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Laboratory Report
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Project Number: GM 6.1.2
Project Title: 1976 Field Studies of Different Formulations of Dimilin and Sevin-4-Oil
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: Larry L. Herbaugh, W. H. McLane, C. R. Stacy, J. A. Finney

Introduction: Each year for the past 3 generations, the gypsy moth, *Lymantria dispar* (L.) has defoliated thousands of acres of forested land in the northeast. Because of this, investigations are continually being carried out to evaluate new materials and new formulations of current registered materials.

Materials and Methods: In 1976 the following materials and formulations were tested for gypsy moth control: Dimilin 25 W/P; Dimilin 3.3 oil + kerosene; Dimilin 3.3 oil + Triton X-190 + water; Sevin-4-oil + kerosene; and Sevin-4-oil + Triton X-190 + water. Each of the materials was aerially applied to 50 acre plots in 3 replicates for evaluation. Table 1 presents the details on chemical concentration and application rate.

Table 1. Details of 1976 formulations field tested.

Material	Chemical Concentration and Application Rate	Remarks
Dimilin (25 W/P)	0.06 lb ai/gal/A	applied at less than 5% foliage expansion
	0.06 lb ai/gal/A 0.06 lb ai/.5 gal/A	Dimilin and water no sticker added
Dimilin 3.3 oil + Kerosene	0.03 lb ai/gal/A	96% Kerosene
Dimilin 3.3 oil + Triton X-190 + water	0.03 lb ai/gal/A	.00007% T X-190
Sevin-4-Oil + Kerosene	0.5 lb ai/qt/A 1.0 lb ai/40 oz/A	16 oz Kerosene 8 oz Kerosene
Sevin-4-Oil + Triton X-190 + water	0.25 lb ai/qt/A	25.00% Sevin-4-Oil .16% T X-190 74.84% water
	0.50 lb ai/qt/A	50% Sevin-4-Oil 1% T X-190 49% water
	1.0 lb ai/40 oz/A	80.0% Sevin-4-Oil 1.6% T X-190 18.4% Water

Plot Establishment: The test plots were established in an area adjacent to the 1975 study area in Clinton County, Pennsylvania. This area met the following requirements: (1) a building population in which there had been no more than 1 year's noticeable defoliation prior to the test year (2) a readily measurable population of egg masses and (3) a predominance of preferred host trees (oaks).

Each 50 acre square plot was laid out by establishing an accessible base corner and plotting boundary lines along compass headings. Markers were placed at all four corners for aircraft guidance. Within each 50 acre plot, 5 subplots (0.1 acre) or sampling units were randomly established. These suplots were representative of the entire plot and served as sampling points throughout the experiment. Sampling points were similarly established in untreated check areas to measure natural conditions.

Application: The application schedule was designed so that material was to be applied when the majority of the larvae were in the late second instar stage and the oak foliage was 50-75% expanded. One exception to this was the early treatment of Dimilin which was applied at early 1st instar when oak foliage was less than 5% expanded.

Materials were mixed and transferred into the aircraft with conventional equipment (mixing tank, pumps, hoses, measuring devices and necessary safety equipment). All mixing and spraying equipment was thoroughly cleaned between each treatment to avoid adulteration. Radio communications were maintained between the ground crew and aircraft giving conditions of wind velocity and materials dispersement.

All plots were treated with a Cessna Ag Truck aircraft equipped with a hydraulic driven impeller pump and a conventional spray system (see Table 2). The airplane dispersed the material in 75 foot swaths at 100 mph as near to tree top level as possible with a ground wind velocity of less than 5 mph.

Table 2. Configuration of spray systems utilized.

Material	No. Nozzles	Orifice
Dimilin 25 W/P		
0.06 lb ai/gal/A (early)	20	D8-45°
0.06 lb ai/gal/A	20	D8-45°
0.06 lb ai/gal/A	10	D8-45°
Dimilin 3.3 oil + Kerosene		
0.03 lb ai/qt/A	20	8002FF
Dimilin 3.3 oil + Triton X-190 + water		
0.03 lb ai/qt/A	20	8002FF
Sevin-4-Oil + Kerosene		
0.5 lb ai/qt/A	19	8002FF
1.0 lb ai/40 oz/A	28	8002FF
Sevin-4-Oil + Triton X-190 + water		
0.25 lb ai/qt/A	26	8002FF
0.5 lb ai/qt/A	20	8002FF
1.0 lb ai/40 oz/A	28	8002FF

Evaluation: Each treatment was evaluated from the standpoint of foliage protection and population reduction.

Foliage protection was determined from visual estimates of the degree of defoliation in each of the sampling plots at the termination of larval feeding. Estimates were all performed by the same crew so that human judgement would remain constant (see Figure 1).

Population reduction was estimated by comparing five 10 minute string counts for larvae in each of the 50 acre plots both pre- and post-spray counts were made 5 different times with the exception of Dimilin 3.3 oil + kerosene, Dimilin 3.3 oil + Triton X-190 + water, and Dimilin 25 W/P which were only counted once approximately 20 days after treatment. Figure 2 shows population change by 10 minute larval counts.

Conclusion: All formulations of Dimilin and Sevin-4-oil + kerosene gave excellent results in larval reduction as shown by the graphs in Figure 2. This pattern carried on through the program with significant foliage protection (Figure 1). Also, there was a substantial decrease in egg masses per acre as shown in Table 3.

The Sevin-4-oil + Triton X-190 + water formulations showed a decrease in egg masses per acre. The application at 0.50 lb ai/qt/A produced egg mass reductions from 1890 to 300, but the same formulation showed an increase in larval counts as shown in Figure 2.

One significant conclusion which shows excellent potential is the Dimilin® 25 W/P application of 0.06 lb ai/gal/A with less than 5% oak foliage expansion. This treatment resulted in significant foliage protection and larval activity reduction based on 10 minute counts. Also, egg masses were reduced from 1587 to 209 egg masses/A.

Table 3. Average egg mass counts in aerial plots in Clinton County, PA.

Material and Dosage	Spray Date	# Spring Egg Masses/A	# Fall Egg Masses/A
Dimilin			
0.06 lb ai/gal/A	4-30-76	1587	209
0.06 lb ai/gal/A	5-23-76	1284	124
0.06 lb ai/.5 gal/A	5-23-76	1694	50
Dimilin 3.3 oil + Kerosene			
0.03 lb ai/qt/A	5-23-76	948	52
Dimilin 3.3 oil + Triton X-190 + water			
0.03 lb ai/gal/A	5-22-76	1426	250
Checks	None	1194	806
Sevin-4-oil + Kerosene			
0.5 lb ai/qt/A	5-21-76	1096	666
1.0 lb ai/40 oz/A	5-23-76	1292	65
Checks	None	1144	1967

Table 3 cont'd.

Material and Dosage	Spray Date	# Spring Egg Masses/A	# Fall Egg Masses/A
Sevin-4-oil + Triton X-190 + water			
0.25 lb ai/qt/A	5-22-76	1176	215
0.50 lb ai/qt/A	5-23-76	1890	300
1.0 lb ai/40 oz/A	5-23-76	907	395
Checks	None	1612	883

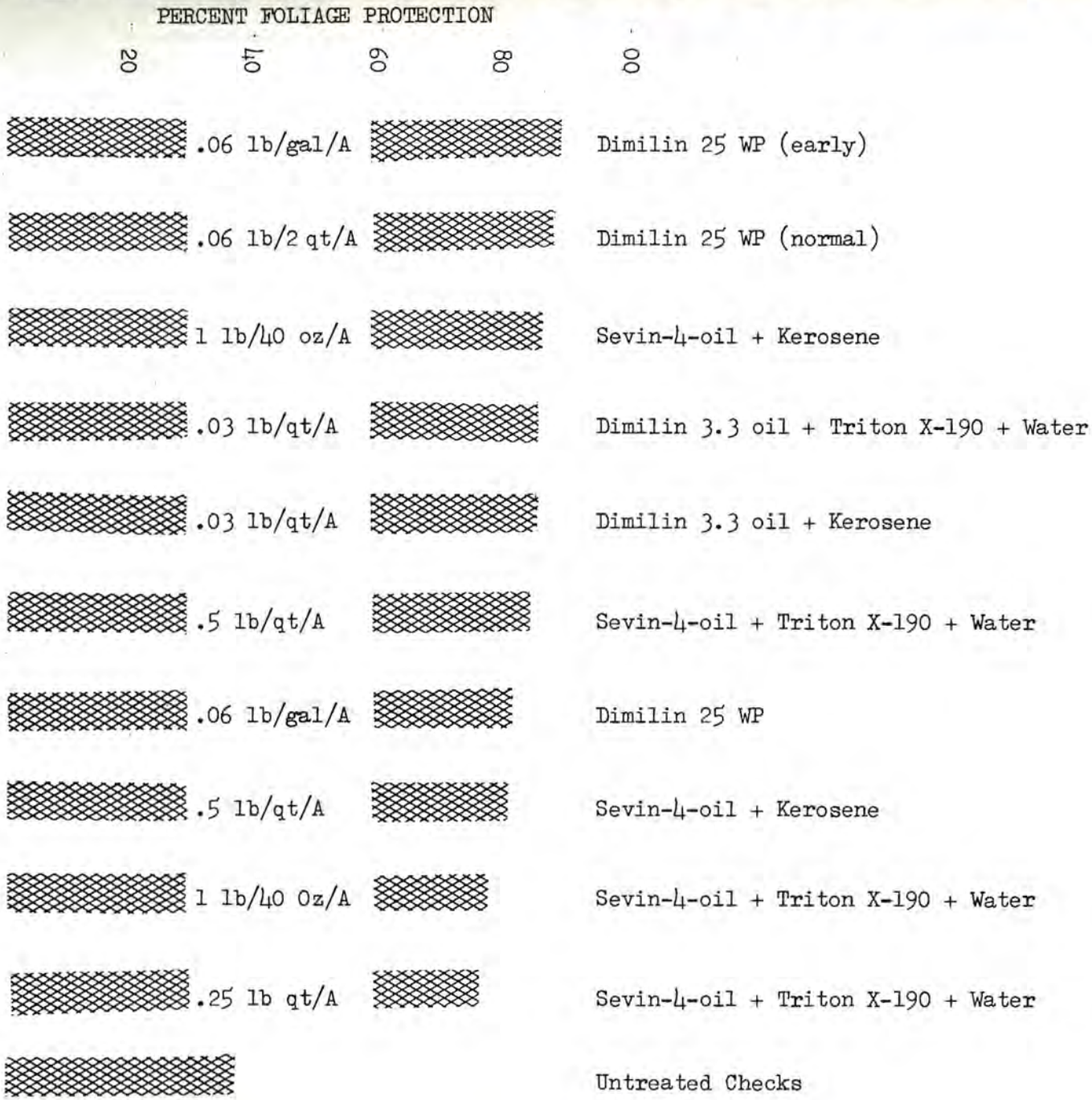
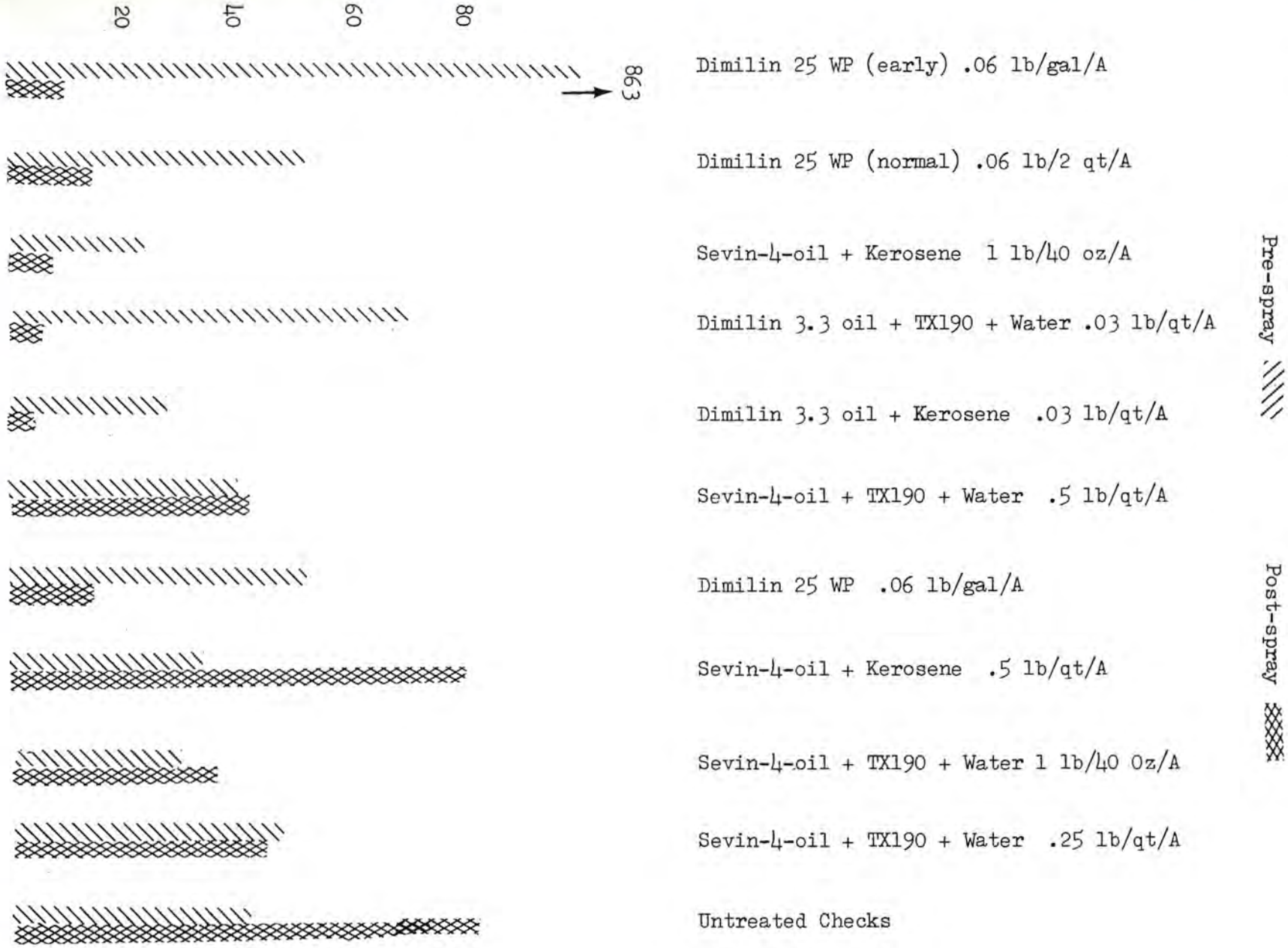


Figure 1. Foliage Protection. Pre-spray defoliation was less than 10%.

