitted by inspection on the logarithmic probability grid. Essential data from these curves have been summarized in Table 5. The materials varied in effectiveness in increasing the toxicity of nicotine sulfate. The least effective was 174, polyethyleneglycol-400 ditriricinoleate. The most effective were 172A, polyethyleneglycol-400 monolaurate; 196, the 600 monolaurate and both samples of the 1000 monooleate, 181 and 181A. The monolaurates also steepened the dosage-response curve, which would make them somewhat more desirable than the monooleate. Number 203, octaethyleneglycol monododecyl ether and 173, polyethyleneglycol-400 monooleate, were next in effectiveness. Number 172, bearing the same name as 172A but from a different source, was much less effective than 172A. Again the difference may be the result of blends of different basic materials. One compound, 194, polyethyleneglycol-300 monolaurate, apparently flattened the slope of the dosage-response curve. Most of the other compounds produced curves parallel to the curve of nicotine sulfate.

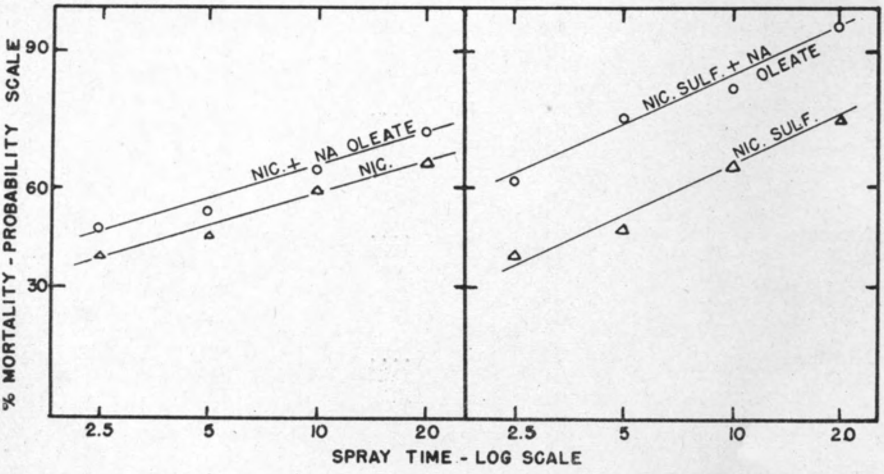

Figure 1. Dosage-response curves for nicotine and nicotine sulfate sprayed on aphids alone and in combination with sodium oleate.

Little is known of the significance of change in slope of the dosage-response curve caused by addition of a penetrating agent. Since the materials used were non-ionic, no chemical change would be expected. Furthermore, use of sodium oleate with nicotine did not change the slope of the dosage-response curve (Figure 1). It seems likely that the change in slope may be a result of a change in coverage of the insects, as indicated by Horsfall (5). Certainly those materials which steepened the slope of the curve appear to be the type desired.

In a majority of the compounds, an increased concentration resulted in increased effectiveness of nicotine sulfate. There were exceptions, however. The toxicity of 0.25 per cent 181A with 0.04 per cent nicotine as sulfate was greater than 0.5 per cent. Materials 193 and 194 at 0.5 per cent with 0.04 per cent nicotine sulfate were no more effective than 0.25 per cent. In the case of 174, the three low concentrations actually reduced the toxicity of nicotine sulfate, and only the two longer spray times increased effectiveness at 0.5 per cent of the compound. Compound 179 and 179A showed a similar effect at the longer spray times. These tests do not indicate the reasons for these results. It seems probable, however, that decreasing surface tension with increased concentrations of the polyethyleneglycol compounds might account for failure of 0.5 per cent to be more effective than 0.25 per cent (8). The interaction between spray time and concentration of 174, 179 and 179A will require further investigation for clarification.

TABLE 5. EFFECT OF POLYETHYLENEGLYCOL DERIVATIVES ON TOXICITY OF NICOTINE SULFATE TO APHIDS AS MEASURED BY THE DOSAGE-RESPONSE CURVE (From data in Table 4)

ERL No.	Wetting Agent	Increased Toxicity of Nicotine[1]	Slope of Curve Relative to Nicotine[2]
183	Tetraethyleneglycol monooctyl ether	7	parallel
203	Octaethyleneglycol monododecyl ether	10	parallel
204	Octaethyleneglycol monocetyl ether	4	parallel
173	Polyethyleneglycol-400 monooleate	10	parallel
172	Polyethyleneglycol-400 monolaurate	7	parallel
174	Polyethyleneglycol-400 ditriricinoleate	1¼	steeper
193	Polyethyleneglycol-200 monolaurate	2	steeper
194	Polyethyleneglycol-300 monolaurate	6	flatter
172A	Polyethyleneglycol-400 monolaurate	>10	steeper
196	Polyethyleneglycol-600 monolaurate	>10	steeper
178A	Polyethyleneglycol-600 monooleate	5	steeper
181	Polyethyleneglycol-1000 monooleate	>10	parallel
181A	Polyethyleneglycol-1000 monooleate	>10	parallel
202	Polyethyleneglycol-1540 monooleate	5	parallel
179	Polyethyleneglycol-600 dioleate	4	steeper
179A	Polyethyleneglycol-600 dioleate	6	steeper
180	Polyethyleneglycol-600 ditallate	4	parallel

[1]Dosage units of nicotine required to equal one dosage unit of nicotine-penetrating agent by interpolation from dosage-response curves.

[2]Relation of slope of curve for nicotine-penetrating agent to slope of curve for nicotine alone.

Toxicity of Some Polyethyleneglycol Compounds to Aphids

Six of the materials were sprayed on aphids at a concentration of 4 per cent. The toxicity of these was compared with that of nicotine sulfate alone as well as nicotine sulfate with 4 per cent of the material (Table 6). All showed some toxicity to aphids when used alone at 4 per cent concentration. All of them also increased the toxicity of nicotine sulfate. The two materials of least toxicity to aphids, 173 (polyethyleneglycol-400 monooleate) and 181 (polyethyleneglycol-1000 monooleate), produced only moderate increases in toxicity of nicotine sulfate but did increase the slope of the dosage-response curve (Figure 2). Number 179 (polyethyleneglycol-600 dioleate) was more toxic to aphids but did not increase toxicity of nicotine sulfate to aphids to any greater extent than 173 and 181. Number 172 (polyethyleneglycol-400 monolaurate), which was not very effective in increasing toxicity of nicotine sulfate in the dosage tests discussed above, was highly effective at the higher concentration used here. It was also highly toxic to aphids when used without nicotine sulfate. Number 180 (polyethyleneglycol-600 ditallate) was of intermediate toxicity to aphids and also of intermediate value in increasing effectiveness of nicotine sulfate. Polyethyleneglycol-1500 monolaurate, 171, was of intermediate toxicity to aphids and increased the toxicity of nicotine sulfate to a greater degree than most of the other materials in this test (Figure 3). It is of interest that 171 contains about 50 per cent of the same material used to make 194 which was not very effective in the tests summarized in Table 5.

