FIELD AND LABORATORY STUDIES OF OIL BASE LIVESTOCK SPRAYS

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By

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PREFACE

Livestock sprays or cattle sprays consist of a large variety of spray insecticides designed for use about the barn and dairy or for direct application to the bodies of animals. Due to the lack of standardization and grading, many of the sprays are cheaply made household insecticides.

In an effort to develop a satisfactory spray the Continental Oil Company established a research fellowship in the Department of Entomology, Oklahoma Agricultural and Mechanical College.

The writer wishes to express his appreciation to the following who aided in this study: Dr. F. A. Fenton, Head of the Department of Entomology, under whose supervision this research was conducted; Dr. Bert H. Lincoln and other officials of the Continental Oil Company for advice and suggestions during the entire research program; Professor H. W. Cave and Mr. N. N. Robb, of the Dairy Department, who provided the animals, pasturage, and the fine cooperation that made possible the field experiments; Dr. T. T. Milby, of the Poultry Husbandry Department, who aided in the evaluation of the data; Dr. D. E. Howell, of the Department of Entomology, for his timely suggestions and planning; and Mr. W. D. Garrett, student in the Department of Entomology, who assisted in the field experiments.

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INTRODUCTION

Field and laboratory experiments to determine the relative values of various insecticidal and repellent materials against the common species of blood-sucking flies, namely: the stable fly, Stomoxys calcitrans (L.), and the horn fly, Haematobia irritans (L.), were conducted at Stillwater, Oklahoma, over the period January 1, 1940, to January 1, 1941.

In the laboratory the toxicity of the various materials was evaluated by the Peet-Grady method (27) while the repellency of these materials was studied by means of a modified method of field repellency testing based on the one-half cow method in which fly counts were made on cattle in the field. This research was endowed by the Continental Oil Company and was carried out under the direction of Dr. F. A. Fenton, Head of the Department of Entomology, Oklahoma Agricultural and Mechanical College. The aim of this research was to develop an efficient and safe livestock fly spray.

The results of this research are discussed in this thesis under several sections, namely: tests for repellency in the field; the effect of temperature and host susceptibility on the abundance of stable flies and horn flies on cattle; materials used in the repellency tests; the evaluation of the materials and formulae used in the field repellency tests; the effect of oil sprays on the body temperature of cows; and a laboratory test procedure for the evaluation of the toxicity of livestock sprays.

Livestock spray formulae at the present time usually consist of a petroleum base in which is incorporated a toxic material or a combination of toxic materials, such as pyrethrum, derris, or synthetic toxicants.

In the development of a livestock spray formula several factors must be studied. The petroleum base is the carrying agent for the toxic and/or repellent materials, and in most cases if it be a proper base it is the chief repelling agent. Sherrick (33), in his review of the standards for livestock sprays, states that the consensus of opinion seems to favor a neutral oil with a viscosity of from 45-55 Saybolt seconds at 100° Fahrenheit for stock spray bases. According to the above author light oil of viscosity 30-35 Saybolt seconds, such as those used in household sprays, is likely to cause burning of the animal's hide because of its solvent and penetrating powers. On the other hand a very heavy oil of high boiling point may cause trouble by blanketing the animal's hide for too long a time. Investigations on the proper type of oil base for fly sprays have been few in number, but real information has been advanced by several investigators, as follows: Fremd (13) concluded that gasoline mixtures were not practicable in fly sprays; Pannewitz (22) stated that the selection of a fly spray base should be based on the Riesenfeld-Bante figures which he lists; Richardson (29) gave a report on studies of mid-continent distillates as bases for pyrethrum extracts in which he lists the toxicity of the distillate at the different boiling ranges; Searls and Snyder (31) reported on the relation of viscosity to drop size and the application of oils by atomization; a proper base for insecticides was listed by Weingard (34).

The toxicity of various insecticidal ingredients in their oil bases is determined by means of the Peet-Grady method of laboratory testing. This method was developed by C. H. Peet and A. G. Grady (26) in 1928, and was revised by Peet (27) in 1932. The Peet-Grady method with various modifications, as given in the Annual Blue Book of Soap and Sanitary Chemicals (2), 1939, is the official method adopted by the National Association of Insecticide and Disinfectant Manufacturers for evaluating liquid household insecticides.

Since there has been no satisfactory method for evaluating livestock sprays, the Peet-Grady method has been applied in the testing of livestock sprays in the laboratory to determine knockdown and kill. This method is not entirely satisfactory due to the lower volatility of the heavier oil bases of the livestock spray types.

The principal toxic elements incorporated in livestock sprays are derris, rotenone, pyrethrum, and organic thiocyanates. Derris and/or rotenone are generally used in combination with pyrethrum. Rotenone in fly sprays was investigated by Badertscher (3). The efficiency of kerosene extracts of derris alone and in combination with kerosene extracts of pyrethrum, against Musca domestica (L.), was compared by Richardson (30), in 1933. Campbell et al (5) conducted comparative tests of derris and pyrethrum. From the dosage mortality curves of pyrethrum sprays on Musca domestica (L.), Hoyer et al (16) found that above 75 mg. of pyrethrum per 100 cc. the toxicity increases, and below this point toxicity decreases. Richardson (28) reported on the insecticidal estimation of the kerosene extract of pyrethrum. The relative toxicity of pyrethrins and rotenone as fly spray ingredients was brought out in the work of Gnadinger and Corl (14). Lethane 384, an organic thiocyanate, in petroleum distillate was compared with pyrethrum and rotenone in petroleum distillate by Murphy and Vandenberg (19) with the results indicating a speedier and more complete knockdown in the case of Lethane 384. Hartzell and Wilcoxon (15) conducted chemical and toxicological studies on organic thiocyanates. A progress report on the investigations of aliphatic thiocyanates as contact insecticides was made by Creighton et al (7).

Petroleum oils are known to have some toxic action on insects. Nelson (21) in investigating a commercial fly spray studied the penetration of a contact oil into the tracheal system of insects. Burdette (4) found that oil droplets from 1 to 10 microns at a concentration of 0.33 cubic centimeter per cubic foot of air increased the temperatures of honeybees and finally caused their death.

