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GRANARY INSECTICIDES

Protection of Stored Grain with Sprays of Pyrethrins–Piperonyl Butoxide Emulsion

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Water-miscible emulsions of piperonyl butoxide and pyrethrins, completely free from mineral oils or objectionable ingredients, can be applied directly to grain to control insects in stored grain. Using a standard concentration of 2% piperonyl butoxide and 0.2% pyrethrins in oil-free emulsion sprays, studies showed satisfactory results from 2 to 12 gallons of emulsion per 1000 bushels of grain. This development opens an immense field of usefulness of a simple spray method, which should meet with complete approval of government agencies concerned with foods.

The protection of stored grain from insect damage is a serious problem, which is even more serious in times of overproduction when it is necessary to store surpluses for long periods. In order to reduce to a minimum the deterioration and losses that result from insects, good methods of handling and storage have aided in holding down insect populations, but alone these are not sufficient to handle the problem. Because grain in storage is always vulnerable to attack by insects (*1*) and the destruction of an insect population already established in grain does not undo the damage done, the primary need is for a material and method that will prevent insects from becoming established in stored grain.

For a number of years, protectant powders (*2, 4, 6*) have been available, which, when mixed intimately with grain, effectively prevent infestations of insects and offer prolonged protection from reinfestations. The active ingredients in these protectants are pyrethrum, long known for its rapid action against insects and its unusual safety, and piperonyl butoxide, a pyrethrum synergist, also of extreme safety. This combination has been most widely used in liquid form as sprays and aerosols, particularly where freedom from toxic hazards (*3*) is an important consideration. The use of the same ingredients applied as liquids to grain would, in some circumstances, have advantages that are not obtained from powders.

In many fields, insecticides are being used in rather highly concentrated form, but at very low rates of application. Such a method of treatment carries a strong appeal for the treatment of stored grains. Tests were undertaken in the laboratory to determine the effectiveness of different sprays applied to grain, the importance of the dosage-concentration relationship, and the effectiveness of a new treatment with oil-free emulsion spray.

Materials and Methods

In comparing oil sprays and water emulsions, soybean oil was used as representative of the more readily available vegetable oils. Ultrasene was used as representative of deodorized petroleum-base oils. These oils were used as diluents for a commercially available oil-base concentrate containing 50 grams of piperonyl butoxide and 5 grams of pyrethrins per 100 ml. of concentrate. The water emulsion was prepared by diluting with water an oil-base emulsifiable concentrate containing 10 grams of piperonyl butoxide and 1 gram of pyrethrins per 100 ml. of concentrate. The protectant powder used for comparative purposes was a commercially available product.

The oil-free emulsions were prepared by adding 20% of an emulsifier, polyoxyethylene (8) stearate (available as MYRJ-45 and also under different trade names from various sources), to 80% of an oil-free concentrate and then diluting with water to give the desired concentration. The oil-free concentrate consists of 75% of piperonyl butoxide, 7.5% of pyrethrins, and 17.5% of inert material, mostly other pyrethrum extractives. The resulting oil-free emulsifiable concentrate (T-647) contained

Table I. Mortality of Rice Weevils

(In shelled yellow corn treated with sprays and powders containing piperonyl butoxide and pyrethrins. Average of two test jars. 50 weevils per jar. Baltimore, Md.)

Concentration %									
Piperonyl butoxide	Pyrethrins	Diluent	Dosage Rate, Gal./1000 Bu.	Piperonyl Butoxide, P.P.M.	Mortality, % 3 days	7 days	30 days	60 days	Population Increase at 60 Days, %
0.5	0.05	Soybean oil	16.8	12.50	34	96	100	93	7
			8.4	6.25	13	70	83	67	32
		Base oil	16.8	12.50	75	92	99	99	0
			8.4	6.25	43	52	60	35	72
		Water emul.	16.8	12.50	8	53	100	97	3
			8.4	6.25	0	24	77	73	6
1.0	0.1	Soybean oil	8.4	12.50	37	95	100	98	3
			4.2	6.25	10	59	80	50	59
		Base oil	8.4	12.50	72	89	96	95	1
			4.2	6.25	11	22	32	19	74
		Water emul.	8.4	12.50	15	65	100	97	3
			4.2	6.25	3	36	80	77	3
0.8	0.05	Asbestol[a]	75 lb.	10.7	24	85	100	100	0
			50 lb.	7.1	8	46	84	87	2

[a] Fibrous talc.