TABLE 6. TOXICITY TO APHIDS OF WETTING AGENTS ALONE AND WITH NICOTINE SULFATE

ERL No.	Concentrations		Mortality, Spray Time, Secs.			
	Nicotine	Wetting Agent	2.5	5	10	20
...	.04	...	32	40	50	65
171	...	4.0	26	32	35	96[1]
171	.04	4.0	65	85	93	100
180	...	4.0	9	22	35	64[1]
180	.04	4.0	45	65	85	100
...	.04	...	31	52	57	67
172	...	4.0	25	45	73	98[1]
172	.04	4.0	59	98	100	100
179	...	4.0	7	37	54	89[1]
179	.04	4.0	46	69	84	100
...	.04	...	60	61	68	71
173	...	4.0	7	19	24	31
173	.04	4.0	46	69	84	100
181	...	4.0	8	13	19	28
181	.04	4.0	73	87	96	100

[1]Injured nasturtium foliage.

A calculation for synergism was made using the method of Wadley (21). This calculation is admittedly only an approximation, because he specifies that dosage-response curves must be parallel. Only one of the adjuncts used alone (171) produced a dosage-response curve parallel to

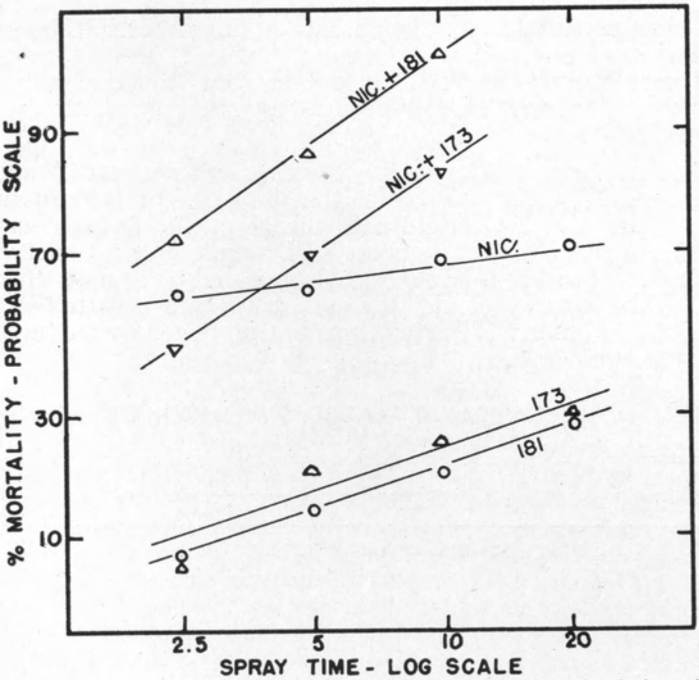

Figure 2. Dosage-response curves for nicotine sulfate and two polyethyleneglycol compounds sprayed on aphids alone and with nicotine sulfate. The concentration of nicotine was 0.04 per cent and of the polyethyleneglycol compounds 4 per cent.

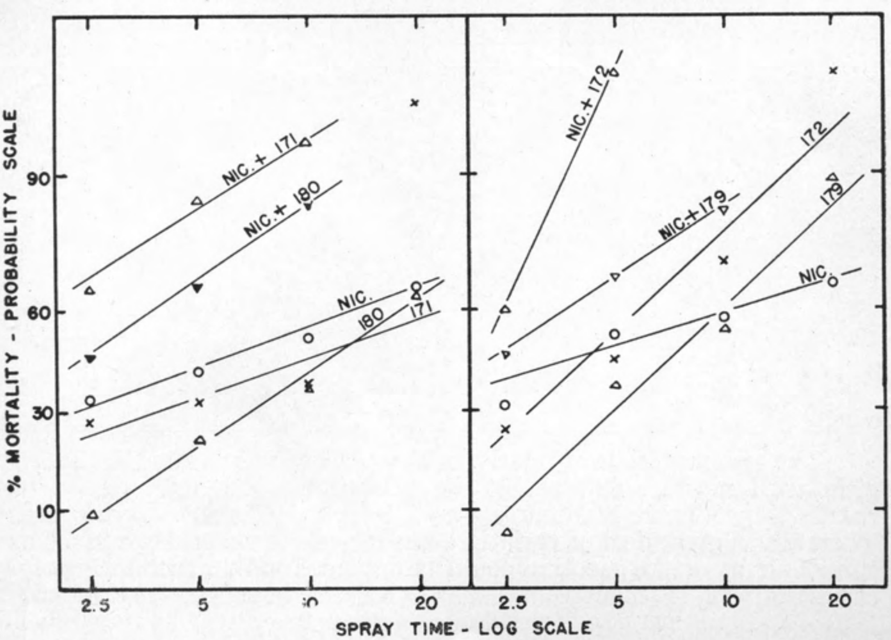

Figure 3. Dosage-response curves for nicotine sulfate and four polyethyleneglycol compounds sprayed on aphids alone and with nicotine sulfate. The concentration of nicotine was 0.04 per cent and of the polyethyleneglycol compounds 4 per cent.

that of nicotine sulfate. In the absence of any other suitable method, parallelism was assumed. The results (Table 7) show that all compounds were synergistic except in low dosages. Since nicotine sulfate plus adjunct produced steeper dosage-response curves, this would be expected.

No attempt was made to apply the later calculation of Wadley (22) to determine log-ratios. Plotting the data on the basis of nicotine sulfate + the nicotine sulfate equivalence of the adjunct served to steepen the dosage-response curves still more. When toxicity of the adjuncts was considered, however, 181 appeared to be most effective in increasing the toxicity of nicotine sulfate to aphids, with 171 a very close second. Number 172 rated third but produced by far the steepest dosage-response curve.