The physiological effects of fly sprays on cattle have been studied to some extent. Freeborn et al (9, 10) studied the relation of flies and fly sprays to milk production. They listed the loss in milk production due to the various species of flies. Melvin (18) carried out physiological studies on the effect of flies and fly sprays on cattle and found that both air temperature and the intensity of the sun influenced the rise in body temperature of oil-sprayed cows. Wilson et al (35), 1933, in studying the effect of 12 repellent sprays on the hides and body temperature of dairy cows concluded that the hand sprayer was the best applicator as the oil did not penetrate and that body areas exposed to direct sunlight were most injured. He further concluded that oils of viscosities of 45-55 seconds caused no injury.

In Soap and Sanitary Chemicals (1), a test of the degree of skin irritation of eight commercial-stock sprays was made by the use of white rats and a spot test technique. These sprays were evaluated as to the extent of injury caused when applied to the rats' skin.

A material that will repel flies is desirable in a livestock spray formula; however to date there has been little research on "biting fly" repellent materials, and those that have been advanced are inefficient. There are several methods to test the degree of repellency of a material to flies. In the laboratory an olfactometer of various designs may be used. A newly proposed method of sandwich bait to test the degree of repellency was developed by Kilgore (17), who used the chemical citronella as a standard insectifuge. Doty (8) devised a comparative test for the degree of repellency of materials by using bait to which was added the spray to be tested. Laboratory techniques, however, are not satisfactory in livestock spray repellency testing. The test insect is the common house fly while the flies to be repelled in the field are of a different species, namely, those with piercing and sucking type of mouth parts. The animals themselves are a limiting factor in regard to laboratory testing for to date it has not been possible to devise a technique which will simulate in the laboratory the attracting qualities of an animal to blood-sucking flies.

Due to the fact that laboratory methods for repellency testing have not been satisfactory, field tests are necessary at least as a final proof of findings. These field tests do have drawbacks, in that they require a herd of cattle and summer weather and therefore place a limitation on investigations.

In a discussion of cattle spray tests, Doty (8) has given an excellent review of the literature on the methods of repellency testing. Cleveland (6) used individual herds applying a different material to each herd and noting the general results. Later actual counts improved this technique. Pearson et al (23) stated that by taking into consideration the normal fly susceptibility of each animal still more consistant results could be obtained. They determined this by taking three-day fly counts prior to the actual spraying and then grouping the animals according to their susceptibility. It was also stated that it was satisfactory to have the cows run free. Modifications of this method were given by Pearson (24), who used this modified procedure in his study of the role of pine oil in cattle fly sprays (25). This modified method differed in that the base oil used in making the sprays was sprayed on cows four days previous to the application of the actual spray. Pearson evaluated the efficiency of the material in question by a comparison of the counts of these two periods. Freeborn and his co-workers (9) were the first to use screened stalls. Freeborn and Regan (10) made comparisons of sprayings in the barn with those in the corral. Marked differences were noted in the counts taken in these two places. They also noted their results were influenced by the species of fly involved. Nelson (20) ran tests both indoors and in the field and mentioned that fly susceptibility of the animals was taken into consideration. He found that an electric sprayer gave better results and less injury than a continuous-type hand sprayer. In his report on the effect of flies and fly sprays on cattle, Melvin (18) used outdoor screened pens and reared and trapped stable and house flies. Dr. H. H. Shepard, of the University of Minnesota, was probably the first one who sprayed one side of the cow using the other side as a control, according to the report of Doty (8). This method was used in the work reported by the R. J. Prentiss Company, in Soap and Sanitary Chemicals (1). Doty (8) used a modified method after Shepard, in which he sprayed one half of the cow including the neck, belly, and legs, while the other half was blanketed with canvass and used as a check. The sprayed animals were staked in the pasture and hourly fly counts were taken over a period of eight hours. Three-day records were replicated three times.

Searls and Snyder (31, 32) discussed the composition of sprays and their application and stated that sprays with a refined kerosene base and enough insecticide to kill flies when hit are effective in keeping cows free from flies during milking time.

TESTS FOR REPELLENCY IN THE FIELD

Field Technique: Procedure

Field repellency tests were conducted at Stillwater, Oklahoma, over the period June 11 to September 13, 1940. Cattle of the Ayreshire, Jersey, and Holstein breeds were used as test animals. After an early morning feeding (Fig. 2), the animals were led to the spraying station and sprayed (Fig. 3). Spraying equipment consisted of a Vestal automatic electric sprayer to which was attached a shell vial in which an exact measurement of spray material was placed (Fig. 4). These vials were kept in a container provided with slots corresponding to the number of the animal (Fig. 5). This provided a more exact and more speedy spraying of the cows. The method of spraying was a modification after Doty's (8) one-half cow technique. One side of the animal was sprayed with toxicants and/or repellents in base oil; the opposite side was sprayed with the base oil alone. A test series was carried out for a minimum of two days so that on the second day of the test, or on alternate days, the base oil, and toxicant-repellent sides of the animal could be reversed. This was done to offset the effect of position of the sprays in regard to the movements of the cow in its orientation to external conditions, such as the prevailing winds or sunlight, and to evaluate their effect on the distribution of the fly population. An equal amount of material, namely 15 ml., was sprayed on each side of the animal, making a total of 30 ml. of spray material per animal. The neck, belly, and legs were sprayed, as well as the sides of the animal. After the animal was sprayed it was staked out in the pasture on a 20-foot tether rope to provide ample grazing. The animals were staked 50 feet apart in two lines (Fig. 6). Fourteen animals were used in the field experiments, 12 being arranged in two strings of six animals each, two animals in each string being sprayed with like materials. Two animals were not sprayed and were used to indicate the fly population trend during the testing period. Fly counts were made simultaneously by two observers at half-hour intervals at which time the species and number of flies were noted for each side of each animal.

Rectal temperatures were taken at 10 a.m., 11 a.m, and 1 p.m., with the initial temperature being taken at the time the animal was sprayed, which was about 8 a.m.