Table II. Mortality of Rice Weevils

(After 7-day exposure in shelled yellow corn treated with sprays and powder containing piperonyl butoxide and pyrethrins. Average of two test jars, 50 weevils per jar. Baltimore, Md.)

Concentration, %					Weevils Introduced at: Mortality, %			
Piperonyl butoxide	Pyrethrins	Diluent	Dosage Rate, Gal./1000 Bu.	Piperonyl Butoxide, P.P.M.	0 day[a]	30 days	60 days	90 days
0.5	0.05	Soybean oil	16.8	12.5	87[a]	14	4	0
		Base oil	16.8	12.5	99	24	10	11
		Emulsion	16.8	12.5	88	94	92	94
1.0	0.1	Soybean oil	8.4	12.5	98	46	2	0
		Base oil	8.4	12.5	91	30	25	46
		Emulsion	8.4	12.5	78	93	87	93
0.8	0.05	Asbestol	100 lb.	14.3	95	99	97	92
		Soybean oil	16.8		4[b]			
		Base oil	16.8		23			
		Water	16.8		1			
		Check	0		0			

[a] Average of 6 jars.
[b] Average of 4 jars.

60% of piperonyl butoxide, 6.0% pyrethrins, and 20% of the emulsifier.

Tests reported in the present paper were made in the laboratory in a room held at a constant temperature of 80° F. and a relative humidity of 60 to 70%, by a jar method previously described (*5*). When grain was treated with liquids, the grain was spread out in an even layer covering an area of 1 square foot. The required dosages were then applied as evenly as possible by means of a DeVilbis atomizer with a minimum of air pressure, usually 2 to 5 pounds per square inch, depending on the type of liquid used. The sprayed sample was thoroughly mixed and placed in 1-pint glass jars. The test insects were then introduced and the jars closed with a 40-mesh brass strainer cloth, held in place by a screw top. The samples were left undisturbed, except when the insects were screened from the test samples for examination.

Comparison of Oil Sprays and Water Emulsions

In critically made studies in the laboratory, it was demonstrated that emulsions are more effective against grain insects than similar concentrations of the active ingredients applied in oils.

A mineral-base oil concentrate containing 50 grams of piperonyl butoxide and 5 grams of pyrethrins per 100 ml. of concentrate was diluted with soybean oil and a deodorized mineral-base oil. An emulsifiable concentrate containing 10% of the synergist, 1% of pyrethrins, and approximately 75% of petroleum oil was diluted with water to give a moderately stable emulsion. Two dilutions of each of the three types of spray were prepared. The concentration of the sprays used and the rate of application to the grain are shown in Tables I and II. Table I shows the results obtained with these sprays applied directly to corn at two dosage rates. The emulsions were less effective than the oil sprays after exposure of 3 and 7 days to rice weevils, but were fully equal to the oil sprays after 30 and 60 days, and were more effective in preventing reproduction in the treated corn. At 30 and 60 days the emulsion treatments were of the same order of effectiveness as a commercially available grain protectant powder.

Table II shows the results of a second series of tests in which quantities of shelled corn were sprayed with the three types of sprays and stored in test jars. Rice weevils were introduced into portions of this treated grain at intervals of 30 days. Similar fresh treatments were included at each interval for direct comparison. The data show that the formulas containing vegetable and petroleum-base oil lost their effectiveness rapidly and were comparatively ineffective 30 days after application to the grain. The emulsion sprays remained effective to the end of the 90-day test period and gave results similar to the treatment with the powder containing piperonyl butoxide and pyrethrins. No reproduction of weevils occurred in the emulsion treatments. For a second series of tests, the mortality of weevils after 30 days of exposure and the rate of increase in the population after 60 days are shown in Table III. In these neither the soybean oil nor the petroleum-base oil sprays gave satisfactory results, except as fresh treatments and during initial exposures to the insects. A 30-day exposure of the weevils to oil spray treatments, made 30 or more days before the

Table III. Mortality of Rice Weevils

(After 30-day exposure and increase in population after 60 days in shelled yellow corn treated with sprays and powder containing piperonyl butoxide and pyrethrins. Average of two test jars, 50 weevils per jar. Baltimore, Md.)