Figure 2. Ten-minute larval string counts.



Project Number: GM 6.1.3
Project Title: Field Testing of Ground Applied Insecticides
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: Larry L. Herbaugh, W. H. McLane, C. R. Stacy, J. A. Finney

INTRODUCTION:

Experimental use permits were received from EPA in early June 1976 for testing Orthene® 75S, Imidan® 50 W/P and Sevin® 80S. In addition, Sevin-4-oil with water and NTN 9306 were tested.

One-acre plots were established in the Bald Eagle State Forest lands in Centre and Clinton Counties, PA. These plots contained a measurable population of gypsy moth eggs and were located in heavily wooded areas representative of camground situations.

The dimensions of these plots were ca. 200 feet square and plots were located on both sides of a small dirt road. Within each one-acre plot, two subplots or sampling units were established and each unit comprised 1/40 of an acre. These 1/40 acre sampling units were used for all evaluations throughout the program.

APPLICATION:

Only a small quantity of each material was needed for application. Therefore, materials were mixed on site using conventional mixing equipment and a "nurse" tank for a water supply. All mixing and spraying equipment was thoroughly cleaned between treatments to avoid adulteration.

The materials were applied with a John Bean Mist Blower mounted on a 1-1/2 ton 4-wheel drive truck at the following rates:

Orthene 75S, Imidan 50 W/P and Sevin 80S	.25 lb ai/5 gal H_2O/A
	.50 lb ai/5 gal H_2O/A
	1.0 lb ai/5 gal H_2O/A
	1.5 lb ai/5 gal H_2O/A
	2.0 lb ai/5 gal H_2O/A
	3.5 lb ai/5 gal H_2O/A

Each of these dosages was replicated 3 times for each material.

NTN 9306	.125 lb ai/5 gal H_2O/A
	.50 lb ai/5 gal H_2O/A
Sevin-4-oil and T X-190 + water	2.0 lb ai/5 gal H_2O/A
	3.5 lb ai/5 gal H_2O/A

EVALUATION:

Each treatment was evaluated from the standpoint of percent foliage protection, larvae reduction using a 15 foot string count, and egg masses/acre reduction.

Foliage protection, larvae reduction and egg mass change are shown in the following table.

Table 1.

Material	Dosage	Protection	Average Larval String Counts Pre-Treatment	Average Larval String Counts Post-Treatment 1/	Avg. # of Spring EM/A	Avg. # of Fall EM/A
Imidan 50 W/P	.25 lb ai/5 gal/A	49.5	20	46	520	5293
	.50 lb ai/5 gal/A	66.7	25	22	313	3880
	1.0 lb ai/5 gal/A	85.4	30	20	306	2293
	1.5 lb ai/5 gal/A	58.0	18	33	600	2293
	2.0 lb ai/5 gal/A	56.5	25	16	1986	3673
	3.5 lb ai/5 gal/A	42.0	33	14	2710	306
Check	-	3.5	19	8	1060	1235
Sevin 80S	.25 lb ai/5 gal/A	11.5	26	5	1000	713
	.50 lb ai/5 gal/A	62.0	22	2	1046	326
	1.0 lb ai/5 gal/A	43.5	23	2	1653	80
	1.5 lb ai/5 gal/A	55.5	22	1	1663	126
	2.0 lb ai/5 gal/A	82.7	26	1	1373	40
	3.5 lb ai/5 gal/A	89.4	25	.5	1046	100
Check	-	5.0	42	40	1225	2045
Orthene 75S	.25 lb ai/5 gal/A	80.0	29	6	606	453
	.50 lb ai/5 gal/A	91.4	25	3	640	526
	1.0 lb ai/5 gal/A	94.0	26	2	480	386
	1.5 lb ai/5 gal/A	94.0	29	0	533	113
	2.0 lb ai/5 gal/A	94.0	22	0	446	33
	3.5 lb ai/5 gal/A	94.0	19	0	360	13
Check	-	7.5	48	51	1060	1235
Sevin-4-oil + T X-190 + H_2O	2.0 lb ai/5 gal/A	77.0	39	6	-	173
	3.5 lb ai/5 gal/A	87.0	35	9	- *	80
NTN 9306	.125 lb ai/5 gal/A	75.3	15	1	953	1320
	.50 lb ai/5 gal/A	73.0	15	2	1520	480
Check	-	2.0	9	10	1033	4646

1/ Post-treatment readings based on 5th day after treatment.

* Plots established after spring hatch.

CONCLUSION:

Table 1 presents results of the various treatments of the insecticides.

Imidan, Sevin and Orthene were applied at rates ranging from 0.25 to 3.5 lb ai/A.

Imidan® 50 W/P applied at 3.5 lb ai/5 gal/A is the only treatment of this insecticide which resulted in reduction of egg masses. Significant increases in egg masses were noted in all the lower dosages. Application rates of 1.5, 2.0 and 3.5 lb ai/5 gal/A revealed an average of 52% foliage protection and 16% larval reduction.

All plots sprayed with Sevin® 80S showed a reduction in egg masses. In the plots receiving the 3 highest dosages, foliage protection averaged 76% and larval activity was reduced ca. 99%.

Ninety-four percent foliage protection and ca. 100% larval reduction was observed in plots sprayed with the 3 higher dosages of Orthene® 75S. All Orthene treatments revealed egg mass reduction.

In comparison, the two new formulations of Sevin-4-oil and T X-190 and water and NTN 9306 gave ca. 82% and 74% foliage protection, respectively and ca. 78% and 87% reduction in larval activity, respectively. Spring egg mass counts are not available for the Sevin-4-oil plots. However, egg mass counts in surrounding areas and other Sevin-4-oil data indicate that both application rates of Sevin-4-oil + the emulsifier and water probably decreased egg masses/acre, whereas only the higher dosage of NTN 9306 showed an egg mass reduction.

Data in Table 1 indicate that Orthene® 75S and Sevin® 80S provided the greatest degree of foliage protection, virtually stopped larval activity on a 15 foot string count and reduced the number of egg masses resulting in a subsequent population reduction.

Project Number: GM 6.1.4
Project Title: Evaluation of Pesticides for Browntail Moth
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: Winfred H. McLane, J. A. Finney

The primary objective of this project is to screen candidate materials against the browntail moth. No candidate insecticides were tested against the browntail moth during this period.

On December 10, 1976 a meeting was held at Truro, MA with the Cape Cod National Seashore Advisory Commission. The Commission is made up of 10 residents who serve in an advisory capacity to the National Park Service. Their opinions are highly regarded by the National Park Service. The Commission has recommended that no control measures be taken against the browntail moth on Seashore lands. Therefore, this line of testing is discontinued pending resolution of the conflict between the USDA control effort and the National Park Service.

Project Number: GM 6.1.5
Project Title: Regulatory Treatments (Laboratory-Field)
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leader: Winfred H. McLane, J. A. Finney

The lack of effective treatment methods for recreational vehicles and mobile homes is a major deficiency in the APHIS gypsy moth regulatory program. Experimental laboratory and field tests are conducted to develop improved techniques for dealing with this and other regulatory problems.

The field test started August 3, 1976 using Top Job, Pine Scent, wax, wax remover, Dimilin, and Light Water and was continued throughout the winter. These tests are being conducted in an infestation near Dighton, MA. Twelve egg masses were treated each month with 3 dosages of each material. Six were covered with black building paper and 6 were left exposed.

Egg masses were also treated in the laboratory with various dilutions of Dowgard and Light Water.

Material	Average 5 Egg Masses Hatch	Check
Dowgard 2.5% + Light Water 2.5%	100	147
Dowgard 5%	158	147
Dowgard 10%	154	147
Dowgard 25%	121	147
Dowgard 50%	2	147
Dowgard 100%	0.3	147
S-20546 5%	96	152
S-20546 10%	72	152
S-20546 25%	0	152
S-20546 50%	0	152

Project Number: GM 6.1.6
Project Title: Field Testing of Juvenile Hormone Analogs for Ovicidal Activity
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leaders: Arthur P. Morris, C. P. Schwalbe

The juvenile hormone analogs, Altozar 4E and ZR-619 5E, have been reported as having ovicidal properties. When gypsy moth egg masses are deposited on recently treated surfaces, hatchability of eggs is reduced. Compounds with this property may be useful for regulatory treatment.

Trunks (boles) of white and red oak trees were sprayed to the point of runoff with water emulsions of Altozar and ZR-619 at 8g/l. Treatments were aged 1, 3, 7, 14, 21 and 28 days before bioassay. At the end of each aging period, 5 or more 3 day old, mated females were imprisoned on each of 5 trees (minimum of 25 replicates/treatment). Two untreated control trees were used for each treatment (minimum of 10 replicates). The egg masses laid were collected during January and February 1977 and stored outdoors in plastic petri dishes until tested for hatchability at 27°C and 56% RH. The number of larvae to emerge from each egg mass was recorded for a minimum of 21 days after first hatch. After 28 days, egg masses were dissected to determine the number of eggs that failed to hatch, that were unembryonated and that were parasitized.

Egg masses from 2 aging periods have been completely examined to date. The data available on the numbers of eggs to hatch, unhatched and parasitized are shown in Table 1. Computations of the percentage of eggs hatched, unhatched and parasitized are shown in Table 2. The numbers of unembryonated eggs are recorded but not shown as they are so small that results in computations would be insignificant.

Neither compound affected the rate of parasitization. Altozar, after 7 days of exposure, showed 24% parasitization of eggs laid on treated trunks versus 35% on untreated trees. However, this difference is not likely caused by treatment since there was no affect after 1 day's aging. Since parasitized eggs do not hatch, the number of those parasitized is subtracted from the total number for each treatment and its control. The corrected totals are shown in Table 2 and were used for the hatched-unhatched data.

Altozar prevented hatch of eggs for 3 days and only 0.7% hatch after 7 days aging.

ZR-619 did not prevent enough hatch to consider it further for regulatory treatments.

Table 1. Egg mass data from juvenile hormone field tests - 1976.

Compound	Days Treatment Aged	Number of Egg Masses		Number of Eggs: Total		Hatched		Unhatched		Parasitized	
		Treat.	Contr.	Treat.	Contr.	Treat.	Contr.	Treat.	Contr.	Treat.	Contr.
Altozar 4E	1	31	13	6127	2327	0	1195	3916	252	2211	880
	3	28	15	5478	3367	0	1993	3858	344	1620	1030
	7	32	10	7485	1986	44	1148	5893	142	1908	696
	14	37	16			905	3131				
	22	25	11			364	820				
	28	25	10			1120	1193				
ZR-619 5E	1	26	12	14979	6935	1681	2689	9209	2464	4089	1782
	3	27	11			1431	1289				
	7	41	14	9477	2810	3442	1184	2470	634	3565	992
	15	33	15			6985	2240				
	21	24	12			624	2031				
	28	25	10			3127	1704				

Table 2. Ovicidal effects of juvenile hormones on hatch and parasitization of egg masses.

Compound	Days Treatment Aged	% of Eggs Parasitized		Corrected* Total # of Eggs		Percentage of Eggs Hatched		Percentage of Eggs Unhatched	
		Treat.	Contr.	Treat.	Contr.	Treat.	Contr.	Treat.	Contr.
Altozar 4E	1	36.09	37.82	3916	1447	0	82.58	100.00	17.42
	3	29.57	30.59	3858	2337	0	85.28	100.00	14.72
	7	24.32	35.05	5937	1290	0.74	88.89	99.26	11.01
	14								
	22								
	28								
ZR-619 5E	1	27.30	25.70	10890	5153	15.44	52.18	84.56	47.82
	3								
	7	37.62	35.30	5912	1818	58.22	65.13	41.78	34.87
	14								
	22								
	28								

* Total numbers of eggs in masses minus parasitized eggs in masses.