At the conclusion of the testing period, which was between 8 a.m. and 2 p.m., each animal was washed with soap and water (Fig. 7) to remove the oil and toxic-repellent materials so that a residue would not be left to enter into the results of the second day of the test series.

The prevalent species of flies were the stable fly, *Stomoxys calcitrans* (L.), and the horn fly, *Haematobia irritans* (L.). A few individuals of Tabanus species were observed as well as some Culicine mosquitoes, but these did not enter into the counts because they did not occur in numbers.

Figure 2
Animals Feeding in the
Holding Pen

Figure 3
Spraying Station

Figure 4
Electric Sprayer with
Shell Vial Attached

Figure 5
Container for Shell Vials

Figure 6
Cows Staked in Pasture

Figure 7
Cows Being Washed at the
End of the Testing Period

THE EFFECT OF TEMPERATURE AND HOST SUSCEPTIBILITY ON THE ABUNDANCE OF STABLE FLIES AND HORN FLIES ON CATTLE

The comparative abundance of stable flies and horn flies on the testing herd was studied during the period June 11, to September 13, 1940, inclusive. It must be pointed out that the records on fly abundance were taken from 14 animals in one pasture and it is not definitely known if this pasture is representative of this area, so far as the abundance of these flies was concerned. Further, the fly counts were made during but a portion of the day, namely, 8 a.m to 2 p.m. These data do, however, give an index of the fly abundance in this pasture which was necessary to evaluate the effect of fly population on the repellency tests.

Figure 1 shows the average number of each of the two species of flies observed per animal for each testing day, the mean temperature for that portion of the day during which observations were made, namely, 8 a.m. to 2 p.m., and rainfall. The curve representing fly population was drawn from a moving three-point average. Due to interruptions in field observations, fly populations are shown in four separate periods rather than as a continuous record.

Comparative Abundance of Both Species.

On June 11, when the tests were begun, there was an average of 46 stable flies per animal. This was the point of greatest abundance of this species in the first period and in fact for the entire season. Figure 1 shows that at no other time was the stable fly as numerous as it was early in June. In the first period an average of 67 horn flies per animal on June 11 was also the high point of the abundance of this species for the season. In contrast to the stable fly, however, this species occurred in large numbers at several other times during the period of the study.

The Effect of Temperature on Fly Abundance.

General field observations showed that during the period when these observations were made, stable flies appeared on the cattle at a later hour than did the horn flies. As a rule stable flies appeared in numbers after 9 a.m. when there was a general increase in temperature. Later in the morning, about 11 a.m., the number of stable flies decreased. This was due at least in part to a further rise in temperature. This relationship is shown in Table 1, which shows the comparative abundance of the stable fly throughout the season's experiments on the unsprayed cows in relation to temperature. This table shows the optimum temperature range for the stable fly in relation to its abundance on the unsprayed cows was from 81° F. to 88° F.

Field observations indicate that the horn flies occurred in numbers early in the morning during the period when these observations were made. Table 2 shows that in this experiment this species reached its greatest abundance at a temperature range of 70° F. to 78° F. However, large numbers were present on the animals up to temperatures as high as 97° F.

While these data are concerned only with the fly population occurring on unsprayed animals, a similar study involving the total population occurring on all animals including those which were sprayed showed a similar trend. The observational period did not include the cooler temperatures before 8 a.m. or in the evening.

Susceptibility[1] of Cattle to Stable Flies and Horn Flies.

The fly population on unsprayed animals was studied over a five-day

1 The term "susceptibility" here implies the comparative attractiveness of cows to flies.

period, namely July 22 to 26, inclusive, to determine the individual susceptibility to Stomoxys calcitrans (L.) and Haematobia irritans (L.) and also to determine whether the unsprayed animals should be used as checks for the base oil and toxic-repellent materials in repellency tests. A total of 560 fly counts was made at one-half hour intervals on 14 animals. The observational totals for each of the fly species on the individual animals are shown in Table 3.

The observational totals of the two species on 14 animals for the five-day period were 6,928 stable flies and 31,115 horn flies or a ratio of approximately one stable fly to five horn flies. The lowest population of stable flies occurred on animal No. 47, on which a total of 347 flies were observed while animal No. 106 attracted the largest number, namely, 666. The difference between these two extremes was 213 flies and the mean population for the entire group was 507 flies. The range in the numbers of horn flies on the several animals is much greater. The difference in the extremes of population ranged from 276 flies on animal No. 120 to 5,566 flies on animal No. 106, or a difference of 5,290 flies. The mean population of the horn flies was 2,645.

From these results it was determined that due to the extreme variation in the animals' susceptibility to the two fly species and additional evidence that an animal's susceptibility to flies varied at different periods, it was impossible to use unsprayed animals as checks in determining the effects of fly sprays.

Figure 1. The Comparative Abundance of Stable Flies and Horn Flies on Cattle; Mean Temperature; and Rainfall. Stillwater, Oklahoma, June-September 1941