TABLE 7. CALCULATION BY WADLEY'S METHOD FOR MEASURING SYNERGISM

ERL No.	Nicotine Equiv.	Mortality		Synergism
		Actual	Estimated	
		(The Compounds in Table 6)		
171	.0134	65	39	++
	.0268	85	50	+++
	.0536	93	64	+++
	.1072	100	73	+++
172	.0273	59	51	+
	.0546	98	63	+++
	.1092	100	74	+++
	.2184	100	82	+++
179	.0278	46	53	——
	.0576	69	63	+
	.1112	84	73	++
	.2224	100	80	+++
180	.0165	45	41	+
	.033	65	54	++
	.066	85	66	+++
	.132	100	77	+++
173	.0101	46	58	—
	.0201	69	63	+
	.0402	84	68	+++
	.0804	100	72	+++
181	.0101	73	58	++
	.0201	87	63	+++
	.0402	96	68	+++
	.0803	100	72	+++

The two materials of relatively low toxicity were tested for synergism with nicotine sulfate by the "titration" technique (5). The results show (Table 8, Figures 4 and 5) a high degree of synergism. There was a great deal of variation, but it was obvious that as much as three-fourths of the nicotine could be replaced with either 173 (polyethyleneglycol-400 monooleate) or 181 (polyethyleneglycol-1000 monooleate), and still increase toxicity.

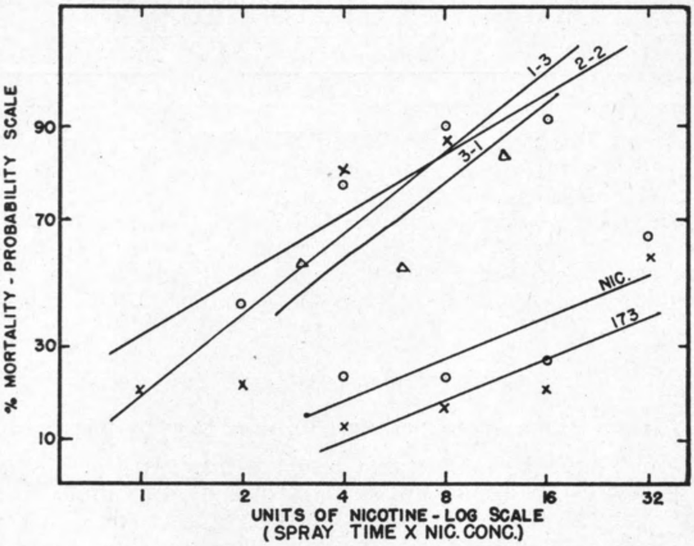

Figure 4. Dosage-response curves for nicotine sulfate and 173 sprayed on aphids alone and in three mixtures (1 part nicotine and 3 parts 173, etc.).

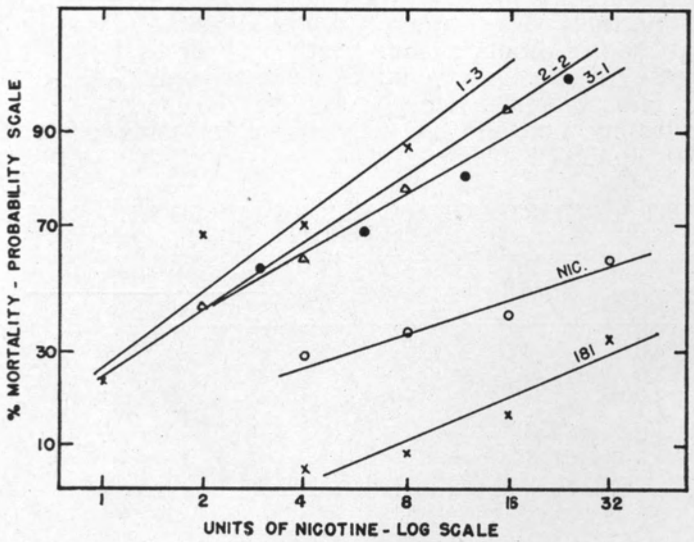

Figure 5. Dosage-response curves for nicotine sulfate and 181 sprayed on aphids alone and in three mixtures (1 part nicotine and 3 parts 181, etc.).

TABLE 8. TOXICITY OF NICOTINE SULFATE WITH TWO WETTING AGENTS BY THE "TITRATION" TECHNIQUE

	Concentration		Mortality, Spray Time, Secs.			
Treatment	Nicotine	Wetting Agent	2.5	5	10	20
Nicotine	.04	. . .	22	22	26	65
173	. . .	4.0	12	15	19	59
Nicotine—173	.01	3.0	19	20	82	88
Nicotine	.02	2.0	43	79	90	91
Nicotine	.03	1.0	56	55	85	100
Nicotine	.04	. . .	29	36	41	59
181	. . .	4.0	7	9	15	35
Nicotine—181	.01	3.0	23	67	70	88
Nicotine	.02	2.0	44	59	79	93
Nicotine	.03	1.0	56	68	82	96

Toxicity of the Compounds to Milkweed Bugs by Injection

It would have been desirable to have had tests of all the compounds as sprays on aphids. The tests of six compounds in Table 6 indicated that they were less than 1 per cent as toxic as nicotine as sulfate. Since aphids were not available, the injection technique was selected in order to learn: (1) how toxic the compounds were when they were introduced into the blood stream and (2) what effect they had on nicotine in the blood stream.

Each chemical was injected into milkweed bugs at 2 per cent and 4 per cent concentration. Of the entire group of compounds used only four were somewhat toxic at these concentrations, and results of a series of injections with them are given in Table 9. It is interesting to note that the two ditallates were relatively toxic by injection but did not increase toxicity by spraying as much as some of the other compounds. The two octaethyleneglycol ethers, 203 and 204, were relatively toxic by injection and 203 was among the more effective when sprayed on aphids (Table 5).

TABLE 9. TOXICITY OF COMPOUNDS TO MILKWEED BUGS BY INJECTION

	Mortality[1] from Compound			
Concentration	170	180	203	204
4.0			83	75
2.8			67	67
2.0	95	69	92	33
1.4	53	62	25	0
1.0	50	67		
.7	53	67		

[1]Average of 3 replicates of 12 adults each.