Project Number: GM 7.1.1
Project Title: Laboratory Screening of Candidate Pesticides Against the Gypsy Moth
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leaders: Winfred H. McLane, J. A. Finney

The main objective of this laboratory screening project is to collect mortality data on registered and experimental compounds potentially useful against the gypsy moth, evaluating the data and selecting suitable materials for field testing.

Material	Company	Dosage	Instar	Weather	% Mortality	Days After Treatment
DPX-5444	DuPont	1 lb ai/gal/A	II		100	6
"	"	0.5 lb ai/gal/A	II		100	5
"	"	0.25 lb ai/gal/A	II		100	5
"	"	0.125 lb ai/gal/A	II		100	5
"	"	0.06 lb ai/gal/A	II		100	6

Material	Company	Dosage	Instar	Aging	% Mortality	Days After Treatment
DPX-5444	DuPont	.25 lb ai/gal/A	II	7	100	3
"	"	.25 lb ai/gal/A	II	15	100	6
"	"	.25 lb ai/gal/A	II	21	90	3
"	"	.125 lb ai/gal/A	II	7	100	4
"	"	.125 lb ai/gal/A	II	15	100	6
"	"	.125 lb ai/gal/A	II	21	80	3
"	"	.06 lb ai/gal/A	II	7	100	7
"	"	.06 lb ai/gal/A	II	15	100	6
"	"	.06 lb ai/gal/A	II	21	67	3

Material	Company	Dosage	Sticker	Weather	% Mortality	Days After Treatment
DPX-5444	DuPont	.5 lb ai/gal/A		1"	100	7
"	"	.5 lb ai/gal/A	3% Chevron	1"	100	4
"	"	.25 lb ai/gal/A		1"	100	7
"	"	.25 lb ai/gal/A	3% Chevron	1"	98	5
"	"	.125 lb ai/gal/A		1"	100	7
"	"	.125 lb ai/gal/A	3% Chevron	1"	99	5
"	"	.06 lb ai/gal/A		1"	98	7
"	"	.06 lb ai/gal/A	3% Chevron	1"	31	5
"	"	.03 lb ai/gal/A		1"	50	7
"	"	.03 lb ai/gal/A	3% Chevron	1"	0	5

Material	Company	Dosage	Instar	% Mortality	Days After Treatment
UC 51762	Union Carbide	1 lb ai/gal/A	II	100	5
"	"	.5 lb ai/gal/A	II	100	5
"	"	.25 lb ai/gal/A	II	100	5
"	"	.125 lb ai/gal/A	II	100	5
"	"	.06 lb ai/gal/A	II	99	5
"	"	.03 lb ai/gal/A	II	98	5
"	"	.015 lb ai/gal/A	II	82	5
"	"	.007 lb ai/gal/A	II	82	5
"	"	.0039 lb ai/gal/A	II	52	5
Check				0	5

A series of tests were conducted comparing wild PA gypsy moth larvae versus laboratory reared NJ gypsy moth larvae (F15) when exposed to Sevin 80S, Orthene 75S and Dylox 80S.

Material	Dosage	Wild	Lab-reared	% Mortality	Days After Treatment
Dylox 80S	.25 lb ai/gal/A		X	100	6
"	.25 lb ai/gal/A	X		100	6
"	.125 lb ai/gal/A		X	95	4
"	.125 lb ai/gal/A	X		77	4
"	.06 lb ai/gal/A		X	82	4
"	.06 lb ai/gal/A	X		63	4
"	.03 lb ai/gal/A		X	34	4
"	.03 lb ai/gal/A	X		22	4
Check				0	4
Orthene 75S	.25 lb ai/gal/A		X	98	5
"	.25 lb ai/gal/A	X		98	5
"	.125 lb ai/gal/A		X	91	4
"	.125 lb ai/gal/A	X		97	4
"	.06 lb ai/gal/A		X	90	4
"	.06 lb ai/gal/A	X		85	4
"	.03 lb ai/gal/A		X	78	4
"	.03 lb ai/gal/A	X		66	4
Check				0	4
Sevin 80S	.25 lb ai/gal/A		X	80	5
"	.25 lb ai/gal/A	X		95	5
"	.125 lb ai/gal/A		X	65	5
"	.125 lb ai/gal/A	X		69	5
"	.06 lb ai/gal/A		X	37	5
"	.06 lb ai/gal/A	X		27	5
"	.03 lb ai/gal/A		X	1	5
"	.03 lb ai/gal/A	X		2	5

Project Number: GM 7.1.2
Project Title: Delayed Effects of Sub-lethal Dosages of Insecticides on Fecundity and Fertility of Gypsy Moth
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leader: Robert G. Reeves

The objectives of this project are to establish LD_{10}, 50, 90 of Dimilin 25 WP, Sevin 80S, Orthene 75S and Dylox 80S for first, second and third instar gypsy moth larvae and to monitor the hatchability of gypsy moth eggs produced from adult moths which were reared as larvae on diet containing insecticides.

For preliminary work, the insecticides were topically applied to diets at 5 concentration levels for each pesticide. The following table shows results of these tests.

SEVIN 80S

% Mortality

ppm	Instar	5-day	7-day
5	I	92	92
	II	58.3	70.8
	III	28	36
4	I	66.6	75
	II	41.6	46
	III	20	44
3	I	41.6	45.8
	II	37.5	45.8
	III	36	40
2	I	25	33.3
	II	16.6	20.8
	III	12	16

ORTHENE

% Mortality

ppm	Instar	4-day	6-day
12	I	100	-
	II	100	-
	III	100	-
6	I	91.6	91.6
	II	95.8	95.8
	III	95.8	96
3	I	95.8	100
	II	89.5	89.5
	III	72	80
1.5	I	91.6	95.8
	II	25	37.5
	III	44	48

DYLOX

% Mortality

ppm	Instar	2-day	8-day
12	I	100	100
	II	66.6	100
	III	36	100
6	I	58.3	100
	II	29.1	100
	III	40	100
3	I	62.3	87.5
	II	45.8	87.5
	III	20	80
1.5	I	29.1	62.5
	II	29.1	87.5
	III	0	0.04

DIMILIN 25 WP 1/

ppm	Instar	8-day
1.5, .75, .385, .19	I	0
	II	0
	III	0

1/ No mortality was observed at these concentrations.

Topical application of insecticides onto the diet has resulted in erratic mortality rates of gypsy moth larvae. Consequently, this technique has been abandoned and the insecticides are now being incorporated during the mixing process of diet preparation.

In preliminary tests, Dimilin at 3 ppm resulted in 100% mortality of first, second and third instar larvae. No mortality was recorded in later tests (see table) at 1.5 ppm. There is no tenable explanation for these inconsistent data at this time. Tests are being repeated.

As a general rule, there is an inverse relationship between insect age and mortality. It is thought that these trends will be clearer and more reproducible when tests are conducted with insecticides incorporated directly into the diet.

Project Number: GM 7.1.7
Project Title: 1977 Field Studies of Dimilin® W-25
Report Period: October 1, 1976 - March 31, 1977
Report Type: Preliminary
Project Leaders: Larry L. Herbaugh, W. H. McLane, J. A. Finney

One function of this laboratory is to field test materials of different formulations at various rates and times of application (insect growth, leaf expansion, etc.) to derive the maximum benefits from compounds used in the control of gypsy moth larvae. Therefore, these tests are designed to establish an economical and satisfactory dosage for regulatory control and develop an eradication treatment for isolated infestations of gypsy moth using Dimilin. Dimilin (TH-6040) N-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzomide has received considerable attention in recent years as an insect growth regulator which inhibits the deposition of chitin in gypsy moth larvae. It is presently registered for controlling gypsy moth larvae by aerial applications of .03 lb ai/A to .06 lb ai/A.

A work plan has been approved for this project and most pre-spray field work is complete. Treatment application is scheduled for after May 15 and results of the evaluations will be the subject of a future Laboratory Report. Highlights of the work plan are presented below.

OBJECTIVES:

1. Determine the activity and compare the efficacy of the following treatments for use in regulatory treatment of hazardous areas:

Material	Dosage-lb/A	Rate-gal/A	No. of Applications	Plot Number	No.of EM/A	No.of Prism Pts.
Dimilin W-25	0.06	1	1	1	820	23
				7	460	30
				30	1800	20
Dimilin W-25	0.06	0.5	1	6	460	30
				14	1520	30
				29	1000	20
Dimilin W-25	0.03	0.5	1	2	366	34
				4	1740	20
				12	3360	20
				19	886	23
				23	533	30
				28	1360	20
Check	none	none	none	3	2906	20
				8	1280	20
				9	1520	20

These treatments will be applied when the majority of gypsy moth larvae are in the second instar and oak leaf expansion is 80% (normal timing).

2. Determine the relative utility for eradication of infestations with the following treatment schedules of Dimilin W-25:

Material	Dosage-lb/A	Rate-gal/A	No. of Applications	Plot Number	No. of EM/A	No. of Prism Pts.
Dimilin W-25	0.06	1	1	1	820	23
				7	460	20
				30	1800	20
Dimilin W-25	0.06	1	2*	10	1146	20
				13	1780	20
				25	680	24
Dimilin W-25	0.03	1	2	11	1636	20
				20	560	30
				27	920	20
Dimilin W-25	0.03	1	3**	16	320	36
				17	293	36
				22	280	36
Dimilin W-25	0.03	.5	2	8	1466	20
				24	793	24
				26	1520	20
Dimilin W-25	0.03	.5	2	3	1040	20
				5	3093	20
				18	546	30
Dimilin W-25	0.03	.5	3	9	1800	20
				15	793	23
				21	680	24
Checks	none	none	none	1	600	26
				2	826	23
				4	1353	20
				5	4160	20
				6	273	36
				7	600	26
				10	960	20

* The first application will be applied when 100% of target insects are hatched or approximately 10 days prior to the second application applied at normal treatment.

** The first application will be applied when 100% of target insects are hatched or approximately 10 days prior to the 2nd application applied at normal time. The third application will be applied when the majority of target insects are in the late 3rd instar.

METHODS:

A healthy field infestation of gypsy moth which has shown noticeable defoliation for only one previous year such as Pike County, PA will be selected for plot establishment.

Compass lines will be used to establish forty 50 acre square plots in a predominantly oak hardwood forest for the program (see Figure 1).

Within each 50 acre plot a centrally located 10 acre unit will be established where all sampling data will be collected throughout the study period.

Each 10 acre plot will be surveyed both pre- and post-spray for egg masses. This egg mass survey will be accomplished using the diagnostic prism method developed by the USFS. Approximate egg mass density was estimated from the average of six 1/40 acre counts within each plot; the number of prism points required for 20% standard error ranged from 20 to 36 per plot.

In addition to egg mass survey, efficacy in each plot appraised for regulatory treatment will be evaluated at peak defoliation by making 10 estimates of foliage protection.

All plots in which the seven treatments are being evaluated for eradication potential will have 200 burlap bands attached to host trees 4" and larger in diameter to monitor late larval and pupal activity within the sampling units. These bands will be placed in the area of each prism point, e.g. if a 10 acre plot has 1000 em/A, it will have 20 prism points and 10 numbered burlap bands in the vicinity of each prism point. Readings from these bands will be recorded 3 times; twice during late larval activity and once after pupation. Egg mass estimates will also be done in these plots.