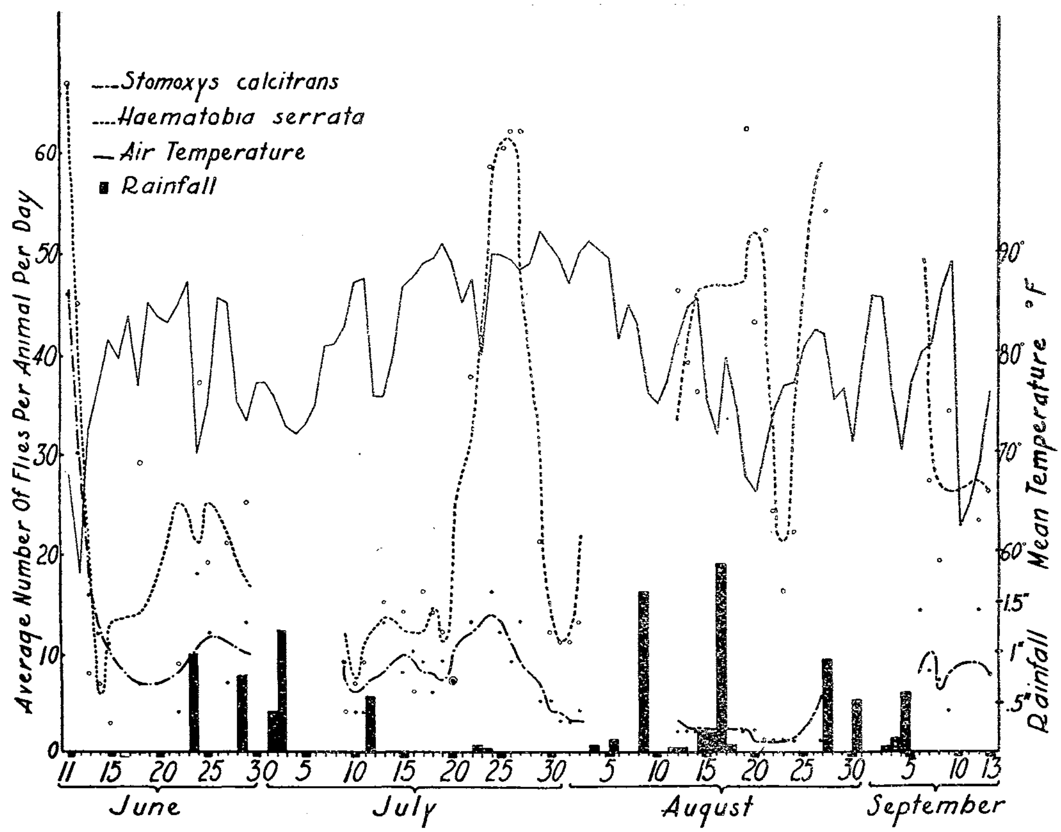

Table 1.--Effect of Temperature on Comparative Abundance of Stable Flies on Unsprayed Cows between 8:00 a.m. and 2:00 p.m. Stillwater, Oklahoma, June-September, 1940

Temperature in Degrees Fahrenheit	Total Number of Flies Observed	Number of Observa-tions	Number of Flies Observed per Observation		
			Maximum	Minimum	Mean
56	2	2	1	1	1.0
60	1	2	1	0	.5
62	0	2	0	0	0
64	1	4	1	0	.25
68	40	8	25	0	5.0
69	17	2	11	6	8.5
70	102	18	50	0	5.7
71	88	30	30	1	14.7
72	162	19	50	0	8.7
73	40	6	23	0	6.7
74	240	22	55	0	10.9
75	93	9	40	0	10.3
76	201	24	40	0	8.4
77	90	9	20	1	10.0
78	161	19	40	0	8.5
79	39	6	15	0	6.5
80	180	32	30	0	5.6
81	126	9	31	1	14.0
82	384	34	41	0	11.3
83	42	8	14	0	5.25
84	768	53	47	0	14.5
85	355	25	38	0	14.2
86	1091	83	44	0	13.1
87	860	61	59	0	13.2
88	1429	98	55	1	15.6
89	467	47	31	0	9.9
90	956	89	35	0	10.7
91	162	20	15	1	8.1
92	1058	105	40	0	10.0
93	400	57	37	0	7.0
94	346	86	19	0	4.0
95	174	40	10	0	4.4
96	252	55	15	0	4.6
97	60	16	8	0	3.8
98	20	10	7	0	2.0
99	1	2	1	0	0.5
100	6	4	2	0	1.5
101	1	2	1	0	0.5
TOTAL	10415	1094	953	12	

Table 2.--Effect of Temperature on Comparative Abundance of Horn Flies on Unsprayed Cows between 8:00 a.m. and 2:00 p.m. Stillwater, Oklahoma, June-September, 1940

Temperature in Degrees Fahrenheit	Total Number of Flies Observed	Number of Observations	Number of Flies Observed per Observation		
			Maximum	Minimum	Mean
56	83	2	50	33	41.5
60	114	2	80	34	57.0
62	77	2	42	35	38.5
64	200	4	75	30	50.0
68	416	8	100	10	52.0
69	240	2	200	40	120.0
70	1123	18	150	20	62.4
71	707	6	250	21	116.8
72	1388	19	300	7	73.1
73	526	6	375	18	87.7
74	1759	22	325	18	80.0
75	619	9	300	7	68.8
76	1943	24	200	4	81.0
77	343	9	100	5	38.1
78	1362	19	300	3	71.7
79	249	6	53	3	41.5
80	1820	32	300	1	56.9
81	303	9	60	2	33.7
82	1746	34	200	1	53.7
83	311	8	90	7	38.9
84	2483	53	170	1	46.9
85	1535	25	300	7	61.4
86	4678	83	430	2	53.4
87	3021	61	300	1	49.5
88	4760	98	300	2	48.5
89	2383	47	250	1	50.7
90	4278	89	205	1	48.1
91	896	20	260	1	44.8
92	4297	105	200	1	40.9
93	2605	57	250	0	45.7
94	3345	86	250	0	38.9
95	1419	40	164	0	35.5
96	2227	55	140	0	40.5
97	911	16	285	1	56.9
98	30	10	6	0	3.0
99	3	2	2	1	1.5
100	53	4	20	10	13.3
101	30	2	17	13	15.0
TOTAL	54283	1094	7099	341	

Table 3.--Comparative Susceptibility of Different Cows to the Stable Fly, Stomoxys calcitrans (L.), and the Horn Fly Haematobia irritans (L.).

	Stomoxys calcitrans (L.)	Haematobia irritans (L.)	
Animal	Observational Totals	Animal	Observational Totals
106	666	106	5,566
44	583	116	4,810
107	573	19	4,163
19	531	44	3,153
64	530	64	2,739
59	528	107	2,593
14	521	30	1,839
116	519	59	1,719
120	472	54	1,699
54	457	47	1,180
73	432	14	590
23	409	23	409
30	360	73	379
47	347	120	276

MATERIALS USED IN THE REPELLENCY TESTS

The materials used in the field repellency tests are listed as to their chemical composition and were supplied by the commercial companies producing these materials.