Five polyethyleneglycol derivatives were selected for dosage tests with alkaloid nicotine by injection. These were (a) compounds which increased toxicity of nicotine (as sulfate) to aphids by spraying:

171 Polyethyleneglycol-1500 monolaurate
172 Polyethyleneglycol-400 monolaurate
181 Polyethyleneglycol-1000 monooleate

(b) compounds which reduced toxicity of nicotine sulfate to aphids by spraying:

184 Tetraethyleneglycol dioctyl ether
185 Tetraethyleneglycol monododecyl ether

Table 10 shows that alkaloid nicotine at 0.2 per cent killed 20 per cent of the injected bugs. None of the five test compounds had any consistent effect on the toxicity of nicotine. The two which may have increased toxicity were 184 and 185. Obviously there was no relation between effect on alkaloid nicotine by injection and on nicotine sulfate by spraying.

TABLE 10. EFFECT OF POLYETHYLENEGLYCOL DERIVATIVES ON THE TOXICITY OF ALKALOID NICOTINE BY INJECTION

Treatment	Concentration		Per Cent Mortality		
	Wetting Agent	Nicotine	1	2	Mean
171	4	.2	25	33	29
	2	.2	17	17	17
	1	.2	25	17	21
	.5	.2	17	8	12
172	4	.2	25	33	29
	2	.2	25	25	25
	1	.2	25	33	29
	.5	.2	17	17	17
181	4	.2	17	17	17
	2	.2	25	33	29
	1	.2	17	25	21
	.5	.2	17	17	17
184	4	.2	33	42	37
	2	.2	25	25	25
	1	.2	17	33	21
	.5	.2	8	33	20
185	4	.2	42	42	42
	2	.2	42	33	37
	1	.2	33	42	37
	.5	.2	17	17	17
Nicotine	..	.2	7	33	20

Eight materials were injected with and without nicotine in two series of tests for synergism. These were (a) compounds which increased toxicity of nicotine sulfate to aphids by spraying:

180 Polyethyleneglycol-600 ditallate
183 Tetraethyleneglycol monooctyl ether
187 Tetraethyleneglycol monocetyl ether
194 Polyethyleneglycol-300 monolaurate

(b) two which had no effect in spray tests:

170 Polyethyleneglycol-300 ditallate
173A Polyethyleneglycol-400 monooleate
189 Tetraethyleneglycol monooctadecyl ether

and (c) one which reduced toxicity in spray tests:
182 *Triton X-100*

The test with the ditallates (Table 11, Figure 6) indicates that the dosage-response curve is probably not parallel with the curve for nicotine, which makes synergism calculations difficult. If it is assumed that the curve for ERL 170 is parallel, and a calculation is made by Wadley's (21) method, there is no evidence of synergism (Table 12).

The test with the other materials (Table 13) showed that 194 reduced the toxicity of nicotine sharply. Four materials, 182, 183, 187 and 189 also reduced toxicity when calculated by Wadley's (22) method. One, 173A, had no effect on the toxicity of nicotine.

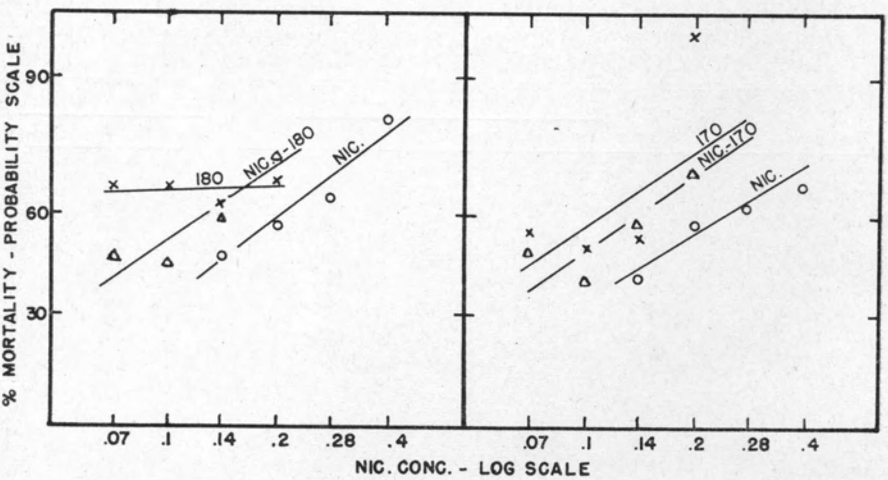

Figure 6. Dosage-response curves for nicotine alkaloid, 170 and 180, alone and in combination, applied to milkweed bugs by injection.

A synergism test with the two ethers, 203 and 204, which were toxic by injection, has been summarized in Table 13a. The dosages were somewhat high for suitable mortality. However, calculation by Wadley's (21) technique indicates antagonism between these materials and nicotine rather than synergism.

In general, these tests showed that the toxicity of the polyethyleneglycol compounds injected into milkweed bugs was relatively low. Furthermore, none of the materials tested had any synergistic effect when injected with nicotine. Only one material, 203 (octaethyleneglycol monododecyl ether), was both highly effective with nicotine in spray tests and toxic by injection. Obviously mortality in the spray tests included any toxicity of this compound applied by that method.

These tests indicate that the effectiveness of these materials when sprayed with nicotine is largely physical. They imply that the synergism shown is the result either of removal of the waxy coating of the insect cuticle or improved penetration through it.