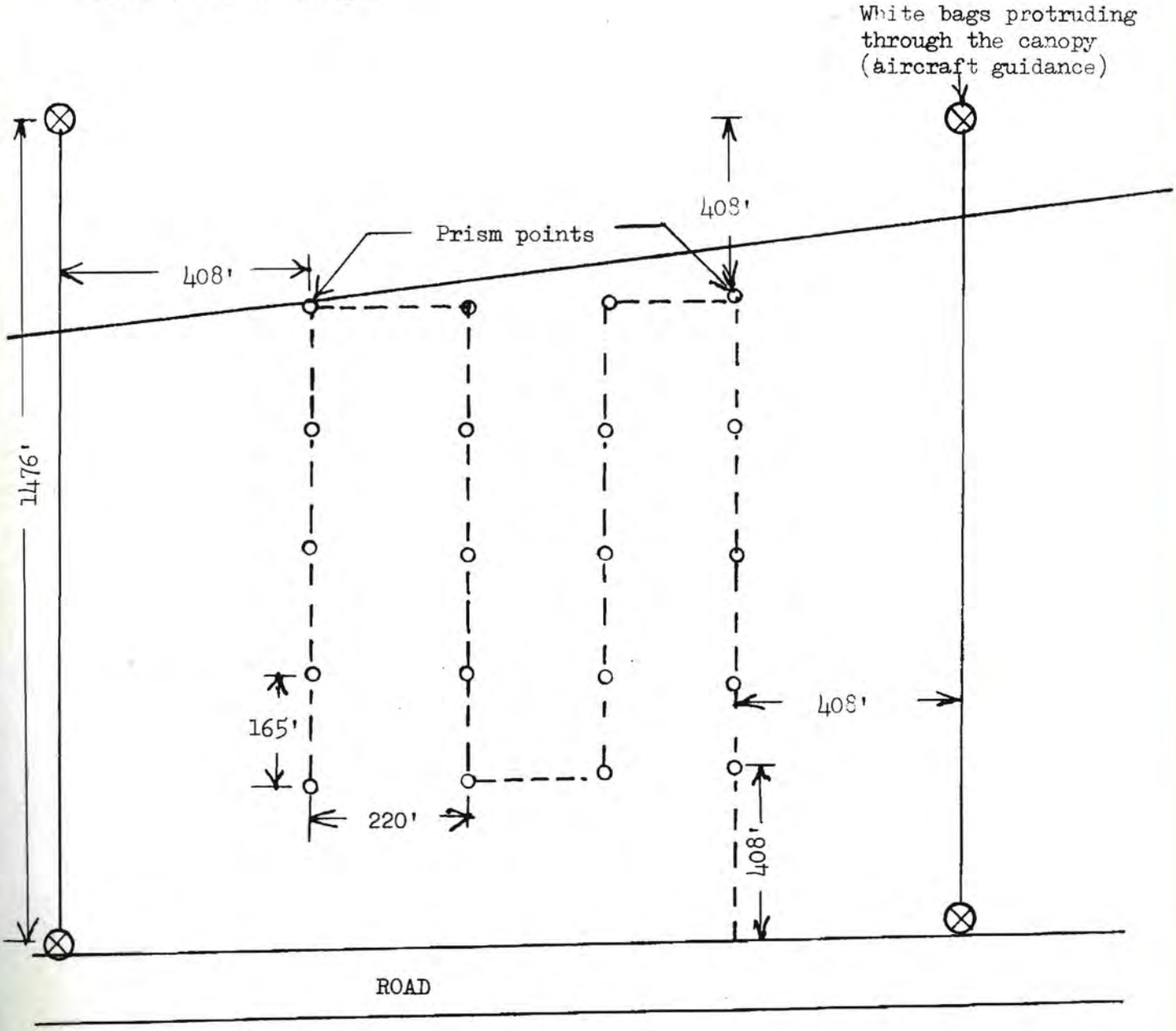
The corners of each 50 acre plot will be marked with double orange flags and small 2"x3" paper tags to identify the plot and corner. A single orange line comprised of 12" or less streamers will mark 2 opposite boundaries of a plot to facilitate location of the inaccessible corners. In the "eradication" plots, a single pink line will lead from an accessible point and connect all prism sampling points located on 10 acres within each 50 acre plot. A total of 200 trees in each plot will be banded with burlap to monitor late larval and pupal activity along these lines in the vicinity of the prism points.

All material tested will be applied aerially using the USDA-APHIS airplane. When feasible, those plots receiving multiple applications will be flown perpendicular to the previous application. Communications will be maintained between a ground spotter man and the spraying aircraft by two way radio.

Treatment efficacy will be expressed in foliage protection and egg mass reduction for objective number 1; late larval and pupal activity and fall egg mass counts will be ranked numerically for objective number 2.

The project will run from January 1, 1977 to December 31, 1977. A preliminary progress report will be included in the September 1977 Laboratory Report with a final recommendation published shortly after termination of the project.

Figure I. Plot Example



Project Number: GM 7.1.9
Project Title: 1977 Field Evaluations of 5 Insecticides Applied by Ground Equipment
Report Period: October 1, 1976 - March 31, 1977
Report Type: Preliminary
Project Leaders: Larry L. Herbaugh, W. H. McLane, J. A. Finney

Each year Animal and Plant Health Inspection Service personnel treat campgrounds, trailer parks and other hazardous sites for the control of gypsy moth larvae. Many of the treatments (largely Carbaryl®) are applied with ground equipment (mist blowers). During the past year two new insecticides have been registered for gypsy moth control: Dimilin® and Orthene®. It is necessary that minimal effective dosages be established to minimize environmental contamination. If dosages can be lowered, treatment cost will also be lowered.

Currently registered materials have only slight contact toxicity and are most effective against early instar larvae. There is a need to develop new materials that have strong contact toxicity on both early and late larval developmental stages.

Test areas have been chosen near Stroudsburg, PA and test plots have been established. Application of the test materials will be made in late May, pending receipt of Experimental Use Permits. For informational purposes, highlights of the Work Plan for this project are presented below.

OBJECTIVES:

1. Determine the minimal effective dosage of Sevin® 80S, Orthene® 75S and Dimilin® 25 WP against gypsy moth larvae when applied with ground equipment.
2. Determine the insecticidal value for gypsy moth control of FMC-45498 (Decis) and Pydrin when applied with ground equipment.
3. Publish test results in such a way that they will be available to all interested personnel.

METHODS:

Materials, dosages and number of plots will be as follows:

Material	Dosage	Number of Plots
Sevin® 80S	0.12 lb ai/5 gal/A	4
"	0.25 lb ai/5 gal/A	2
"	0.5 lb ai/5 gal/A	2
"	1.0 lb ai/5 gal/A	2
"	1.5 lb ai/5 gal/A	2
"	2.0 lb ai/5 gal/A	2

Material	Dosage	Number of Plots
Orthene® 75S	0.12 lb ai/5 gal/A	4
"	0.25 lb ai/5 gal/A	2
"	0.5 lb ai/5 gal/A	2
"	1.0 lb ai/5 gal/A	2
"	1.5 lb ai/5 gal/A	2
"	2.0 lb ai/5 gal/A	2
Dimilin® 25 WP	0.001 lb ai/5 gal/A	4
"	0.002 lb ai/5 gal/A	4
"	0.005 lb ai/5 gal/A	4
"	0.01 lb ai/5 gal/A	4
"	0.03 lb ai/5 gal/A	4
"	0.06 lb ai/5 gal/A	4
FMC-45498 (Decis)	0.003 lb ai/5 gal/A	4
"	0.006 lb ai/5 gal/A	4
"	0.012 lb ai/5 gal/A	4
"	0.025 lb ai/5 gal/A	4
"	0.05 lb ai/5 gal/A	4
"	0.10 lb ai/5 gal/A	4
Pydrin	0.003 lb ai/5 gal/A	4
"	0.006 lb ai/5 gal/A	4
"	0.012 lb ai/5 gal/A	4
"	0.025 lb ai/5 gal/A	4
"	0.05 lb ai/5 gal/A	4
"	0.10 lb ai/5 gal/A	4
Checks		10

One hundred and ten plots will be established along remote roads in primarily oak forest containing a measurable population of gypsy moth eggs (Pike County, PA). Plots will be located on State lands and will be 200 feet wide and 200 feet long with a road dividing them. Within each one acre plot, two subplots or sampling units will be established, each comprised of a 33 foot square or 1/40 acre; one will be placed on each side of the road. Ribbons will be placed near the road at both ends of the 200 foot plots with tags identifying each plot. The two study areas will be delineated with white ribbon and tagged for identification.

Treatments will be applied with a John Bean mist blower when the majority of larvae are 2nd and 3rd instar. Materials will be mixed on site with conventional mixing equipment and a "nurse" tank of water. Mixing and spraying equipment will be thoroughly cleaned between treatments to avoid adulteration.

Each treatment will be evaluated from the standpoint of foliage protection and population reduction. Emphasis will be on larval reduction and will be quantified by one 25' string count in each subplot. One pre-spray count will be made in each plot and 2 post-spray counts, one after 5 days and again after 10 days.

Foliage protection will be estimated by visual examination of the degree of defoliation in all sampling subplots just prior to treatment and again at the termination of larval feeding.

In early spring, egg masses will be counted in each subplot to determine the number of egg masses/acre before treatment. After foliage drop, egg masses will be counted in each subplot to determine the number of post-treatment egg masses/acre. These data will be used to calculate the population reduction.

Project Number: GM 6.2.2
Project Title: Responses of Male Gypsy Moths to Traps as Affected by the Rate of Pheromone Release
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: Charles P. Schwalbe, E. C. Paszek

Appendix 1 is a manuscript which has been submitted for publication in the Journal of Environmental Entomology.

Additional work with the (+) enantiomer of disparlure (which was tested in this project) will be conducted under projects 7.2.1 and 7.2.3.

Project Number: GM 6.2.3
Project Title: Periodicity of Pheromone Release from Female Gypsy Moths
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leader: Charles P. Schwalbe

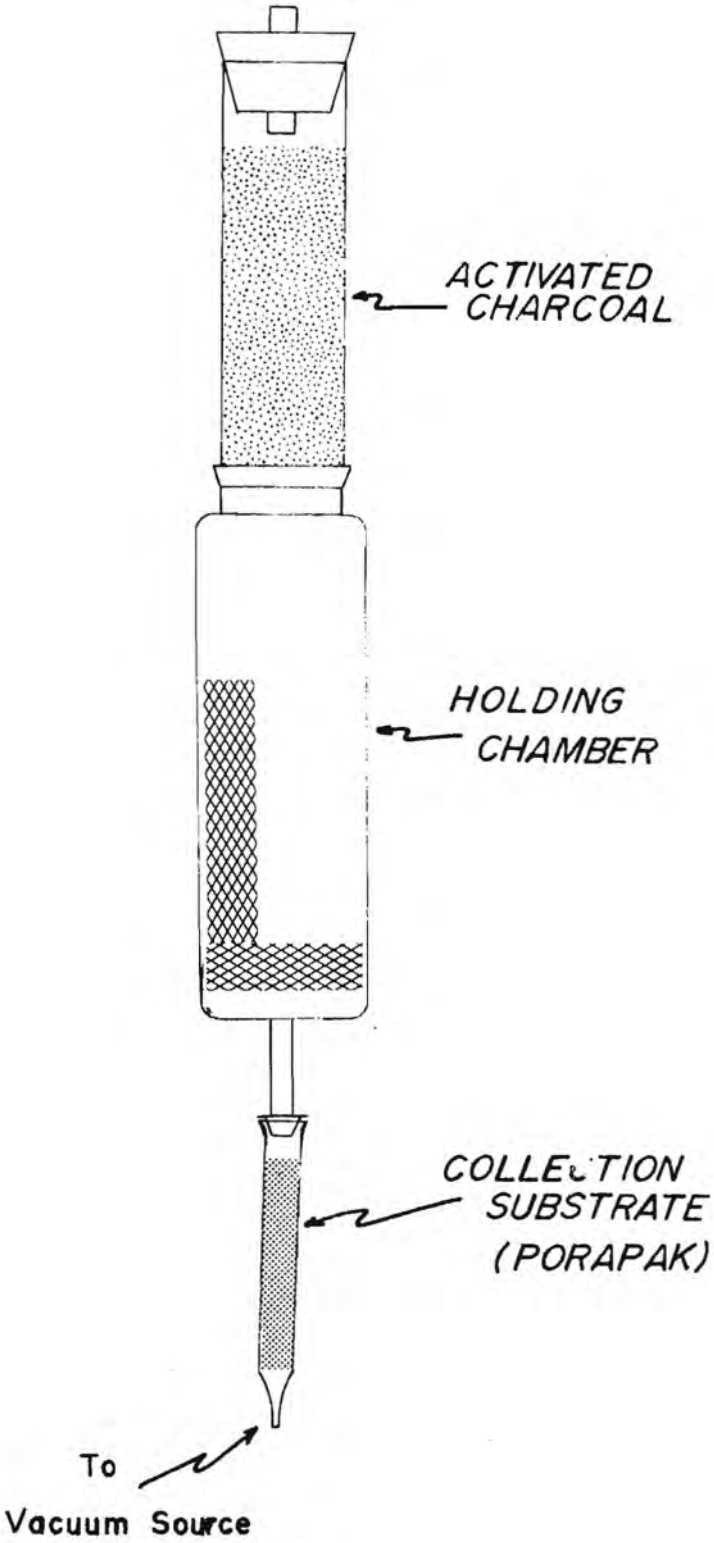
Most effort during this period has been directed toward development of an apparatus useful for collecting pheromone from female gypsy moths. The design adopted is illustrated below. Room air passes through a 25x95 mm column of activated charcoal, over the female moth held in a chamber 27x 85 mm and into a column of activated Porapak Q. The Porapak (.35 g) is held in a glass medicine dropper tube which is connected by tubing to a vacuum manifold. The manifold has 20 ports and air flow through each collection unit is controlled by individual needle valves. The Porapak tubes can be replaced at intervals without disturbing the insect. The following tests were conducted to develop and demonstrate the collection apparatus.

In test 1, known amounts of disparlure (2.5 ug) were applied to .35 g Porapak Q in medicine dropper tubes. After air drying for several hours, the disparlure was eluted with 2.5, 5.0 or 10.0 ul nanograde hexane. The solvent was evaporated to 1.0 ml for GLC analysis. Results are given in Table 1. Recovery was similar (90-92%) when elution volumes were 2.5, 5.0 or 10 ml. Therefore, collection tubes in all subsequent experiments were eluted with 2.5 ml. Recoveries were lower (70-84%) when pheromone was added directly to hexane. It is thought that some of this material was lost during evaporation of the samples to 1.0 ml for analysis.

Table 1. Efficiency of Elution of Disparlure from Porapak Q with Varying Volumes of Hexane.