The Base Oil.

The livestock base oil used in this research was developed by the Continental Oil Company, of Ponca City, Oklahoma. An analysis of this oil lists the following: I. B. P. 484 F, Endpoint 714 F; Gravity A. P. I. 34.7; Viscosity S. S. U. at 100 calculated from modified Ostwald 45.9; Unsaturates 6 percent.

Pyrethrum.

Pyrocide 20, Deodorized-Clarified, a product of McLaughlin-Gormley-King Company, Minneapolis, Minnesota, is guaranteed to contain 2.0 grams pyrethrins per 100 cc. (Seil Method), equivalent to 2.5 percent pyrethrins by weight. It is stabilized and standardized pyrethrum concentrate containing a special antioxident used to prevent deterioration of the pyrethrin content; it is made up with a special deodorized mineral base called Deo-Base, and is clarified by means of chilling to remove any of the nontoxic resins extracted from pyrethrum flowers.

Pyrin is the trade name of the pyrethrum concentrate of John Powell and Company, New York, New York. Pyrin contains .8 grams (.96 percent) pyrethrins and 8.4 grams (10.4 percent) normal-isobutyl-undecylenamide, a small percentage of pyrethrum extractives other than pyrethrins, and the balance being oil falling in the kerosene boiling range 350°-500° F.

Derris-Rotenone.

Protex No. 10 L. S. is a concentrate manufactured by the Whitmire Research Corporation, of St. Louis, Missouri, containing from 1.0 gram to 1.25 grams per 100 cc. of Derride ($C_{20}H_{16}O_6$ M.P. 163 C.), Toxicarol ($C_{23}H_{22}O_7$ M.P. 232 C.), Tephrosin ($C_{23}H_{22}O_7$ M.P. 198 C.), Deguelin ($C_{23}H_{22}O_6$ M.P. 171 C.), along with traces of rotenone and semi-volatile substances of Derris resins.

Derex Standard and Derex Rotenone Free are products of the U. S. Industrial Chemicals Company, New York City, New York. Derex Standard contains 1.7 percent rotenone, 9.4 percent other ether extractives. The balance is Dihydropyrone (butyl-mesityl oxide oxalate). Derex Rotenone Free contains 15 percent ether extractives of Derris (other than rotenone), and the balance is Dihydropyrone (butyl-mesityl oxide oxalate).

Chemical No. 325 is a product of the United States Rubber Company, Passaic, New Jersey. Chemical No. 325 is a 5 percent solution of derris resinate in Chemical No. 96 (halogenated hydrocarbon produced synthetically).

Thiocyanates.

Lethane 384 and Lethane 384 special are compounds of Röhm and Haas Company, Philadelphia, Pennsylvania. Lethane 384 is a solution of beta butoxy betá thiocyano diethyl ether standardized at 50 percent by volume with a highly refined hydrocarbon oil. Lethane 384 special is a mixture of organic thiocyanates including beta butoxy betá thiocyano diethyl ether and the thiocyano ethyl esters of higher fatty acids standardized at 50 percent thiocyanate content by volume with a highly refined hydrocarbon oil.

Other Insecticide Compounds.

K-58 and K-383 are compounds of the Dow Chemical Company, Midland, Michigan. K-58 is a technical grade of B- (p-ter. butyl phenoxy) ethanol. K-383 is a technical grade of B- chloro B- (2,4,6- trichlorophenoxy) diethyl ether.

Essenol No. 183 A and Protessenol are compounds of Dodge and Olcott Company, New York, New York. Essenol No. 183 A has the approximate composition as follows: Tertiary Alcohols, 15 percent; Secondary Alcohols, 21 percent; Phenol Ethers, 39 percent; and Cyclic Ketones, 25 percent. Protessenol C. S. Concentrate is a combination material. It contains one-third of the Essenol No. 183 A and two-thirds of a Derris extract (Protex Derris).

Chemicals No. 15, No. 96, No. 250, No. 102, and No. 461 are products of the United States Rubber Company, Passaic, New Jersey. Chemicals No. 15 and No. 96 are halogenated hydrocarbons produced synthetically. The exact chemical structure is not known. Chemical No. 250 is seed Cedrus Oil Atlantica. Chemical No. 102 is dimethyl cyano pyrroline. Chemical No. 461 is rubber distillate.

Pine Oil is a steam-distilled pine oil.

EVALUATION OF THE MATERIALS AND FORMULAE USED IN THE FIELD REPELLENCY TESTS.

The several materials and formulae have been evaluated as to their repellency to stable flies and horn flies by the statistical analysis of variance.[2] The technique for the repellency testing has been described under the heading "Testing for Repellency in the Field". The total fly population occurring on all animals sprayed with like materials at a given fly count period has been used in the calculations; the base oil has been compared with the unsprayed sides of the animals. All other materials and formulae have been compared with the base oil and the data represent the difference in the number of flies occurring on the check or base oil side

2 G. W. Snedecor, Statistical Methods, pp. 171-197, Collegiate Press, Inc., Ames, Iowa, 1937.

when compared with the test or toxic-repellent sprayed sides of the same animals. In the case of the base oil compared with the unsprayed sides, the base oil is the test side and the unsprayed sides are the check sides.

In this statistical analysis, account has been taken of the variation in the number of flies between days and the variation in the number of flies between the fly counts.

The Statistical Analysis of Variance: Example

(The difference in the number of stable flies occurring on the unsprayed sides of the animals when compared with the base-oil sides of the same animals.)