TABLE 11. TEST FOR SYNERGISM, INJECTION OF ALKALOID NICOTINE AND TWO TOXIC PENETRATING AGENTS

Treatment	Concentration: Nicotine	Concentration: Wetting Agent	Per Cent Mortality: 1	2	3	Mean
Nicotine	.4	...	75	92	83	83
	.28	...	58	83	50	64
	.2	...	42	67	58	56
	.14	...	17	58	67	47
180	...	2.0	58	92	58	69
	...	1.4	33	83	67	62
	...	1.0	33	75	92	67
	...	.7	42	67	92	67
Nicotine—180	.2	1.0	75	75	75	75
	.14	.7	25	83	67	58
	.1	.5	25	58	50	44
	.07	.35	25	83	33	47
Nicotine	.4	...	92	67	75	78
	.28	...	83	75	58	72
	.2	...	83	75	42	67
	.14	...	92	7	25	41
170	...	2.0	100	92	92	95
	...	1.4	83	25	50	53
	...	1.0	50	33	67	50
	...	.7	83	17	58	53
Nicotine—170	.2	1.0	58	83	75	72
	.14	.7	58	67	50	58
	.1	.5	33	58	33	41
	.07	.35	42	67	42	50

TABLE 12. CALCULATION FOR SYNERGISM BETWEEN POLYETHYLENE-GLYCOL-300 DITALLATE AND ALKALOID NICOTINE

Concentration: Nicotine	Concentration: 170	Nicotine Equivalent Conc.[1]	Mortality: Actual	Mortality: Estimated	Synergism
.07	.35	.15	50	45	—
.1	.5	.22	41	54	—
.14	.7	.3	58	62	—
.2	1.0	.43	72	71	—

[1]Nicotine equivalent of polyethyleneglycol-300 ditallate estimated at .23.

Effect of Type of Nicotine and of Alkaline and Ionic Materials on Nicotine by Injection

Since alkaline materials were shown by deOng (3) to have an effect on the toxicity of nicotine applied in sprays, it was of interest to study them by injection. Accordingly, alkaloid nicotine and nicotine

TABLE 13. SYNERGISM TEST, ALKALOID NICOTINE WITH POLYETHYLENEGLYCOL DERIVATIVES INJECTED INTO MILKWEED BUGS

Treatment	Concentration: Nicotine	Concentration: Wetting Agent	Per Cent Mortality: Replicate 1	Replicate 2	Mean
Nicotine	.4	...	83	83	83
	.28	...	75	75	75
	.2	...	42	58	50
	.14	...	33	42	38
173A	...	2.0	0	0	0
	...	1.4	0	0	0
	...	1.0	0	0	0
	...	.7	0	0	0
194	...	2.0	0	0	0
	...	1.4	0	0	0
	...	1.0	0	0	0
	...	.7	0	0	0
Nicotine—173A	.2	1.0	75	67	71
	.14	.7	42	25	34
	.1	.5	17	17	17
	.07	.35	25	0	12
Nicotine—194	.2	1.0	17	50	33
	.14	.7	17	33	25
	.1	.5	8	17	12
	.07	.35	0	0	0

Treatment	Nicotine	Wetting Agent	Replicate 1	Replicate 2	Replicate 3	Mean
Nicotine	.4	...	67	67	75	70
	.28	...	50	42	67	53
	.2	...	17	33	33	28
	.14	...	17	17	8	14
182	...	2.0	25	8	8	14
	...	1.4	17	67	8	31
	...	1.0	50	33	0	28
	...	.7	33	25	8	22
183	...	2.0	42	8	0	17
	...	1.4	8	58	0	22
	...	1.0	0	25	0	8
	...	.7	8	42	0	17
Nicotine—182	.2	1.0	33	33	58	41
	.14	.7	17	25	33	25
	.1	.5	8	17	17	14
	.07	.35	50	17	17	28
Nicotine—183	.2	1.0	50	42	42	44
	.14	.7	8	33	25	22
	.1	.5	8	25	33	22
	.07	.35	0	25	17	14

Treatment	Nicotine	Wetting Agent	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
Nicotine	.4	...	83	83	83	75	81
	.28	...	67	75	75	67	71
	.2	...	58	83	50	50	60
	.14	...	17	83	33	33	29

TABLE 13. (Continued) SYNERGISM TEST, ALKALOID NICOTINE WITH POLYETHYLENEGLYCOL DERIVATIVES INJECTED INTO MILKWEED BUGS

Treatment	Concentration		Per Cent Mortality				
			Replicate				
	Nicotine	Wetting Agent	1	2	3	4	Mean
187	...	2.0	0	8	8	25	10
	...	1.4	0	25	8	0	8
	...	1.0	8	0	0	0	2
	...	.7	0	0	8	0	2
189	...	2.0	0	8	8	17	8
	...	1.4	0	8	8	0	4
	...	1.0	0	25	8	0	8
	...	.7	0	25	0	8	8
Nicotine—187	.2	1.0	33	50	42	58	46
	.14	.7	33	25	33	67	39
	.1	.5	25	75	25	50	44
	.07	.35	8	50	8	42	27
Nicotine—189	.2	1.0	33	75	50	42	50
	.14	.7	25	17	42	33	29
	.1	.5	8	17	50	58	33
	.07	.35	8	8	17	42	19

TABLE 13a. SYNERGISM TEST, ALKALOID NICOTINE WITH POLYETHYLENEGLYCOL ETHERS INJECTED INTO MILKWEED BUGS

Treatment	Concentration		Per Cent Mortality		
			Replicate		
	Nicotine	Wetting Agent	1	2	Mean
Nicotine	.4	...	100	100	100
	.28	...	100	100	100
	.2	...	92	92	92
	.14	...	83	75	79
203	...	4.0	92	100	96
	...	2.8	100	100	100
	...	2.0	67	83	75
	...	1.4	58	50	54
204	...	4.0	92	100	96
	...	2.8	100	92	96
	...	2.0	100	100	100
	...	1.4	83	83	83
Nicotine—203	.2	2.0	100	100	100
	.14	1.4	75	100	88
	.1	1.0	50	58	54
	.07	.7	67	58	62
Nicotine—204	.2	2.0	100	100	100
	.14	1.4	92	92	92
	.1	1.0	67	83	75
	.07	.7	75	25	50

Calculations by Wadley's Technique

Treatment	Nicotine Equivalent	Mortality Actual	Mortality Estimated	Synergism
Nicotine—203	.34	100	98	—
	.24	88	95	—
	.17	54	87	—
	.12	62	73	—
Nicotine—204	.4	100	99	—
	.28	92	97	—
	.2	75	92	—
	.14	50	79	—

sulfate were tested with and without sodium hydroxide (Table 14, Figure 7). Nicotine sulfate was slightly more toxic than the alkaloid. The original data indicate that addition of sodium hydroxide reduced toxicity of the alkaloid and increased toxicity of the sulfate. However, when the Wadley (21) calculation was made and the toxicity of the sodium hydroxide considered, there was no evidence of synergism. Furthermore, sodium hydroxide seemed to flatten the slope of the dosage-response curve when used with the alkaloid.