No. Replicates	Substrate	Fortify	Elution Volume	ug Recovery	% Recovery
5	.35 g Porapak	2.5 ug	2.5 ml	2.29	92
5	.35 g Porapak	2.5 ug	5.0 ml	2.25	90
5	.35 g Porapak	2.5 ug	10.0 ml	2.24	90
5	.35 g Porapak	2.5 ug	10.0 ml	2.27	91
5	2.5 ml hexane	2.5 ug	-	1.76	70
5	5.0 ml hexane	2.5 ug	-	2.05	82
5	10.0 ml hexane	2.5 ug	-	2.12	84
5	10.0 ml hexane	-	-	-	0
1	1.0 ml hexane	12.5 ug	-	13.75	110

PHEROMONE COLLECTION APPARATUS



In another experiment, the effect of air flow rate through the collection system on release of disparlure from Hercon wicks and efficiency of collection by Porapak was tested. Hercon wicks were suspended in the holding chamber by fine wire and air flow was adjusted to 50, 100, 200 and 300 ml/min (V=10, 20, 40, 60 cm/min respectively). Two Porapak tubes were connected in series on each collection unit. The experiment was run for 6.5 hours. No disparlure was collected in tubes with 50 or 100 ml/min flow rate (Table 2). Limit of sensitivity was ca. 100 ng/sample. It appears that emission of disparlure from Hercon wicks increases with increasing flow rate. All released pheromone is held in the first collection tube; none was detected in the second tube. The holding chambers were not rinsed and analyzed in this test. In tests conducted hereafter, flow rate was adjusted to at least 200 ml/min.

Table 2. Effect of Air Velocity on Release of Disparlure from Hercon Wicks and Efficiency of Collection by Porapak.

	ug Disparlure Collected		
Flow Rate(ml/min)	Porapak 1	Porapak 2	Total
50	-	-	-
100	-	-	-
200	1.15	-	1.15
300	1.49	-	1.49
300	2.17	-	2.17

In a third test, collection units were set up with 2 Porapak tubes in series. Hercon wicks were suspended in the holding chambers and flow rate was held at 200 ml/min for 24 hours. Porapak was eluted for analysis and the holding chambers were rinsed with 10 ml hexane. Disparlure distribution is given in Table 3. Again, no pheromone was found in the second Porapak tubes but the rinses of the holding chambers yielded ca. 1.4 ug pheromone. Twenty-seven and 30% of the total released disparlure was recovered from the holding chambers.

Table 3. Breakthrough Test. Distribution of Disparlure Collected in Porapak Tubes Connected in Series.

Pheromone Source	Holding Chamber	$Porapak_1$	$Porapak_2$	Total
Hercon	1.43	3.93	-	5.36
Hercon	1.47	3.43	-	4.90
Hercon	-	-	-	-

An experiment was conducted to determine distribution of disparlure in collection units of various designs. The designs are described in the footnotes to Table 4. Air flow in this test was held at 200 ml/min for 24 hours. Results are presented in Table 4. Forty percent more pheromone was collected in Porapak tubes on the "new" holding chambers, compared to the "old" (6.42 ug vs. 3.80 ug). The rinses from the "old" holding chambers could not be analyzed for disparlure because of inter-

Table 5. Adsorption of Disparlure by Holding Chambers Treated with Sylon® and Untreated.

Sample	Porapak	ug Disparlure Recovered Holding Chamber	Teflon Disc	Total
Check 1	2.271	3.187	3.516	8.974
Check 2	2.985	2.593	1.795	7.373
Check 3	1.776	1.593	3.384	6.753
Check 4	1.119	1.119	3.041	5.279
Check 5	1.418	1.011	3.992	6.421
Check 6	3.358	2.164	1.697	7.219
$\bar{x}$	2.154	1.944	2.904	7.003
+ Sylon 1	4.022	1.414	3.663	9.099
+ Sylon 2	4.304	0.934	1.923	7.161
+ Sylon 3	3.678	1.832	3.571	9.081
+ Sylon 4	2.835	1.359	2.990	7.184
+ Sylon 5	4.155	1.553	1.087	6.795
+ Sylon 6	1.786	1.717	5.231	8.734
$\bar{x}$	3.463	1.468	3.077	8.009

Using the described collection apparatus and techniques, ca. 50 virgin female F_{14} laboratory-reared moths have been tested. Collection periods ranged from several hours to 3 days. In no instance has disparlure been detected. With the available techniques, collection efficiency is at least 70% and the sensitivity of analysis is ca. 200 ng/sample. Obviously, these insects are emitting very small amounts of disparlure (less than 100 ng/day). Field collected eggs are currently being reared and these "wild" moths will be tested. Also, electron capture GLC methods are being evaluated to improve sensitivity of analysis.

fering peaks, presumably originating from the plastic syringe parts. Twenty percent of the disparlure accounted for in the "new" units was in the holding chambers. Based on these data, the all-glass holding chambers were adopted for future use.

Table 4. Distribution of Disparlure in Holding Chambers of Two Designs.

	ug Disparlure Collected			
	Holding Chambers			
Pheromone Source	Old [1]	New [2]	Porapak	Total
Hercon	-	Sample lost	6.74	6.74
Hercon	-	1.49	7.06	8.55
Hercon	-	1.46	5.47	6.93
Blank	-	0	0	0
$\bar{x}$		1.48	6.42	7.40
Hercon	? [3]	-	3.53	3.53
Hercon	?	-	3.89	3.89
Hercon	?	-	4.00	4.00
Blank	?	-	0	0
$\bar{x}$			3.80	3.80

[1] These holding chambers were constructed by gluing luer-lock fitting from a disposable syring to the glass holding chamber.

[2] These chambers were all glass in design.

[3] Disparlure could not be quantified in the holding chamber rinses because of unidentified interfering peaks.

In test 5, a Supelco GLC column coating product, Sylon® was tested to determine if adsorption of airborne disparlure by glassware could be reduced. All glassware was coated with Sylon and washed with methanol and hexane. Teflon discs containing 10 ug disparlure were suspended in the holding chamber; air flow was 200 ml/min for 48 hours. Thereafter, all components were rinsed or extracted and analyzed. From data in Table 5, it appears that coating glassware with Sylon generally improves recovery and reduces adsorption to the holding chamber. In this experiment, 18.3 and 27.7% of the recovered pheromone was found in the treated and untreated holding chambers respectively. Only 70-80% of the disparlure introduced into the system was recovered.

Project Number:	GM 6.2.8
Project Title:	Field Evaluation of Controlled Release Formulation of Disparlure for Mating Disruption
Report Period:	October 1, 1976 - March 31, 1977
Report Type:	Interim
Project Leaders:	Charles P. Schwalbe, E. C. Paszek

A draft manuscript has been prepared for publication in the Annals of the Entomological Society of America. Upon finalization of the manuscript, it will be included as a Final Report in the next Laboratory Report.

Project Number: GM 7.2.5
Project Title: Emission Characteristics of Hollow Fibers
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: Charles P. Schwalbe, L. Mundy, D. H. Simser

This test was conducted to determine if Conrel fibers offer a reliable and calibrated system for dispensing disparlure from survey traps. The supposed advantages of the fiber system is that hollow fibers release pheromone at a constant rate for the duration of the flight season and, if fiber length is properly adjusted, the full amount of material within each fiber is utilized.

Conrel fibers (20 mil or .508 mm ID) extruded from PET were cut to lengths of 2.0 cm and filled (by capillary action) with either 100, 75, 50 or 25% racemic disparlure in hexane. In one test, one end was sealed with epoxy glue and in another series, both ends were left open. Fibers were attached to a narrow block of wood in such a way that the open ends were not in contact with anything. The fibers were then held at 25°C and 65% RH and emission was monitored by measuring recession of the meniscus from the open end(s) with an ocular micrometer and stereo-microscope. These length measurements were then converted to volume by $V = L\pi r^2$. Emission was measured from 15 of each of the following treatments.

	% Disparlure		
	100	75	50
one end open	x	x	x
both ends open	x	x	

Table 1. Volume (mm3) of disparlure and/or hexane released from 20 mil PET Conrel fibers held at 25°C and 65% RH.

	One end closed			Both ends open	
Day	100%	75%	50%	100%	75%
0	0	0	0	0	0
2	.03463	.48607	1.39138	.01681	.91746
3	.04597	.61164	1.60403	.02532	1.00005
4	.05751	.69670	1.65061	.03362	1.02075
5	.06764	.73923	1.64859	.06602	1.01427
7	.08303	.79189	1.65669	.10754	1.04080
9	.10025	.82227	1.67694	.11929	1.06125
11	.10612	.82632	1.64656	.20253	1.04566
15	.11969	.83644	1.65669	.20638	.99422
19	.12455	.83644	1.65669	.22157	1.07968
22	.12597	.83644	1.65669	.24952	1.08940
25	.13306	.83647	1.65669	.26086	1.10459

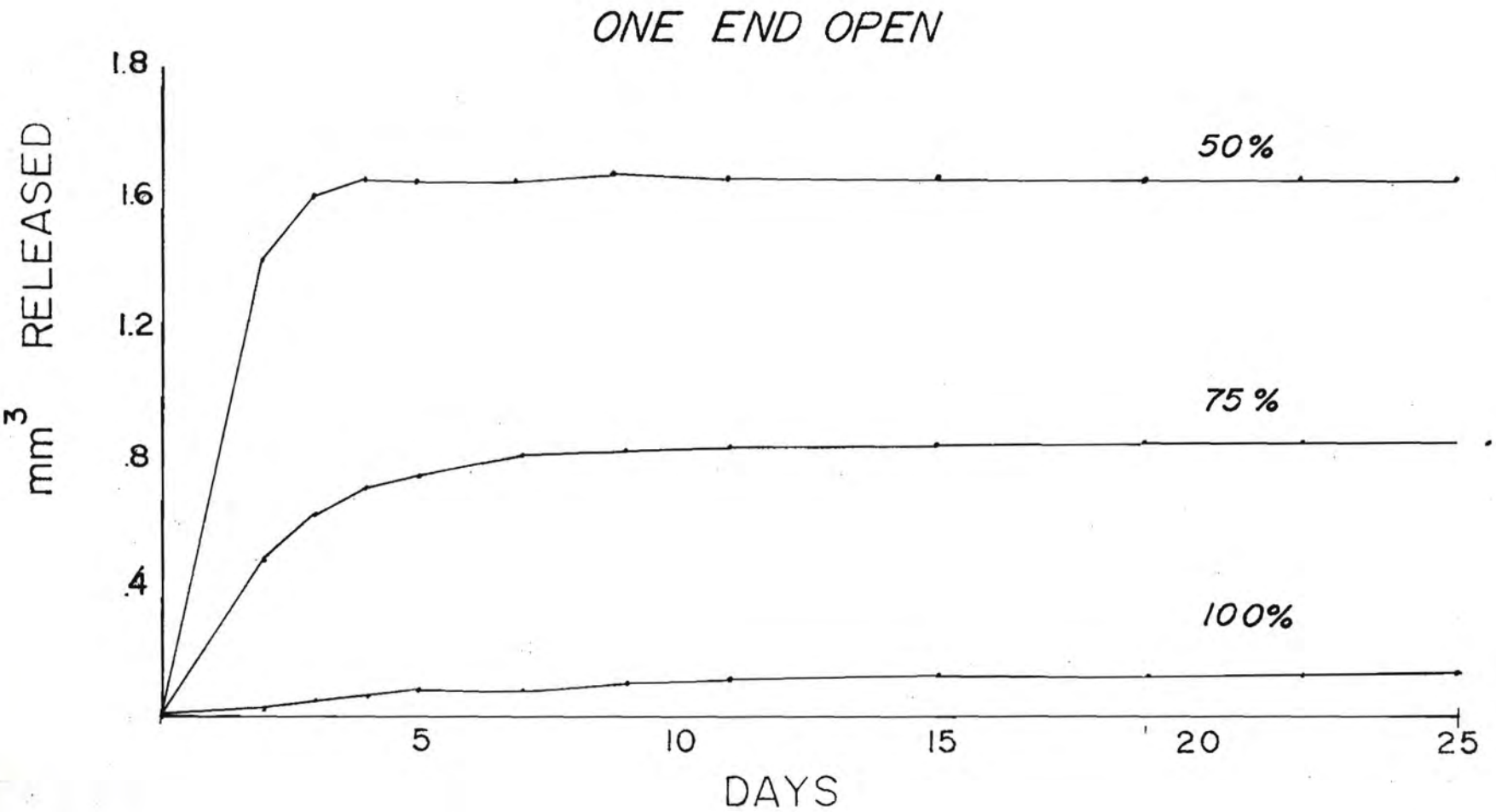
From the data presented in Table 1, it can be seen that fibers containing 100% disparlure continue to emit at a relatively constant rate over 25 days. Approximately 100 µg disparlure was released in 25 days from the closed fibers and 211 µg was released in 25 days from the fibers with both ends open. After 25 days, only ca. 3% and 6% of the disparlure was evaporated in the closed and open fibers, respectively.