Concentration, %				Age of Treatment When Weevils Introduced						
				0 Day[a]		30 Days		60 Days		90 Days
Piperonyl butoxide	Pyrethrins	Diluent	Dosage Rate, Gal./1000 Bu.	% dead 30 days	% increase 60 days	% dead 30 days	% increase 60 days	% dead 30 days	% increase 60 days	% dead 30 days
0.5	0.05	Soybean oil	16.8	92	3	28	0	18	65	5
		Base oil	16.8	100	5	24	2	18	58	14
		Emulsion	16.8	99	0	100	0	100	0	100
1.0	0.1	Soybean oil	8.4	99	3	62	2	9	106	2
		Base oil	8.4	99	2	55	0	50	54	66
		Emulsion	8.4	99	0	100	0	97	0	100
0.8	0.05	Asbestol	100 lb.	100	0	100	0	100	2	100
		Soybean oil	16.8	12	72	...	. .	...	...	...
		Base oil	16.8	47	141	...	. .	...	...	...
		Water	16.8	5	382	...	. .	...	...	...
		Untreated	...	2	459	...	. .	...	...	...

[a] Average of 6 jars.

Table IV. Effect of Concentration of Active Ingredients in Oil-Free Emulsions Applied Directly to Wheat

(Average of four paired replications by jar method against rice weevils. Total of 400 insects per treatment. Baltimore, Md.)

Concentration of Active Ingredients, %		Mortality of Rice Weevils					
Butoxide	Pyrethrins	Dose, gal./ 1000 bu.	Mort., %	Dose, gal./ 1000 bu.	Mort., %	Dose, gal./ 1000 bu.	Mort., %
2.0	0.2	1.56	32	2.25	72	2.9	89
1.0	0.1	3.12	44	4.50	74	5.8	86
0.5	0.05	6.24	43	9.00	68	11.6	86
0.33	0.033	9.36	42	13.50	75	17.4	88
Untreated control			0				

Treatments within each column are equal in amount of active ingredients applied.

Table V. Mortality of Grain Infesting Insects from Special Oil-Free Emulsifiable Concentrate (T-647)

(14-day exposure, dilution and application rates balanced to maintain same deposit of active ingredients per 1000 bushels. Wheat moisture 13.5%. Kansas State College 1954–1955)

		Mortality, 14 Days, %					
Dilution with Water	Gal./ 1000 Bu.	Rice weevil	Saw-toothed grain beetle	Lesser grain borer	Gran. wvl.	Tribolium	Flat grain beetle
1 : 14	2	74.2	77.8	99.0	84.7	2.0	96.9
1 : 29	4	83	72.0	94.9	91.0	3.0	90.0
1 : 44	6	80.8	60.4	99	78.0	3.1	95.9
1 : 59	8	79.8	78.0	98.9	73.2	3.1	92.9
1 : 74	10	86.2	75.8	100.0	88.0	4.0	96.0
1 : 89	12	73.7	55.6	98.0	68.7	1.1	80.4
Water	6	5.9	4.0	19.3	9.9	1.0	10.0

insects were added, did not give a satisfactory increase in mortality of the insects.

The brief effective period of the oil treatments was probably due to the physical properties of the oil sprays. Soybean oil did not penetrate the seed coat readily but tended to dry on the surface of the kernels in the form of a film which tended to bind the kernels together. This drying action of the oil probably accounts for the short effective life of the treatment. The petroleum-base oil, on the other hand, penetrated the seed coat readily, possibly carrying much of the active ingredient beneath the surface where insects did not readily come into contact with it. In contrast, the emulsions broke on the surface of the grain, after which the water was withdrawn by the grain or the air, leaving the active ingredients deposited on the surface where they were readily contacted by the insects.