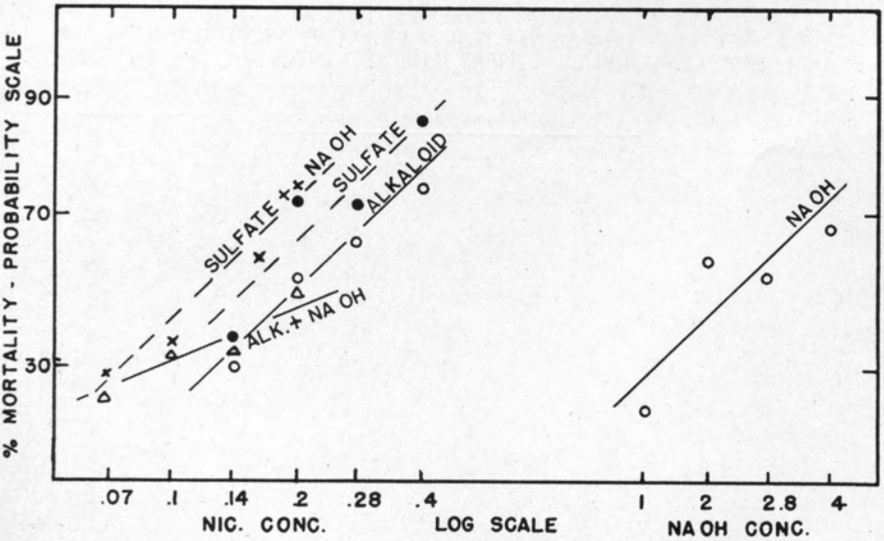

Figure 7. Dosage-response curves for nicotine alkaloid, nicotine sulfate and sodium hydroxide, and both forms of nicotine with sodium hydroxide applied to milkweed bugs by injection.

A similar type of experiment was made using sodium oleate. The results (Table 15, Figure 8) show that sodium oleate had little effect on the sulfate and reduced toxicity of the alkaloid.

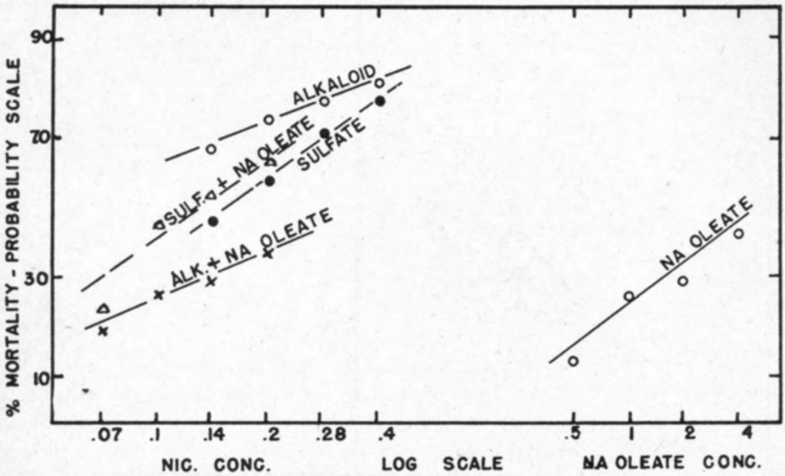

Figure 8. Dosage-response curves for nicotine alkaloid, nicotine sulfate, and sodium oleate, and both forms of nicotine with sodium oleate applied to milkweed bugs by injection.

In the third test, the ammonium soap *Blendene* and non-ionic *Triton X-100* were used. Neither had any effect on the toxicity of nicotine sulfate (Table 16, Figure 9). *Blendene* did not affect the toxicity of the alkaloid. *Triton X-100* flattened the slope of the dosage-response curve in the test with the alkaloid.

TABLE 14. DOSAGE TESTS OF ALKALOID AND SULFATE NICOTINE INJECTED WITH SODIUM HYDROXIDE

Treatment	Concentration		Per Cent Mortality		
	Nicotine	NaOH	1	2	Mean
Alkaloid	.4	..	75	75	75
	.28	..	58	67	62
	.2	..	42	63	52
	.14	..	17	42	30
Sulfate	.4	..	83	92	87
	.28	..	75	67	72
	.2	..	67	75	72
	.14	..	50	25	37
Sodium hydroxide	..	4.0	75	58	66
	..	2.8	58	50	54
	..	2.0	67	50	58
	..	1.4	42	0	21
Alkaloid—sodium hydroxide	.2	2.0	42	58	50
	.14	1.4	33	33	33
	.1	1.0	42	25	33
	.07	.7	50	0	25
Sulfate—sodium hydroxide	.2	2.0	58	92	75
	.14	1.4	67	50	58
	.1	1.0	50	25	37
	.07	.7	50	8	29

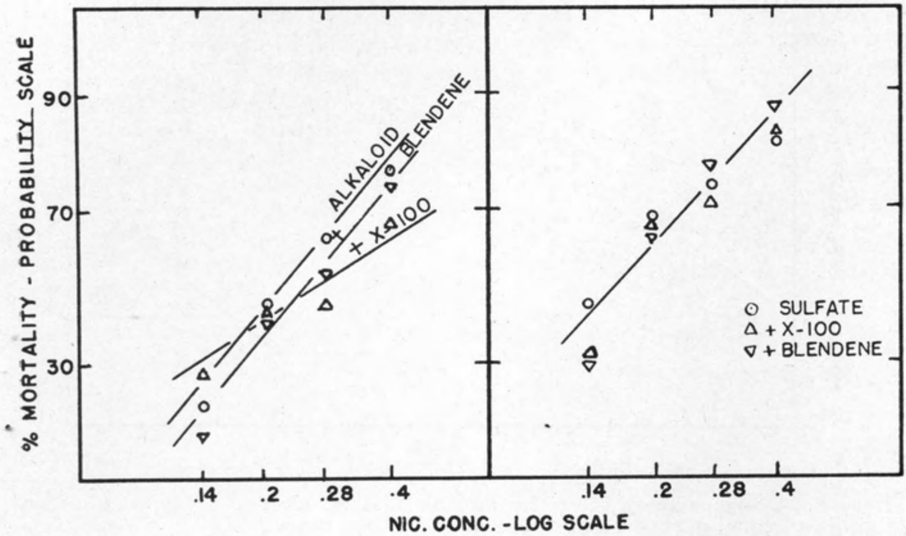

Figure 9. Dosage-response curves for alkaloid nicotine and nicotine sulfate, injected into milkweed bugs alone and in combination with BLENDENE and TRITON X-100.