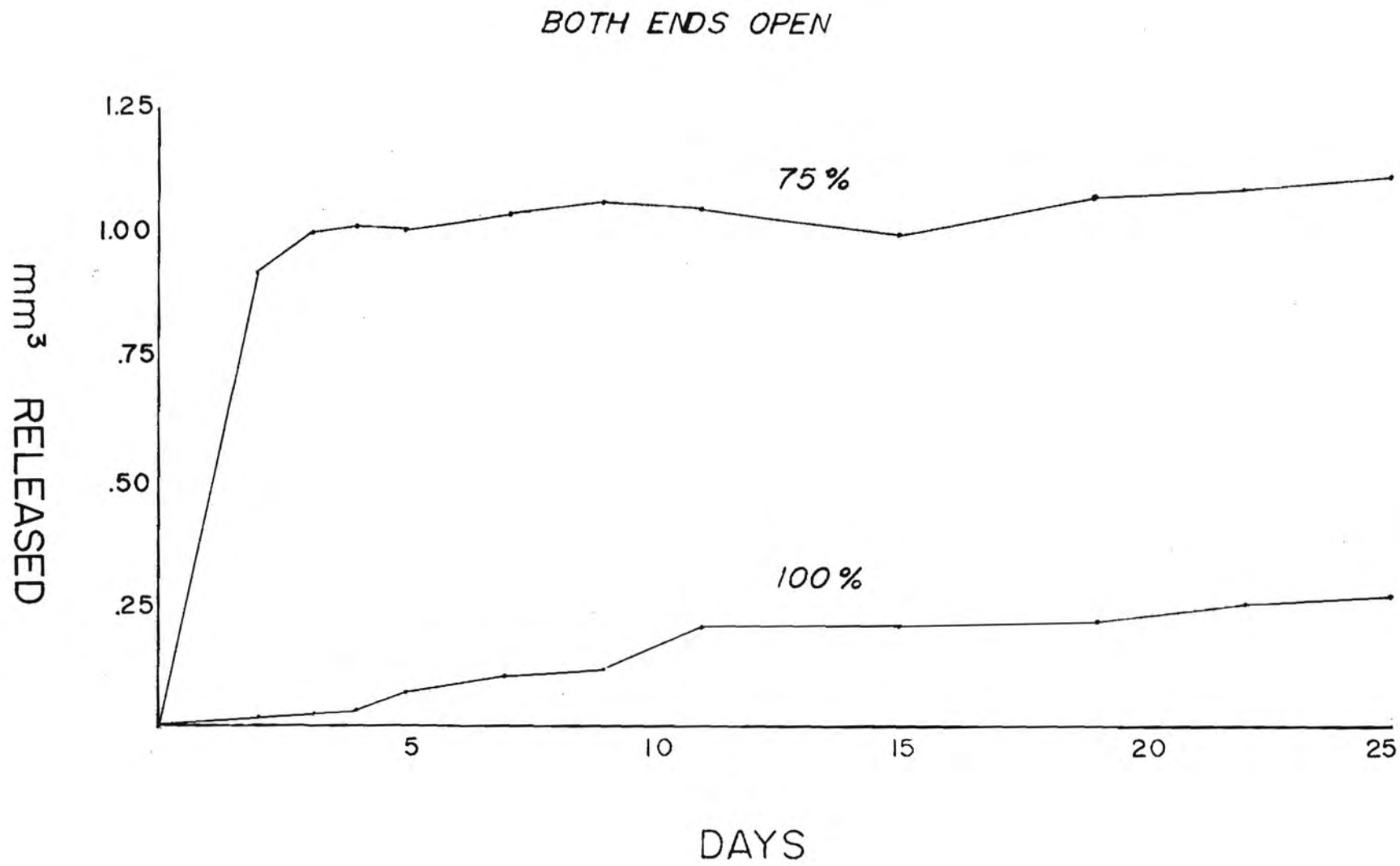
The fibers containing disparlure diluted in hexane (50% and 75%) were difficult to read because bubbles formed within them. These fibers released very small amounts of disparlure; the menisci did not recede further after 7 days. The early "burst" of emission from fibers is the result of hexane evaporation.

These data are plotted on Figure 1 to illustrate that emission curves from fibers containing diluted disparlure and sealed on one end level off after 7-9 days. Evaporation from fibers does not occur when the meniscus of the disparlure column is at least 0.5 cm from the open end. Alternatively, when both ends of the fiber are left open (Figure 2), the hexane evaporates within 3 days and disparlure release continues for 25 days. This is because the pheromone column "moves" to an open end of the fiber by capillary action.

Similar experiments were performed earlier with 8 mil and 20 mil (ID) fibers, and similar observations of the release characteristics of Conrel fibers were confirmed.

Although constant emission is achieved when fibers are loaded with 100% disparlure, the system is not useful for efficiently dispensing small amounts of material. The meniscus of the disparlure column in 20 mil fibers only recedes at .026 mm/day. A "50 day" fiber would only be 1.31 mm long.


ONE END OPEN
1.8
1.6
1.2
.8
.4
mm3 RELEASED
50%
75%
100%
5
10
15
20
25
DAYS


BOTH ENDS OPEN
1.25
1.00
.75
.50
.25
mm³ RELEASED
75 %
100 %
5
10
15
20
25
DAYS

Project Number: GM 7.2.6
Project Title: Emission of Disparlure from Cotton Wicks
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leaders: Charles P. Schwalbe, D. H. Simser, L. Mundy

Expensive and scarce (+) disparlure will be used in extensive trapping studies this season and the intervals at which test wicks (pheromone dispensers) are to be replaced must be determined. The purpose of this study was to determine the patterns of disparlure emission from cotton wicks.

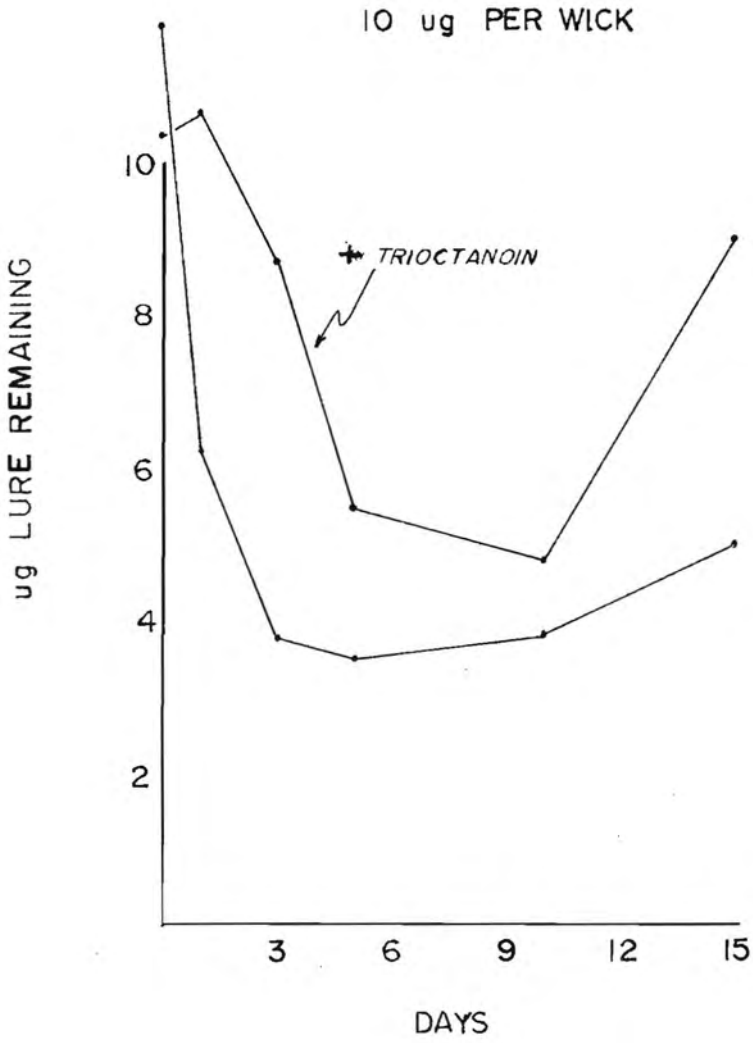
The emission of disparlure from cotton wicks to which had been added 200 µl each of the following formulations was tested. There were 10 replicates per formulation per time interval.

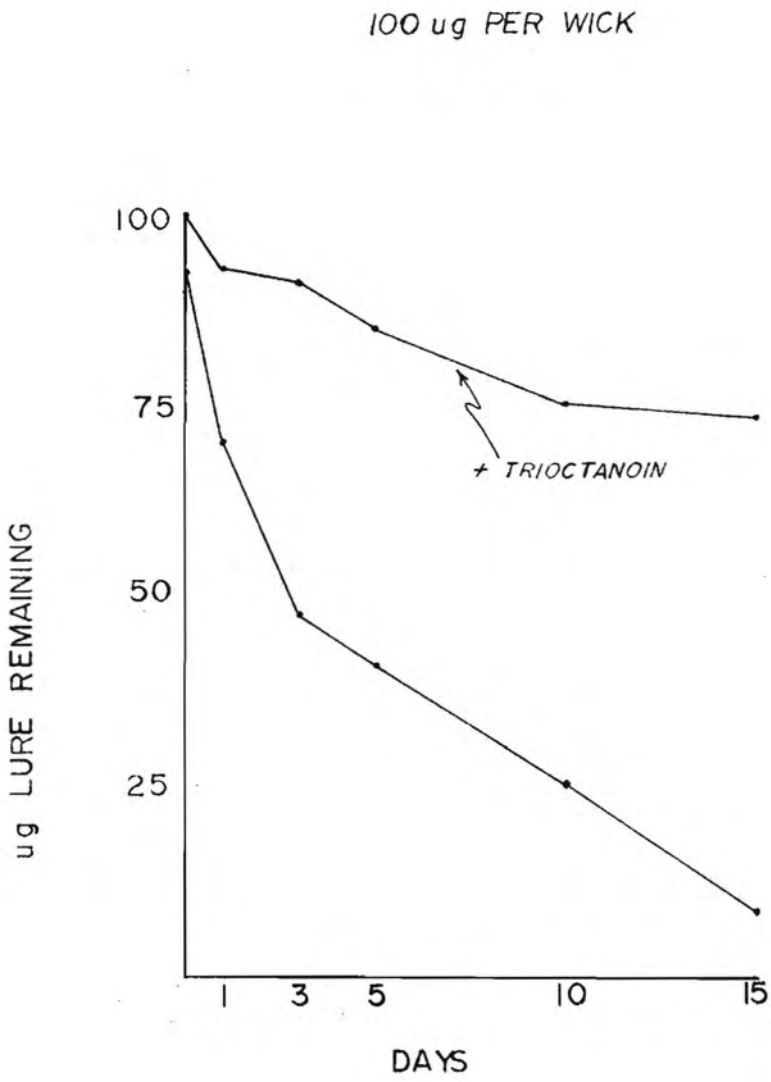
TREATMENTS

ug (+) disparlure	mg trioctanoin
10	-
100	-
1000	-
10	2
100	2
1000	2

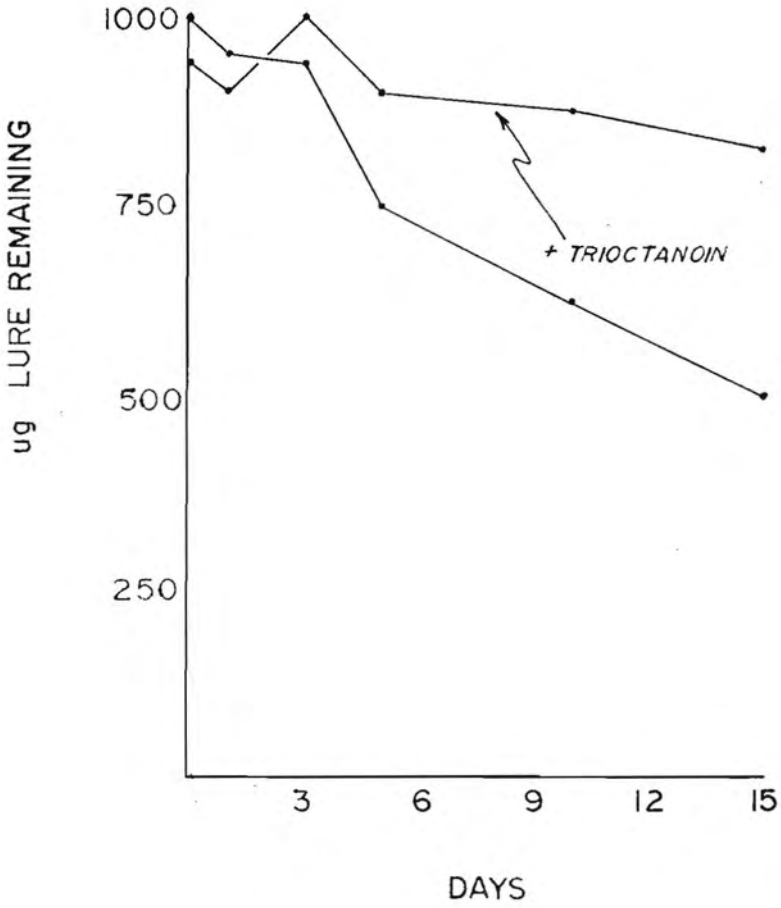
Thirteen wicks (2 of each formulation and one blank) were threaded onto a copper wire and suspended within a delta trap; no adjacent wicks were in contact. Two hundred microliter volumes of each treatment were applied to each wick and traps were suspended in an environmental chamber (26°C, 60-70% RH). A total of 30 traps were used. At time intervals of 0, 1, 3, 5, 10 and 15 days, 5 traps were randomly chosen, the wicks were removed and placed into 15 ml centrifuge tubes containing 5 ml hexane for GLC analysis (Tracor 222 Gas Chromatograph). The 10 µg treatments had to be concentrated for analysis. Residual disparlure was calculated by comparison of peak heights with those from a known standard. Results are presented in the following graphs.