Counts	Aug. 12	Aug. 13	Aug. 14	Aug. 19	Aug. 20	Aug. 21	Total
1	5	9	8	5	-1	0	26
2	11	9	15	4	4	7	50
3	4	4	0	-1	0	4	11
4	12	9	5	-1	-1	1	25
Total	32	31	28	7	2	12	112

Sum of squares = 1006

Correction Term = $\frac{(\text{Sum}^2)}{N}$ = 522.67

Sum of squares between days = $\frac{\text{Sum}^2 + \text{Sum}^2}{N \text{ (counts)}} = \frac{(32^2 + 31^2 \text{-------} + 12^2)}{4} = 741.5$

Sum of squares between counts $\frac{\text{Sum}^2 + \text{Sum}^2}{N \text{ (days)}} = \frac{(26^2 + 50^2 \text{-----} + 25^2)}{6} = 653.67$

Sum Squares Error = Total Sum Squares - (Sum Squares between days + Sum Squares between counts) = 133.5

Mean = 4.65 ± .61 $(\sigma M = \frac{\sigma}{\sqrt{N}} \quad \frac{2.98}{\sqrt{24}} = .61)$

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	Standard Deviation
Total	23	483.33	21.01	4.58
Bet. Days	5	218.83	43.77	
Bet. Counts	3	131	43.67	
Discrepence	15	133.5	8.9	2.98

The actual t value of 7.65 is much greater than that t value at 15 degrees of freedom necessary to place the results in the 1 percent level which is 2.947; therefore, the base oil at two hours was highly significant in repellency to the stable fly over the unsprayed sides.

Table 4 lists the formulae showing a significant repellency over the base oil at two, three, and five hours after the cows were sprayed. This table also compares the repellency of the base oil with that of the unsprayed sides of the animals. With the exception of the Continental base oil, the formulae show a significant repellency to only one fly species. The formulae showing a highly significant repellency to the stable fly for a period of five hours were the Continental base oil at 100 percent and the formula Derex Rotenone Free 0.5 percent - Dihydropyrone 7 percent - Pyrocide 20 5 percent - Base oil 87.5 percent.

The formula U. S. Rubber Chemical No. 15 5 percent - Base oil 95 percent shows a significant repellency to the stable fly for five hours.

Continental base oil shows a highly significant repellency to the hornfly for a period of five hours after spraying. The formulae Dow K-58 3 percent - Pyrocide 20 5 percent - Base oil 92 percent, and Derex Rotenone Free 0.5 percent - Dihydropyrone 7 percent - Pyrocide 20 5 percent - Pine Oil 5 percent - Base oil 82.5 percent, show a highly significant repellency to the horn fly at three hours and a significant repellency to the horn fly at two and five hours after the animals were sprayed.

The following formulae show a significant repellency to the horn fly for two hours: Lethane 384 5 percent - Base oil 95 percent; Pyrocide 20 5 percent - Base oil 95 percent; Pyrin 5 percent - Base oil 95 percent.

Table 4.--Comparative Repellencies of Different Cattle Sprays Against the Stable Fly, (*S. calcitrans*), and the Horn Fly, (*H. irritans*), as Determined by Analysis of Variance, Stillwater, Oklahoma, 1940

Materials - Formulae		*Stomoxys calcitrans* (L.) 2 Hours Mean	3 Hours Mean	5 Hours Mean	*Haematobia irritans* (L.) 2 Hours Mean	3 Hours Mean	5 Hours Mean
Continental Base Oil		4.67 ± .61**	3.36 ± .6**	2.92 ± .61**	129.75 ± 7.0**	103.28 ± 8.09**	85.85 ± 30.93**
Derex Rotenone Free Dihydropyrone Pyrocide 20 Base Oil	0.5% 7.0% 5.0% 87.5%	6.5 ± 1.25**	5.38 ± 1.02**	4.09 ± .85**	2.25 ± 3.06		
U. S. Rubber No.15 Base Oil	5.0% 95.0%	3.13 ± .93*	2.83 ± .74*	2.38 ± .75*	2.5 ± 1.62		
Dow K-58 Pyrocide 20 Base Oil	3.0% 5.0% 92.0%	5.67 ± 3.67			2.0 ± .75*	2.06 ± .54**	1.67 ± .75*
Derex Rotenone Free Dihydropyrone Pyrocide 20 Pine Oil Base Oil	0.5% 7.0% 5.0% 5.0% 82.5%	4.75 ± 2.16			2.25 ± .44*	2.83 ± .65**	2.19 ± .69*
Lethane 384 Base Oil	5.0% 95.0%	.35 ± .56			2.55 ± .87*	2.4 ± 1.06*	1.95 ± 1.73
Pyrocide 20 Base Oil	7.0% 93.0%	1.88 ± 1.1			1.54 ± .68*	.33 ± .5	
Pyrin Base Oil	5.0% 95.0%	.25 ± .51			4.83 ± 1.94*	7.67 ± 6.35	

*Significant
**Highly significant

The following spray formulae did not show a significant repellency to either of the two fly species over the base oil at two hours.

Lethane 384, 3 percent; Lethane 384 special, 1 percent; Pyrocide 20, 2 percent; Continental Base Oil, 94 percent.

Pyrocide 20, 5 percent; Continental Base Oil, 95 percent.

Lethane 384 special, 5 percent; Continental Base Oil, 95 percent.

Dow K-383, 3 percent; Continental Base Oil, 97 percent.

Derex Standard, 0.5 percent; Dihydropyrone, 7 percent; Lethane 384, 3 percent; Continental Base Oil 89.5 percent.

Derex Standard, 0.5 percent; Dihydropyrone, 7 percent; Lethane 384, 3 percent; Pine Oil, 5 percent; Continental Base Oil, 84.5 percent.

Derex Standard, 1 percent; Dihydropyrone, 0.5 percent; Lethane 384, 3 percent; Continental Base Oil, 95.5 percent.

U. S. Rubber Chemical No. 250, 5 percent; Continental Base Oil, 95 percent.

Essenol No. 183 A, 3 percent; Protex Derris, 6 percent; Lethane 384, 1 percent; Continental Base Oil, 90 percent.

U. S. Rubber Chemical No. 325, 5 percent; Continental Base Oil, 95 percent.

U. S. Rubber Chemical No. 96, 5 percent; Continental Base Oil, 95 percent.

U. S. Rubber Chemical No. 102, 5 percent; Continental Base Oil, 95 percent.

U. S. Rubber Chemical No. 102, 1 percent; (Lethane 384, Base Oil 1-20) 99 percent.

U. S. Rubber Chemical No. 461, 1 percent; (Lethane 384, Base Oil 1-20) 99 percent.

U. S. Rubber Chemical No. 461, 5 percent; Continental Base Oil, 95 percent.

Pine Oil, 10 percent; Continental Base Oil, 90 percent.