TABLE 15. DOSAGE TESTS OF SODIUM OLEATE INJECTED INTO MILKWEED BUGS WITH NICOTINE ALKALOID AND NICOTINE SULFATE

Treatment	Concentration		Per Cent Mortality		
	Nicotine	Oleate	1	2	Mean
Alkaloid	.4	..	83	83	83
	.28	..	75	83	79
	.2	..	75	75	75
	.14	..	67	67	67
Sulfate	.4	..	75	83	79
	.28	..	67	75	71
	.2	..	58	58	58
	.14	..	42	50	46
Sodium oleate	..	4.0	33	50	41
	..	2.0	25	33	29
	..	1.0	8	42	25
	..	.5	0	25	12
Alkaloid—sodium oleate	.2	2.0	58	17	37
	.14	1.4	33	25	29
	.1	1.0	33	17	25
	.07	.7	17	17	17
Sulfate—sodium oleate	.2	2.0	75	50	63
	.14	1.4	58	50	54
	.1	1.0	58	33	45
	.07	.7	25	17	21

TABLE 16. DOSAGE TESTS OF WETTING AGENTS INJECTED WITH NICOTINE ALKALOID AND NICOTINE SULFATE

Treatment	Concentration		Per Cent Mortality		
	Nicotine	Wetting Agent	1	2	Mean
Alkaloid	.4	..	83	75	79
	.28	..	50	75	63
	.2	..	42	50	46
	.14	..	17	25	21
Alkaloid—*Triton X-100*	.4	.5	75	58	67
	.28	.35	42	50	46
	.2	.25	58	33	45
	.14	.175	33	25	29
Alkaloid—*Blendene*	.4	.5	75	75	75
	.28	.35	50	58	54
	.2	.25	67	17	42
	.14	.175	25	8	16
Sulfate	.4	..	83	83	83
	.28	..	67	83	75
	.2	..	67	67	67
	.14	..	33	58	45
Sulfate—*Triton X-100*	.4	.5	92	75	84
	.28	.35	75	67	71
	.2	.25	75	58	67
	.14	.175	17	42	30
Sulfate—*Blendene*	.4	.5	92	83	88
	.28	.35	83	75	79
	.2	.25	67	58	63
	.14	.175	17	42	30

These results cannot be interpreted as contradicting the results of Richardson (14). His conclusions were based on penetration of the insect and not on the injection procedure. When the results of injections are compared with the results of spraying in Tables 1 and 2, it is obvious that here, too, the sodium oleate and *Blendene* acted to aid penetration of the nicotine in the spray tests.

It should also be noted that Levine and Richardson (7) found that potassium bicarbonate, potassium chloride and sodium bicarbonate increased the time of paralysis of American roaches after injection with nicotine. Sodium chloride had no effect. Barratt and Horsfall (1) have shown that small amounts of inorganic salts affect the permeability of spores to fungicides. This may account for the observation of Levine and Richardson. In our tests no such result was noted following injection of sodium hydroxide. The relatively high toxicity of the sodium hydroxide at the dosages used may have obscured any effect of the alkali on penetration within the insect.

DISCUSSION

The data obtained in the spraying tests showed that some of the polyethyleneglycol compounds applied to aphids in sprays increase the toxicity of nicotine sulfate. This is an extension of the work of Wigglesworth (25) in which nicotine was applied with the undiluted compounds. The tenfold increase in toxicity with the more effective materials seems large enough to be of practical value. There remains the necessity of providing some sort of rating of the value of the compounds and of discussing the relation between chemical composition and effectiveness.

The screening tests (Table 3) offer some basis for comparison. There was, however, a single dosage of nicotine sulfate and of each of the test compounds. The only numerical comparison possible is by ratio of probit mortality for nicotine sulfate and for nicotine sulfate plus the test compound. These probit ratios have been listed in Table 17. Since the ratios in 1949 were considerably higher than in 1948, the order of effectiveness has been ranked separately for the two years.

TABLE 17. SUMMARY OF SPRAY TESTS ON *Aphis rumicis* Linn.

	Screening Tests Table 3			Dosage Tests—Table 5		Synergism Test Table 7
		Rank				
ERL No.	Probit Ratio	1948	1949	Increased Toxicity of Nicotine	Slope of Curve Relative to Nicotine	Rank
170	1.02[1]					
171	1.10[1]	5				2
172	1.12[1]	3		7	parallel	3
173	1.13[1]	1.5		10	parallel	5
174	1.06[1]	7.5		1.3	steeper	
173A	1.04[1]	9.5				
178A	1.26[2]		2	5	steeper	
179	1.03[1]	11		4	steeper	6
179A	1.23[2]		3.5	6	steeper	
180	1.06[1]	7.5		4	parallel	4
181	1.13[1]	1.5		>10	parallel	1
181A	1.16[2]		8	>10	parallel	
182	.92[1]					
183	1.08[1]	6		7	parallel	
184	.85[1]					
185	.94[1]					
187	1.11[1]	4				
189	1.04[1]	9.5				
193	1.15[2]		9	2	steeper	
194	1.19[2]		6.5	6	flatter	
172A	1.23[2]		3.5	>10	steeper	
196	1.20[2]		5	>10	steeper	
202	1.28[2]		1	5	parallel	
203	1.14[2]		10	10	parallel	
204	1.19[2]		6.5	4	parallel	

[1]Tests carried out in 1948.
[2]Tests carried out in 1949.

All of the materials found more effective in screening tests, with the exception of 187, were included in the dosage test (Table 5). In general, this test agreed with the screening test, that is, the same materials were most effective in both. The important deviations which were high in the screening test and low in the dosage test were 202, 178A and 179A. The one low in the screening test and high in the dosage test was 203.

Ranks on the basis of the synergism test (Table 7) have been assigned in Table 17. While these are important, it should be remembered that in the synergism test the compounds were used at 4 per cent concentration, far greater than the 0.5 per cent top concentration in the dosage tests.