In each case, the addition of 2 mg trioctanoin as keeper retarded disparlure evaporation from the wicks. Release was constant for 5, 10 and 15 days from the 10, 100 and 1000 µg (+ trioctanoin) wicks, respectively. During those periods, release rate was ca. 0.9, 5.0 and 11.7 µg/day from the 10, 100 and 1000 µg wicks, respectively. Linear release was generally shorter when trioctanoin was not added, and the amount of disparlure evaporated was greater. Constant release was observed for 1-3, 3 and 15 days from the 10, 100 and 1000 µg wicks, respectively. During those periods, 2, 17.3 and 33.3 µg/day was emitted. The 100 µg wicks released at a constant rate of 3.3 µg/day from 3-15 days after initial application. The tests with wicks containing 10 µg will be repeated. The apparent increase of disparlure after 5-10 days cannot be reconciled.


10 ug PER WICK
TRIOCTANOIN
ug LURE REMAINING
10
8
6
4
2
3
6
9
12
15
DAYS


100 ug PER WICK
100
75
50
25
ug LURE REMAINING
+ TRIOCTANOIN
1
3
5
10
15
DAYS

1000 ug PER WICK



Project Number: GM 6.3.1
Project Title: Percent Hatch of *L. dispar* Eggs as Affected by Precooling and Storage Techniques
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: John Allen Tanner and L. F. Kennedy

The currently used precooling storage technique has never been properly tested. Embryo mortality was a problem which limited the production of gypsy moth in this laboratory. This experiment investigates the possibility of reducing mortality by modifying these storage techniques.

Methods:

The following precooling (15°C, varied RH, 24L:0D) techniques were examined.

No. Egg Masses	Container	Covering	Position
50	250 ml urine cup	4 layers plastic wrap	upright (control)
"	"	"	inverted
"	"	open	upright
"	"	"	inverted
"	16 oz unwaxed Dixie®container	plastic closure	upright
"	"	"	upright

Prior to refrigeration, open urine cups were covered with four layers of plastic. Egg masses from one 16 oz Dixie® container were placed into a urine cup and covered with plastic wrap. The remaining Dixie® cup was placed into refrigeration. All urine cups were inverted and refrigerated at 4°C for 150 days. After refrigeration, 25 masses from each group were incubated (22°C, 95-99% RH, 14L:10D). This test was replicated three times.

Results:

The mean hatch rate was similar for all treatments. Eggs survived refrigeration in Dixie® cups. Dixie® cups are less expensive and easier to obtain than the currently used urine cups.

Project Number: GM 6.3.3
Project Title: Percent Hatch of L. dispar Eggs as Affected by Surface Sterilization with Clorox® and Captan®, Temperature and Relative Humidity
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: John Allen Tanner, L. F. Kennedy

Eggs were normally surface sterilized with a .1% solution of Clorox® and a 10% solution of Captan® to suppress fungal growth. This treatment has recently been replaced by a 10% Formalin solution (Shapiro and Bell, 1976). The eggs were then incubated at 26.7°C, 95-99% RH and 14L:10D photoperiod to insure rapid and uniform hatch. The objective of this investigation was to determine the effects of surface sterilization with Clorox® and Captan® and incubation temperature and relative humidity on the percent hatch of eggs.

METHODS:

This experiment was developed in conjunction with the preparation of eggs for use in colony maintenance. Nine 100-egg samples were randomly selected from a large group of dehaired egg masses. A second group of samples was taken after the eggs had been treated with a .1% Clorox® solution and a third group of samples was taken after the eggs were treated with a 10% solution of Captan®. Three 100-egg samples from each treatment were incubated at 22°C, 95-99% RH and 14L:10D photoperiod, the remaining samples were incubated under 26.7°C, 60-70% RH and 14L:10D photoperiod. Percent hatch was based on the number of embryonated eggs. The test was replicated 10 times over a period of time.

RESULTS:

The low colony hatch was not caused by treatment of eggs with Clorox® and Captan® (Table 1). The temperature and relative humidity conditions of incubation slightly affected the hatch rates (Table 1). Eggs incubated at 22°C and 95-99% RH produced a higher percent hatch than eggs incubated at 26.7°C and 95-99% RH and 26.7°C and 60-70% RH respectively.

Table 1. Mean hatch rates of gypsy moth eggs as affected by surface sterilization and incubation temperature-relative humidity combination.

Temperature	Relative Humidity	Treatment		
		Dehaired	Clorox	Clorox+Captan
22.0°C	95-99%	45.9	54.2	46.9
26.7°C	95-99%	39.2	41.7	38.9
26.7°C	60-70%	31.4	30.6	22.8

REFERENCES:

Shapiro, M. and R. A. Bell, 1976. Gypsy Moth Mass Rearing: Evaluation of Method for Surface Sterilization of Eggs and/or Pupae, April 1976 - September 1976. Laboratory Report, Gypsy Moth Methods Development Laboratory: 50-53.

Project Number: GM 6.3.4
Project Title: Gypsy Moth Mass Rearing: Evaluation of Methods for Surface Sterilization of Eggs and/or Pupae
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: Martin Shapiro, R. A. Bell

It was previously shown that immersion of eggs in 10% Formalin for 60 minutes had no detrimental effect upon larval hatch and development (see April-September 1976 Laboratory Report). Moreover, a 30 minute Formalin treatment had a greater viricidal effect than Clorox but was not 100% effective.

In all previous tests, egg masses were used which contained "natural" virus. Unfortunately, the actual amount of virus associated with the egg masses could not be determined. Therefore, a series of tests were initiated to (1) determine a dose-mortality response for gypsy moth nucleopolyhedrosis virus (NPV) and (2) compare the effectiveness of Formalin and Clorox as viricides. To accomplish this, it was necessary to devise methods for artificially infecting egg masses with known NPV concentrations.

Inoculation of egg masses with known NPV concentrations

The initial problem was that of contaminating the surface of egg masses with suspensions of polyhedral inclusion bodies (PIBs). Since a water suspension of PIBs will not adhere to egg masses, an effective sticker was sought. Triton X-100, a commonly used surfactant, was used as a sticker at 1% (v/v). Dilutions of PIBs were made in Triton X-100 and 1 ml of virus material was absorbed by the egg masses. After treatment, the masses were air dried and maintained at 26°C. Upon hatching, neonates were placed on standard hornworm diet and were incubated at 26°C. Virus mortality was assessed and surviving larvae were reinfested in 16 ounce Dixie containers plus diet. Mortality readings were taken until pupation.

One-half of each egg mass was removed after drying, rinsed in running tap water for 60 minutes and then dried. After drying, the masses were handled as previously described. The purpose of this test was to determine the effectiveness of Triton X-100 as a sticker, e.g., whether a water rinse would remove Triton X-100 containing PIBs.

Virus concentrations of $5x10^2$-$5x10^7$/ml were used (Table 1).

Table 1. Effectiveness of Triton X-100 as a sticker for gypsy moth NPV.

PIB Concentration (ml)	% Virus Mortality	
	No water rinse	Water rinse
0	0	0
$5x10^2$	0	0
$5x10^3$	0	2.2
$5x10^4$	6.2	26.6
$5x10^5$	0	22.2
$5x10^6$	0	42.0
$5x10^7$	100	100.0

From these data, it would appear that Triton X-100, at 1%, is inhibitory to gypsy moth NPV. Only at very high virus concentrations, i.e. 5x107 PIB/ml, was any activity observed. Water rinsing apparently removed excess Triton X-100 but it was not known whether all virus activity had been retained.

Since Cardinal and Smirnoff (1973) successfully used "Chevron" as an adhesive for gypsy moth NPV on egg masses, this material was evaluated for our studies. The efficiency of sticking and spreading was greatly enhanced when Triton X-100 was added in very low amounts. Moreover, water rinsing did not remove the material from the egg surface nor reduce viral activity.

A bioassay was run, utilizing PIB concentrations from 5x10/ml to $5x10^6$/ml on second instar larvae (Table 2).

Table 2. Effectiveness of Chevron-Triton X-100 as a sticker-spreader for gypsy moth NPV. 1/

PIB Concentration/ml	Percent Virus Mortality 2/
0	0
5x10	0
$5x10^1$	16
$5x10^2$	33
$5x10^3$	59
$5x10^4$	100
$5x10^5$	100
$5x10^6$	100

1/ PIB suspensions diluted in Chevron-Triton X-100, placed on egg mass and air dried.

2/ Virus assayed against 2nd instar larvae; 26°C.

From Tables 1 and 2 it is quite apparent that Triton X-100 (1%) was inhibitory to GM NPV ($LD_{50}=5x10^6$ PIB) in comparison to the Chevron-dilute Triton X-100 ($LD_{50}=5x10^3$ PIB). The dose-mortality response now serves as a baseline for evaluation of Clorox vs Formalin disinfection.

Evaluation of Clorox vs Formalin as a viricide

In this series of tests, Formalin and Clorox were diluted 1:10 and masses were treated for 30 or 60 minutes and then rinsed for 30 or 60 minutes. Neonates were infested on virus-free diet and mortality readings were taken. Data are summarized in Table 3.

Clorox was effective as a viricide at NPV concentrations up to $5x10^5$/ml/egg mass. At higher virus concentrations ($5x10^6$), Clorox was ineffective. Formalin, however, was effective at the highest NPV concentration used.

Table 3. Effectiveness of Formalin and Clorox in suppression of NPV on gypsy moth egg masses.

	Virus Induced Larval Mortality				
		Formalin		Clorox	
Treatment	0	30	60	30	60
No virus	0	0	0	0	0
Virus					
$5x10^3$	68	0	0	0	0
$5x10^4$	95	0	0	0	0
$5x10^5$	100	0	0	0	0
$5x10^6$	100	0	0	76	82

A second series of tests were conducted utilizing Clorox and Formalin (1:10 dilution, 30 or 60 minutes) and the highest NPV concentration (Table 4). This test confirms the earlier findings that Formalin was a more effective viricide than Clorox at high virus concentrations. As a result of these tests, Formalin (1:10, 60 minutes) is now routinely employed in the APHIS mass rearing facility and has work very effectively.

Table 4. Comparison of Formalin and Clorox in suppression of gypsy moth NPV.

Treatment	% Virus-Induced Larval Mortality				
	Control	Formalin		Clorox	
		30	60	30	60
Egg masses w/o inoculated virus	0	0	0	0	0
Egg masses treated with $5x10^6$ PIB/ml/mass	100	0	0	100	96

Reference

J. A. Cardinal and W. A. Smirnoff, 1973. The experimental introduction of nuclear polyhedrosis against Porthetria dispar L. (Lepidoptera: Lymantriidae) in the forest. Phytoprotection, 54 (1): 48-50.

Project Number: GM 6.3.5
Project Title: Gypsy Moth Mass Rearing: Evaluation and Development of More Economical Diets and More Efficient Techniques for Rearing and Handling Larval Gypsy Moths
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leaders: Robert A. Bell and O. T. Forrester

Simplified Wheat Germ-Casein and Wheat Germ-Soybean Formulations

Several formulations which contained only wheat germ and casein or soy protein as the only nutrient source were tested against the modified tobacco hornworm diet for rearing gypsy moths. Insects were aggregately reared from neonates to 4th instar larvae in 3 ounce transparent plastic cups (ME-3; Sweetheart Plastics) with paper lids at 10 larvae/cup. Larvae were then transferred to 16 ounce unwaxed Dixie cups with transparent plastic lids (12 larvae/cup); each container was provided with about 90 ml of diet. Larvae were allowed to terminate feeding and pupate in the 16 ounce cup. After pupation was completed, pupae were sexed, weighed and placed in additional 16 ounce cans for adult emergence. Records were kept on time to pupation and adult emergence, pupal and egg mass weights and adult yield. Generally, two strains (i.e., a wild and colonized strain) were tested simultaneously. Exceptions to this procedure are noted in subsequent tests.

The simplified high wheat germ diet compared favorably with the modified hornworm diet (Table 1). There were no differences in performance between the wild and colonized strains reared on either diet except for developmental time. Time to pupation was about 4 days longer for the wild strain. Slightly, but not significantly, heavier pupae were obtained on the hornworm diet which also resulted in slightly higher egg mass weights. In view of these findings, further investigations were carried out using primarily the high wheat germ diet (Table 2). The cost of the high wheat germ diet is $0.33/liter compared to $0.50 for the modified hornworm diet.