Protessenol, 9 percent; Lethane 384, 1 percent; Continental Base Oil, 90 percent.

THE EFFECT OF OIL SPRAYS ON THE BODY TEMPERATURE OF COWS

One of the important points to be considered in the study of a livestock spray is the effect of that spray on the animal's body temperature. Previous experiments have brought out several important points in regard to the effect of oil sprays on the body temperature of dairy cows.

The oils used by Melvin (18) were of viscosity 43 sec. and 41 sec. at 100° F. These oils when sprayed at a rate of 50 ml. per cow twice daily for a period of one week caused no noticeable rise in the sprayed cow's body temperature over that of the controls when tested in stalls in a milking barn at inside air temperature of 27° C. or when staked out in the field at air temperature above 90° F. The rectal temperatures were recorded at two-hour intervals day and night during the inside trials and at two-hour intervals during the daylight hours in the field trials. In the field trials four groups of producing cows, one group of dry cows, and two groups of 18-month old heifers were used. In a test for the margin of safety, heifers, although sprayed with an excess amount of oil (200 ml.) daily for one week, showed no difference in the average body temperatures from those of the controls and there was but 0.4° F. difference in average body temperature in two groups of moderately producing cows sprayed in like manner. The air temperature ranged from 71.6° F. to 79.2° F. An equal number of animals were used as controls.

Wilson (35) found that the body temperatures of dairy cows sprayed at 6:00 a.m. with 60 ml. of petroleum oils of varying viscosity and degree of refinement were slightly higher during the four-day trial with the average air temperature at 86° F. The increase in the body temperature of the sprayed cows was obtained by comparing the hourly temperature of the unsprayed cows with the same cows when sprayed.

Freeborn (11) conducted experiments in a psychrometric chamber on two producing cows of the same lactation period. The maximum environmental temperature known as the pyrexial point was found to be between 80° F. and 85° F., with the animals functioning normally at 80° F. These animals were sprayed with 40 ml. of a medium-blend oil, viscosity (S.S.U. at 100° F.) of 40 sec., unsulphonated residue 89 percent, twice daily for ten days. As a result of this spraying, the pyrexial point was lowered approximately 5° F. After a 10-day rest period the animals were sprayed with 40 ml. of straight-cut oil of viscosity 54 sec. (S.S.U. at 100° F.), unsulphonated residue 93 percent. The body temperature rose slowly until on the eighth day of the trial the temperature was 102° F. and on the ninth and tenth day 102.3° F. The recovery was rapid during the recuperation period. In later tests Freeborn (12) found that the amount of water evaporated by way of the cow's skin was approximately the same at 85° F. and 115° F. He ruled out the effect of lack of water evaporation as a factor in the cows body temperature increase. It was Freeborn's belief that rise in body temperature of oil-sprayed cows was due to a chemical combination with body tissues and that oils with a low unsulphonated residue are certain to produce unfavorable reactions in a shorter time and with more intense results.

According to Freeborn (12) the pyrexial point varies in breeds of cattle as follows:

Room Temperature Degrees F.	Body Temperature Degrees F.	
	Holstein	Jersey
75	102.3	101.5
80	103.3	101.5
85	103.8	103.1

The Jersey cows have a higher pyrexial point than Holstein.

The known safe range for livestock oils is viscosity 45-55 Saybolt seconds and unsulphonated residue above 90 percent, according to Sherrick (33). Melvin (18) used oils of a lower viscosity than the safe range, but the sprayed cows did not show increase in body temperature from that of the controls. Freeborn (11) in the psychrometric chamber trials used one oil not in the safe range that resulted in the lowering of the pyrexial point of the test cows. In a second test using straight-cut oil of safe range, the increase in body temperature was but 0.3° F. above normal on the ninth and tenth day of the test. Wilson (35) using oils of varying viscosities and degree of refinement found a slight increase in the average body temperature of sprayed animals over those same animals when not sprayed.

In the field tests of 1940, the cows (Jersey and Ayreshire heifers) were sprayed with 30 ml. of the Continental base oil having a viscosity of 45.9 sec. (S.S.U. at 100° F.) and a high percentage of unsulphonated residue. The cows were sprayed at 8:00 a.m. and rectal temperatures of the sprayed animals and two unsprayed controls were recorded at the time of spraying or at the time the animal was staked out in the field. Additional body temperatures were recorded at 10 a.m., 11 a.m., and 1 p.m.

Table 5 shows that the average temperature increase of the sprayed cows was approximately the same as the average temperature increase of the unsprayed controls when the number of animals entering into the average is considered. Further, the greatest temperature increase of the unsprayed check is approximately the same as the average temperature increase of the sprayed cows. This indicates, as Freeborn (12) stated, that cows have a maximum environmental temperature or pyrexial point, and that body temperature increases follow in both sprayed and unsprayed animals when the air temperature increases beyond that temperature at which the animal functions normally.

Livestock sprays properly applied cause no injurious increase in a cow's body temperature if the base oil of that spray is within the viscosity range of 45-55 Saybolt seconds and has at least 90 percent unsulphonated residue.

Table 5.--A Comparison between the Average Temperature Increase of Sprayed and Unsprayed Cows; The Greatest Temperature Increase of Unsprayed Cow; Air Temperature of the Various Test Days.