It is probably fruitless to attempt to rank the materials in the order of their effectiveness on the basis of the data available. Nevertheless, they can be grouped, chiefly on the basis of the dosage tests.

a. Highly effective materials

181A	Polyethyleneglycol-1000 monooleate
172A	Polyethyleneglycol-400 monolaurate
196	Polyethyleneglycol-600 monolaurate
173	Polyethyleneglycol-400 monooleate
203	Octaethyleneglycol monododecyl ether

b. Effective materials

172	Polyethyleneglycol-400 monolaurate
179A	Polyethyleneglycol-600 dioleate
183	Tetraethyleneglycol monooctyl ether
194	Polyethyleneglycol-300 monolaurate
178A	Polyethyleneglycol-600 monooleate
202	Polyethyleneglycol-1540 monooleate

c. Materials which may fall in groups a or b

171	Polyethyleneglycol-1500 monolaurate
187	Tetraethyleneglycol monocetyl ether

On the basis of these tests and particularly because of the steeper dosage-response curves, 172A and 196 appear to be first choice for increasing toxicity of nicotine sulfate. It will be noted that 172 was placed in a different category than 172A. Although these materials bear the same name, they differ in biological effect. Likewise 173A and 173B were much less effective than 173.

Wigglesworth (25) reported that undiluted octaethyleneglycol monocetyl ether was more effective than the tetraethylene derivative in removing wax from the cuticle of an insect. Unfortunately we had no direct comparison between the two materials in the same series of tests. If the probit ratios in Table 17 can be considered comparable for the two years, our results were similar. However, as noted above, other compounds were far more effective.

There were two series of compounds available in sufficient numbers to encourage comparison between chemical constitution and effectiveness. The log ratios (22) of the nicotine sulfate alone and with the adjunct were calculated from curves drawn from the data in Table 4. In the cases in which the curves were not parallel, the degree of synergism was estimated (21). A value of one was assigned for each plus (estimated), and totalled with the log ratios that could be calculated. The results are given in Table 18. In the case of the monooleates, effectiveness reached a peak at a molecular weight of 864 and then declined. With the monolaurates, effectiveness increased with increase in the length of the ethyleneglycol chain. It is obvious that the principal effect of length of this chain is on the hydrophilic and lipophilic balance. The more effective molecules contain "the suitable balance of hydrophilic and lipophilic characteristics" which Beament (2) concluded were required for efficiency in penetrating the wax layer of insects.

TABLE 18. RELATION OF COMPOSITION TO SUM OF LOG RATIOS

ERL No.	Wetting Agent	Molecular Weight	Sum of Log Ratios
173	Polyethyleneglycol-400 monooleate	664	12.8
178A	Polyethyleneglycol-600 monooleate	864	17.5
181A	Polyethyleneglycol-1000 monooleate	1264	13.2
202	Polyethyleneglycol-1540 monooleate	1804	6.6
179A	Polyethyleneglycol-600 dioleate	1128	10.1
193	Polyethyleneglycol-200 monolaurate	382	1.4
194	Polyethyleneglycol-300 monolaurate	482	7.9
172A	Polyethyleneglycol-400 monolaurate	582	12.0
196	Polyethyleneglycol-600 monolaurate	782	14.0

SUMMARY

The experiments of Wigglesworth (25) on the effect of polyethyleneglycol derivatives on penetration of nicotine through the cuticle of insects suggested a more detailed study of these compounds for this purpose.

A series of 30 polyethyleneglycol derivatives was provided by the Eastern Regional Research Laboratory.

All materials were tested by spraying *Aphis rumicis* Linn. with nicotine sulfate and the test material.

Toxicity of the wetting agents was determined by injecting them into milkweed bugs, *Oncopeltus fasciatus* Dal. Effect of some of the polyethyleneglycol compounds on the toxicity of alkaloid nicotine was also determined by injection.

Preliminary tests were made by spraying ionic and non-ionic wetting agents with nicotine and nicotine sulfate. Sodium oleate increased the toxicity of nicotine as sulfate more than that of the alkaloid. Ammonium linoleate had no effect on toxicity of sulfate and decreased

toxicity of the alkaloid. The ammonium soap *Blendene* increased the toxicity of the alkaloid to a much greater extent than the sulfate. The quaternary ammonium *Ammonyx Q* and the non-ionic *Igepal 300* had little effect on toxicity of either form of nicotine.

Nineteen polyethyleneglycol derivatives at 0.5 per cent concentration increased the toxicity of 0.04 per cent nicotine in tests on aphids. Six compounds had little effect and five reduced toxicity.

Seventeen of the more effective materials were used in a dosage test at 0.0625, 0.125, 0.25 and 0.5 per cent concentration with 0.04 per cent nicotine sulfate in spray tests on aphids. Four steepened the slope of the dosage-response curve. Five increased the toxicity of nicotine sulfate by tenfold or more. These were polyethyleneglycol-1000 monooleate, the 400 and 600 monolaurates, polyethyleneglycol-400 monooleate and octaethyleneglycol monododecyl ether.

A spraying test with six of the materials at 4 per cent concentration with and without nicotine sulfate showed that all were toxic to aphids but that there was significant synergism with nicotine.

Only four of the compounds were toxic to milkweed bugs by injection at 4 per cent concentration.

Fifteen derivatives selected from effective and ineffective materials, as determined by spraying, were injected into milkweed bugs with alkaloid nicotine. None increased the toxicity of nicotine when used in this way.

It seems evident that the synergism demonstrated in spraying tests was the result of improved penetration of the insect cuticle.

Injection of alkaloid and sulfate nicotine with sodium hydroxide showed that this base did not increase toxicity of the sulfate when a correction was made for toxicity of the hydroxide. Sodium oleate and the ammonium soap *Blendene* had no effect on toxicity of either form of nicotine. The non-ionic *Triton X-100* had no effect on the sulfate but flattened the slope of the dosage-response curve for the alkaloid.

Effectiveness of the monooleates in sprays with nicotine sulfate first increased and then decreased with increase in length of the ethyleneglycol chain. With the monolaurates, effectiveness increased with increase in length of the chain.

The tenfold increase in the toxicity of nicotine applied by spraying with the more effective of these polyethyleneglycol derivatives seems large enough to be of practical value.

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