Relationship of Concentration and Dosage

Previous work with dry treatments, and preliminary tests with emulsions sprayed directly on grain, showed that within rather wide limits, there was little difference between the effectiveness of high and low concentrations of various formulations, provided equal amounts of active ingredients were evenly applied on the grain. Table IV shows results of such a test in which four emulsions, differing greatly in concentration and prepared from the new oil-free emulsifiable concentrate, were applied at dosage rates to give the same amounts of active ingredients on the grain. Each formula was tested at three dosage rates.

In a similar experiment six different dilutions of the oil-free emulsifiable concentrate were tested with six grain-infesting insects at dosages required to give equal amounts of active ingredients on the grain (Table V). There were no consistent differences in effectiveness of the treatments that could be attributed to the dilutions used. The experiments show that only very small amounts of liquid are needed to effect control of insects in grain, and that there is no advantage in using larger amounts when the material is properly distributed throughout the bulk of the grain.

Conventional and Oil-Free Emulsions

The new oil-free combination of piperonyl butoxide and pyrethrins was compared to the regular petroleum-base material in emulsion sprays at four dosage rates on samples of wheat. The final dilutions of each of the sprays contained 2.0% of piperonyl butoxide and 0.2% pyrethrins; the regular emulsion contained 15.3% of petroleum oil. The results of this comparison (Table VI) show that the oil-free emulsion is fully as effective as the conventional emulsion containing petroleum oil. At rates of 2.92 gallons per 1000 bushels, the mortality from the oil-free spray was 63%, as compared to 65% for the regular emulsion. At 4.5 gallons per 1000 bushels, the regular emulsion gave 92% mortality, while the oil-free emulsion gave 98% mortality. The tests demonstrate that it is possible to spray grain effectively without having petroleum oil present in the sprayed deposits.

Comparison of Two Emulsifiers

Because of the extensive use of emulsifier Atlox 1045A in sprays containing piperonyl butoxide and pyrethrins for a wide variety of needs, other than in sprays on grain, its biological effectiveness was compared to that of emulsifier MYRJ-45, which has been found safe for use by the baking industry. Rice weevils were used as the test insects. Table VII reports the results of these tests, and shows that there is no essential difference between the effects of the two emulsions.

Considering these and other laboratory studies, test results show that MYRJ-45 is highly efficient in the oil-free sprays on grain. Of equal importance is the fact that MYRJ-45 is regularly used as a bread softener by commercial bakers. This use affords distinct evidence of its safety and readily suggests its suitability for use in oil-free sprays containing piperonyl butoxide and pyrethrins.

Effect of Conditions of Application

Different Rates from Single Concentration A dilution of 1 volume of oil-free emulsifiable concentrate (T-647) with 29 volumes of water gives a concentration of 2.0% piperonyl butoxide and 0.2% pyrethrins. This emulsion was tested at four rates of application against six species of grain insects (Table VIII). At 4 gallons per 1000 bushels, the effectiveness against the adult lesser grain borers and the flat grain beetles was satisfactory, but better performance is needed against the other species of insects. At the rate of 5 gallons per 1000 bushels, the mortalities of all insects except Tribolium were 100%. These results do not take into consideration the repellency of the materials, which is a marked factor for all species of grain insects. Tribolium is a secondary invader, and depends upon and thrives primarily on the frass and fragments left by other insects. Moreover, when the primary invaders are not successfully controlled suitable conditions are provided for a build-up of Tribolium in whole grain.

Effect of Screenings Added to Stored Wheat The presence of more than normal amounts of broken kernels and other screenings in stored grain is known to be favorable for the development of insects. It seemed desirable to add known amounts of such screenings to the grain in order to determine the effect upon treatments with the special oil-free emulsifiable protectant. Using an emulsion that contained 2% piperonyl butoxide and 0.2% pyrethrins, rates of 4 and 8 gallons per 1000 bushels were applied to infested grain samples that contained 0, 3, 6, and 9% screenings by weight. In Table IX, it will be noted that the higher rate of application was sufficient to overcome this handicap, but the lower rate of 4 gallons per 1000 bushels was not sufficiently effective when more than 3% screenings were present. This would not be serious in elevators, which normally run under 3%, but in some farm storages it could be a factor of importance. The tests serve as a reminder that grain should be clean when treated, to help prevent a build-up of infestations of grain insects.