Date	Average Temp. Increase °F. Sprayed Cows	Number of Cows Sprayed	Average Temp. Increase °F. Unsprayed Cows	Number of Cows Unsprayed	Average Temp. Increase °F. Sprayed over Unsprayed Cows	Greatest Temp. Increase °F. Unsprayed Cow	Air Temp. of Test Period 7 a.m.-1 p.m. Degrees F. High	Low	Range	Mean
Sept. 12	1.36	10	.15	2	1.21	1.1	80	48	32	69
July 19	2.53	12	1.44	2	1.08	2.0	98	78	20	91
July 30	2.44	12	1.55	2	.89	1.8	100	76	24	91
Aug. 24	1.44	10	.6	2	.84	.9	87	69	18	77
July 20	2.98	12	2.15	2	.83	3.3	98	78	20	89
July 29	1.8	12	1.15	2	.65	.9	100	80	20	92
July 16	2.54	11	1.95	2	.59	2.7	95	75	20	88
Aug. 27	.74	12	.2	2	.54	.6	88	72	16	82
Aug. 1	3.13	8	2.6	2	.53	3.2	96	74	22	87
July 31	2.51	12	2.05	2	.46	2.2	96	76	20	90
Sept. 7	2.66	11	2.2	2	.46	2.3	90	68	22	81
Sept. 6	2.13	11	1.75	2	.35	2.2	87	69	18	80
Sept. 13	1.53	10	1.35	2	.18	1.8	86	54	32	77
Aug. 23	1.2	11	1.1	2	.1	1.4	87	68	19	77
July 15	2.21	12	2.3	2	.09*	3.0	93	74	19	87
July 17	1.9	12	2.0	2	.1 *	2.4	95	78	17	89
Aug. 22	1.0	12	1.15	2	.15*	1.1	84	63	21	75
Aug. 2	2.64	8	2.8	2	.16*	3.5	98	76	22	90
June 21	.95	4	1.68	4	.73*	2.1	89	74	15	83
July 13	.79	12	1.65	2	.86*	1.7	81	66	15	76
July 18	2.17	12	3.05	2	.89*	3.1	95	78	17	89
June 20	.78	4	1.75	4	.97*	2.0	72	87	15	84

*Indicates temperature decrease

A LABORATORY TEST PROCEDURE FOR THE EVALUATION OF THE TOXICITY OF LIVESTOCK SPRAYS

In the introduction to this thesis it was stated that to date there had been no satisfactory nor official method for the evaluation of livestock sprays. The Peet-Grady method, which was devised for the evaluation of household sprays, has been applied in the evaluation of livestock sprays. Due to the lower volatility of the heavier oil bases of the livestock spray types, it has not been satisfactory in the testing of livestock sprays to use the official control insecticide which is made up of household sprays.

In an effort to devise a method whereby the efficiency of the toxic elements incorporated in a livestock spray could be compared with the killing power of like toxic agents in a household spray type oil base a modified test procedure was devised as follows: The toxic elements of the livestock spray, namely, Lethane 384, 3 percent; Lethane 384 special, 1 percent; Pyrocide 20, 2 percent, were incorporated in a base oil of the household spray type (94 percent). The Peet-Grady method was followed in the testing of this household type spray and the official test insecticide. The livestock spray was tested in like manner except that in addition to tests of ten minutes exposure, other tests were made with an increased period of exposure, namely, 15, 20, 25, and 30 minutes. These increased exposure tests were made to determine that period necessary to equal the killing efficiency obtained by a like amount of the same toxic elements incorporated in household type oil base when compared with the official test insecticide.

As shown in Table 6, a 25-minute period of exposure of the livestock spray was necessary to obtain the kill caused by the same toxic elements in base oil of the household spray type.

Table 6.--The Exposure Period Necessary for a Livestock Spray to Equal the Killing Efficiency of Like Toxic Agents Incorporated in Household Spray Type Oil Base as Determined by the Peet-Grady Method, Stillwater, Oklahoma, 1940.

Exposure Minutes	Sample	Percent Mortalities	Average Percent Mortality	Rating Percent Kill-O.C.I.
10	Official Test Insecticide	59, 41, 47, 40	47	---
10	Lethane 384 3%, Lethane 384 special 1%, Pyrocide 20 2%, Household Type Base Oil 94%	71, 65, 66, 71	68	+ 21
10	Lethane 384 3%, Lethane 384 special 1%, Pyrocide 20 2%, Livestock Base Oil 94%	60, 59	60	+ 13
15	" " " " " "	72, 58	65	+ 18
20	" " " " " "	68, 50, 56	58	+ 11
25	" " " " " "	71, 64	68	+ 21
30	" " " " " "	72, 65, 70	69	+ 22

SUMMARY

In the testing for the repellency of various spray formulae and the Continental base oil to stable flies and horn flies on cattle staked in the field, a modified test procedure was used in which one half of the cow was sprayed with base oil and the opposite side sprayed with toxic-repellent materials included in that base oil. This made possible the evaluation of the materials as to their repellent action to flies since animal susceptibility to both of the fly species varied considerably and one cow could not be used as a check against a second cow in repellency testing.

From the season's field records, taken over a daily period from 8 a.m. to 2 p.m. from one pasture and 14 cows, it was determined that the stable fly was most numerous at temperatures between 81° F. and 85° F. The greatest abundance of horn flies occurred at a temperature range of 70° F. - 78° F., although this species occurred in appreciable numbers at higher temperatures up to and including 97° F. Both species were more numerous at the start of the field experiments. The stable fly was never as abundant as the horn fly which frequently occurred in comparatively large numbers.

As a result of an evaluation of the spray formulae and base oil by the statistical analysis of variance method, it was found that out of 19 toxicant-repellant materials included in 24 different spray formulae that only two of them showed a significant repellency to stable flies over the base oil for a period of five hours after the cattle were sprayed. Two formulae showed a significant repellency to horn flies over the base oil for a period of five hours after the cows were sprayed. Three formulae showed a significant repellency to hornflies over the base oil two hours after the cows were sprayed. The base oil showed a highly significant

repellency to both fly species for a period of five hours after the cattle were sprayed when compared with unsprayed cow sides.

Livestock sprays composed of an oil base of 45-55 sec. viscosity and 90 percent in sulphonated residue do not cause an increase in the body temperature of cows when sprayed in amounts not greater than 40 ml. per cow other than that temperature increase which follows as a result of the pyrexial point being exceeded caused by an increase in air temperature.

A modified test procedure of the Peet-Grady method indicated that a 25-minute exposure was necessary for toxicants incorporated in the livestock base oil to equal the killing efficiency of equal amounts of the same toxicants in household type base oil at the standard 10-minute exposure.

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