Table VI. Mortality of Rice Weevils in Wheat Sprayed with Two Types of Emulsions

(Average of three paired replications by jar method. Each figure represents 300 weevils. Baltimore, Md.)

Active Ingredients[a] in Concentrate, %		Petroleum Oil in Emulsion, %	Dosage Rates, Gal./1000 Bu. Mortality, %			
Butoxide	Pyrethrins		1.56	2.25	2.92	4.5
Oil-free 60	6.0	None	6	48	63	98
Regular 10	1.0	15.3	18	33	65	92
Untreated control				0		

[a] Both concentrates diluted to give emulsions containing 2.0% piperonyl butoxide and 0.2% pyrethrins.

Table VII. Mortalities of Rice Weevils in Wheat, Following Exposures of 7 Days to Spray Treatments of Four Pyrenone Emulsions

(Each spray contained 2.0% piperonyl butoxide and 0.2% pyrethrins, but with different emulsifiers, and were applied at different rates per 1000 bushels. Baltimore, Md.)

Emulsifier	Used, %	Base Oil, %	Dosage Rates Gal./1000 Bu. Mortality, % 1.56	2.25	2.92	4.5	Est. Gal. for Ld_{90}
MYRJ-45	0.67	0	6	48	63	98	3.7
	1.0	1.4	8	34	72	98	3.5
Atlox 1045A	2.45	1.4	12	41	67	96	3.9
	2.5	14.9	18	33	65	92	4.2

Portion of Grain Sprayed It is not expected that a properly applied spray would actually come in contact with a major portion of the individual kernels of grain. Carefully conducted studies made in the laboratory have shown that this is not

Table VIII. Mortality of Grain-Infesting Insects from Special Oil-Free Emulsifiable Concentrate (T-647)

(Diluted 1 to 29 and applied at rates of 4, 5, 6, and 8 gallons per 1000 bushels of clean wheat of 13.5% moisture content. Exposure 14 days. Kansas State College 1954-1955)

Rate Applied, Gal./1000 Bu.	Mortality of Insects, % Rice weevil	Saw-toothed grain beetle	Lesser grain borer	Granary weevil	Tribolium	Flat grain beetle
4	63	78.6	100	69.7	3.9	98.9
5	100	100	100	100	7.0	100
6	100	100	100	97.0	1.0	99
8	100	100	100	100	17	100
Untreated	5.5	9.4	4.5	17.4	2.0	10.5

Table IX. Mortalities of Grain-Infesting Insects from Special Oil-Free Emulsifiable Concentrate (T-647)

(At recommended 1 to 29 dilution, applied at 4 and 8 gallons per 1000 bushels of wheat having 13.5% moisture content, and to which screenings had been added on a per cent by weight basis. Exposure 14 days. Kansas State College 1954–1955)

Amount of Screenings, %	Gal./1000 Bu.	Mortality of Insects, % Rice weevil	Saw-toothed grain beetle	Lesser grain borer	Granary weevil	Tribolium	Flat grain beetle
None	4	63	78.6	99	69.7	3.9	98.9
3	4	66	55.7	96.0	86	4.0	95.8
6	4	47.5	25.8	93.0	60.6	1.0	92.6
9	4	32	18.8	86.6	27.0	2.0	89.8
None	8	100	100	100	100.0	17	100
3	8	100	99	100	100	14.4	99
6	8	98.0	96	98	100	7	99
9	8	93.9	82	100	97	4	98
Water	8	5.5	6.9	5	16.9	1	8.4

necessary. At the usual rates of application (4 to 5 gallons per 1000 bushels), it is not possible to cover the entire surface area of the grain treated. Tests were made on shelled corn in which $^1/_2$, $^1/_4$, $^1/_8$, and $^1/_{16}$ of the test sample was sprayed with the dose required for the entire sample, then returned to and mixed with the unsprayed portion of the sample. The treated portion was sprayed on only one side of the kernels. When tested against rice weevils, a somewhat lower mortality was obtained when only $^1/_{16}$ of the sample was treated. When as much as $^1/_8$ of the sample was treated, the results were fully as good as when the entire sample was treated. In practice, spray should be applied to grain as thoroughly as possible, but it would seem equally important to provide for thorough mixing of the grain after the spray is applied, so that distribution of the treated kernels among the untreated ones is assured. For adequate mixing under conditions of actual use, the spray should be applied to grain at the lower end of the hiker or elevator. This takes full advantage of the auger or tumbler action for obtaining good distribution of the sprayed kernels.

Addition of Water to Grain During early experimental treatments, it was thought that the use of water sprays on grain might be objectionable because of a possible increase in the moisture content of the grain. A more careful consideration of the amounts of moisture added by emulsifiable sprays emphasized that the amount added is of little significance in relation to the amount of moisture usually present in grain—for example, 5 gallons (42 pounds) per 1000 bushels of wheat (60,000 pounds) is equal to 0.07%, a negligible amount that is soon adjusted through normal evaporation.

Summary and Conclusions

Tests made in the laboratory with sprays containing combinations of pyrethrum and piperonyl butoxide applied directly to grain showed that: (1) Water emulsions were more satisfactory and more lasting in their effect than sprays containing petroleum oil or vegetable oil as diluents. (2) Emulsion sprays were of the same order of effectiveness as dry protectant powders. (3) The concentration of an emulsion used to obtain a given deposit of active ingredients is not important. (4) Emulsions prepared from oil-free concentrates were as effective as those prepared from conventional concentrates containing petroleum oil. (5) The emulsifier MYRJ-45 was as effective biologically as Atlox 1045A. (6) Treatments were more effective when applied to screened grain than when applied to grain containing screenings. (7) An oil-free emulsion containing 2% of piperonyl butoxide and 0.2% of pyrethrins applied at the rate of 5 gallons per 1000 bushels of grain was adequate for the protection of stored grain.

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PESTICIDE SAFETY EVALUATION

Toxicity of Trinitrobenzene-Aniline Complex, a Rodent Repellent

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Complexes of trinitrobenzene have been suggested as promising rodent repellents by the work of Welch and Duggan (*6*) and Welch (*5*). One such repellent is the trinitrobenzene-aniline complex (TNBAC), which was proposed for use in orchards and shelterbelt plantings to decrease the damage from rabbits during the winter months when feed becomes scarce. If solutions of TNBAC are either painted or sprayed on the trunks of trees in the fall, one application is effective throughout the dormant season. With this type of application the hazard to personnel handling the material is primarily one of dermal contact, and time of exposure is relatively brief.

Compounds of the nitrobenzene series are known as potent methemoglobin formers and may produce an effect on the blood-forming organs, especially the bone marrow (*2*, *4*). Aniline is a potent methemoglobin former and is considered very toxic in pure solution (*2*). The chemistry of the complex of trinitrobenzene (TNB) and aniline is not fully understood; however, some of the physical characteristics of the individual components are not found in the complex.

DeWitt, Welch, and Bellack (*1*) have published criteria for a rodent repellent which include the requirement of safety for use. In preliminary tests at another laboratory where determinations of oral minimum lethal dose and studies of eye and dermal irritation were conducted, TNBAC was shown to have a low order of toxicity. Detailed toxicological studies, including comparison with TNB, were undertaken at Hazleton Laboratories to evaluate the safety of TNBAC more extensively.

Oral Administration to Albino Rats

Procedure Male albino rats, weighing between 100 and 170 grams were intubated with a 5.0% weight per volume suspension of anhydrous TNBAC or TNB in 0.5% methylcellulose and observed for 7 days. Signs of toxicity were noted, and mortality was recorded daily. All survivors were sacrificed, and gross autopsies were performed at the completion of the study. The acute oral