A second series of experiments were carried out in which the wheat germ level in the high wheat germ diet was varied from 4-14% whereas the casein was held constant at 2.5%. To keep the total weight of the diet formulations constant, non-nutritive cellulose was used. The results of these tests (Table 3) are expressed in terms of growth response as measured by the larval weight at 28 days following infestation of the neonates. In this test, the neonates were placed in translucent plastic cups (Sweetheart Plastics; XE6) with paper lids at 10 larvae/cup and reared through larval development without a change of diet. Results show that the optimal level of wheat germ is ca. 8%; higher levels did not result in better growth. Also, mainly because of their faster rate of larval development, the NJF_{15} colonized strain was slightly heavier than the wild strain.

In the second part of this experiment, the wheat germ was held constant at 12% and the casein level was varied from 1 to 3%. Growth was fairly comparable in all the formulations (Table 4). Since the wheat germ contains about 25% protein, it is reasonable to assume that it alone can supply the insect with all or a major part of the protein requirements. Tests are in progress in which gypsy moths are being reared on a wheat germ diet without any supplemental protein. Also, tests are in progress to determine the minimal protein requirements for this insect.

Comparison of Various Milk and Soybean Proteins

Since milk protein varies greatly in cost, i.e., from $0.70/lb for crude industrial casein or skim milk powder up to over $2.00/lb for highly purified casein, a test was conducted to compare the development of gypsy moths on diets formulated with various grades of casein. Also included in this test was Supro 610, a soybean protein product obtained from the Ralston Purina Company, St. Louis.

Although the most rapid growth was obtained with the more expensive, highly purified caseins (Teklad and ICN Hi-Nitrogen), good growth and development was also obtained with the crude caseins and soybean protein. Generally speaking, the more refined caseins allowed the insect to reach the pupal stage about a day earlier than the other protein sources. As a protein supplement, there seems to be no economic justification for using the more highly refined proteins.

Agar Substitutes

Since agar comprises over 50% of the total dietary cost, considerable effort has been made to reduce the agar content or to find suitable alternative gelling agents. One advantage of the high wheat germ diet is that it can be formulated with 25% less agar than used in the previous diets and still produce a strong gel which results in a considerable reduction in cost. A series of experiments were carried out with formulations that contained carrageenan (HWG) at 1, 1.2 and 1.5%, HWG + locust bean gum at 1 + 0.5% and agar + locust bean gum (1 + 0.5%). These formulations were compared with the standard high wheat germ diet with 1.5% agar.

The results showed that the diets formulated with HWG produced gels that were as good or better than those produced with agar. Growth and development of larvae reared on diets with HWG vs agar were comparable. Concentrations of HWG above 1%, or addition of locust bean gum, did not significantly improve the gelling properties of the diet or growth response of the larvae. The cost of the high wheat germ diet with 1% HWG is about $0.24/liter or $0.86/gallon. The physico-chemical properties of HWG, however, are different than that of agar. Thus, HWG gels at a much higher temperature. When using HWG, it is advisable to hold the processed diet at a minimum of 80°C with frequent stirring during holding and dispensing while minimizing exposure of diet to the air. Moreover, HWG should be dissolved in very hot or boiling water and nutrients or dry ingredients added while the water-HWG mixture is above 80°C.

Effect of pH

This test involved the high wheat germ-casein and high wheat germ-soy protein formulations with and without microbial inhibitors (methyl paraben and sorbic acid). The purpose of the test was to determine if pH of the diet affected development and incidence of microbial contamination. The diet was prepared in 1 liter batches in a Waring Blendor, and the pH adjusted by addition of concentrated HCl or 4 M KOH. PH was determined on hot blended diet using a Beckman SS-3 pH meter. Previous studies had shown little difference in pH readings obtained from dispersed ingredients in cold water and hot (90°C) blended diet. Diet was poured into 6 ounce (Sweetheart XE6) plastic cups, infested with 10 larvae/cup and reared through larval development without changing the diet. Containers were inspected daily for microbial contamination. Larvae from the various treatments were sexed and weighed at 27 days after infestation. The pH of the different treatments ranged from 3 to 8.

Elimination of the anti-microbial agents from the diet resulted in contamination of all diets with a pH of 4 or above. Larvae grew poorly at pH levels below 4. Anti-microbial agents were ineffective in suppressing contamination at a pH in excess of 6.5. Contamination was greater on the wheat germ-casein than on the wheat germ-soy protein. Growth of the larvae was retarded at pH 7 or above in both diets. It is concluded that a pH ranging from 4.5 to 6.5 is most satisfactory for rearing the gypsy moth with contamination problems being minimal toward the more acid end of this pH range. However, since the natural pH of both diets falls within a range of 5.3-5.8, no considerable advantage can be gained by either increasing or decreasing the pH level.

Effect of Temperature and Cooking Time on Diet

To determine whether the temperature to which nutrients were exposed during diet preparation affected nutritive quality of the diet, the following series of tests were carried out. Diets (modified hornworm vs high wheat germ) were prepared in a 1 gallon Waring Blendor. Nutrients (dry ingredients) were added to the water-agar mixture at 100, 90, 80 and 70°C. Another preparation was autoclaved at 15 psi and 250F for 30 minutes. Diet was poured into 6 ounce (XE6) cups, infested at 10L/cup and the insects were reared to pupae without a change of diet. In another test designed to simulate diet preparation conditions in a mass rearing context, nutrients were added to the water-agar mixture and the diet was held at 80°C for 30, 60, 90 and 120 minutes before pouring.

With the hornworm diet, growth response was poor even in the control so this portion of the test will have to be repeated. Thus the results mentioned here concern only the high wheat germ diet. There was little difference in the effects of different temperatures or cooking time on larval growth. In fact, blending ingredients in at a higher temperature (90-100°C) may have improved the diet particularly with regard to water holding properties. Although further studies need to be conducted on this matter, it appears that the gypsy moth can tolerate considerable variations in nutrient level. Nutrient loss due to temperature during diet processing appears to be less significant than that lost via hydro-

lysis and oxidation during rearing. It is suspected that the additional wheat germ involved in the high wheat germ diet protects against deterioration of the diet during the prolonged rearing period. The presence of raw sugar (sucrose) which is used in the hornworm diet enhances oxidative browning. Future efforts in improving the diet need to be aimed at evaluation of bulking constituents for enhancement of water binding properties, anti-oxidants, improved containers (for optimal moisture retention) and possible use of vacuum processing of the diet to prevent excessive oxidation if this turns out to be a significant factor.

Sex Ratio and Sex vs Time of Hatch

It has been reported that in the gypsy moth there is a sex difference in the time of larval hatch. During the past several months we have been studying this behavior to determine if we can take advantage of it to obtain predominantly male larvae for infesting production stock for the sterile male program. The potential advantages in terms of reduced rearing costs for the sterile male technique are considerable. Females, in contrast with males, consume 3-4 times as much diet and require proportionally more container and shelf space for rearing not to mention the additional labor cost of rearing the un-needed females.

To date, our studies have concentrated in 3 areas: (1) to determine the extent of sexual differentiation in time of hatch in colonized vs wild strains, (2) to determine the degree of natural variation between individual egg masses and (3) to exploit the sexual differentiation phenomenon for mass production of the desired sex. It is worth mentioning here that techniques for production of predominantly female larvae is also a desired goal since Martin Shapiro of this laboratory has determined that the females produce 2-3 times more NP virus inclusion bodies than males.

To determine sex differences in time of hatch, egg masses were selected at random, individually dehaired and surface disinfected with 10% formalin. Generally, 50 or 100 eggs were randomly removed from each mass and placed in individual one ounce plastic cups. Those larvae that hatched each day were removed and placed in XE6 containers at 10/cup. Larvae were reared up to the final larval instars (usually 26 to 28 days after infesting) and the number of male and females scored.

The hatching profiles in terms of sex ratio vs time of hatch for a colonized and 2 wild strains are shown in Figures 1 and 2a and b. The colonized (NJF15) strain (Figure 1) showed a predominance of females hatching during the first 2 days of the hatch period; on the third day and beyond, most of the larvae were males. Although the overall sex ratio approached 50:50, during the latter half of the hatching period (i.e., after the second day), the sex ratio was about 60:40 in favor of males. Although the data are not presented here, we have noticed considerable variation between egg masses; in some the ratio exceeds 80:20 during the latter half of the hatching period. However, in other cases, the sexual pattern is reversed and males predominate during the

the early hatch period. We are pursuing the matter further to determine the potential for selection of strains with the desired hatching patterns.

Considerable sex ratio distortion occurs in certain wild populations which may be useful as an indicator of population quality. For example, egg masses (Figure 2a) collected from Dighton, MA yielded a preponderance of males throughout the hatching period (overall sex ratio = 60:40 ♂♂:♀♀). The Dighton area was heavily infested with gypsy moths and suffered high mortality from NP virus and parasites. By contrast, egg masses collected near Stroudsburg, PA yielded sex ratios and a sex vs time of hatch profile similar to that of the NJ colonized strain (Figure 2b). The PA strain was taken from an area with low virus infection and little parasitism. The Dighton strain showed poor growth response on artificial diets whereas the PA strain showed excellent growth response. Further studies with the PA strain are in progress.

From the studies conducted to date, the prospects of rearing a predominantly male or female population by infesting larvae that hatch either earlier or later in the hatch period appear encouraging. It is conceivable that techniques may be developed shortly wherein two-thirds of the insects reared would consist of either sex.

Table 1. Performance of a colonized (NJF_{15}) vs wild (PA) strain of gypsy moth reared on two artificial diets.

	Modified Hornworm		High Wheat Germ	
	NJF_{15}	PA(wild)	NJF_{15}	PA(wild)
Days to pupation(♂♂)	30.9(1.4)[1]	34.4(2.1)	31.6(2.1)	35.4(2.0)
Days to pupation(♀♀)	35.0(2.0)	38.9(4.0)	35.8(2.7)	40.1(3.3)
Days to adult(♂♂)	43.6(1.5)	47.1(2.5)	43.8(1.4)	48.1(2.0)
Days to adult(♀♀)	45.6(2.0)	49.7(3.5)	46.2(2.7)	50.2(3.1)
Pupal wt (g) ♂♂	.62(.10)	.55(.06)	.59(.06)	.53(.06)
Pupal wt (g) ♀♀	1.8(0.38)	1.9(0.30)	1.6(0.3)	1.7(.41)
Egg mass wt (mg)[2]	449(71.7)	484(119.8)	408(90.6)	416(52.8)
Adult yield %	86.4	77.1	76.7	84.0

[1] Figures represent mean values with standard deviations in parentheses. Results based on observations of 80-100 insects/treatment except for egg masses where N = 5 samples/treatment.

[2] To estimate number of eggs/mass, multiply weight by 1.33.

Table 2. Composition of high wheat germ diet used for rearing gypsy moth.

	Source	Amt/liter
Wheat germ	Niblack Foods	120 g
Casein	Milk Specialties	25 g
Salt mix, W.	ICN	8 g
Sorbic Acid	ICN	2 g
Methyl paraben	Tenneco Chemicals	1 g
Vitamin premix #26862	Hoffman LaRoche	5 g
Agar [1]	Moorehead and Co.	15 g
Total cost/liter = $0.33 (with HWG = 0.24)		

[1] Gelcarin HWG (Marine Colloids) at 10g/L (or 1%) may be used instead of agar.

Table 3. Effect of concentration of dietary wheat germ on larval growth of wild and colonized gypsy moths (N = 80-100 insects/treatment).

Diet	Concentration of W. germ	Casein	Cellulose	LARVAL WT (G) 1/ DAY 28 NJF15 ♂♂	NJF15 ♀♀	PA (wild) ♂♂	PA (wild) ♀♀
1	4%	2.5%	8%	.71 (.07)	1.92 (.20)	.58 (.06)	1.70 (.17)
2	6%	2.5%	6%	.89 (.10)	2.30 (.08)	.67 (.04)	2.04 (.05)
3	8%	2.5%	4%	.90 (.14)	2.70 (.26)	.78 (.08)	2.54 (.45)
4(std)	12%	2.5%	-	.70 (.02)	2.50 (.28)	.66 (.04)	2.30 (.02)
5	14%	2.5%	-	.75 (.03)	2.70 (.20)	.66 (.04)	2.42 (.14)

1/ Figures represent the $\bar{x}$ weights and standard deviations (in parentheses) of 4 samples of larvae (N = 10/sample).

Table 4. Effect of level of dietary casein on larval growth of wild and colonized gypsy moths (N = 80-100 insects/treatment).

Diet	Concentration of Casein	W. germ	Cellulose	LARVAL WT (G) - DAY 30 NJF15 ♂♂	NJF15 ♀♀	PA (wild) ♂♂	PA (wild) ♀♀
1	1.0%	12.0%	1.5%	.78 (.02)	2.8 (.41)	.78 (.02)	2.7 (.26)
2	1.5%	12.0%	1.0%	.76 (.02)	3.1 (.29)	.77 (.06)	2.7 (.44)
3	2.0%	12.0%	0.5%	.85 -	3.2 (.18)	.76 (.04)	2.9 (.35)
4(std)	2.5%	12.0%	-	.73 (.03)	2.7 (.03)	.78 (.05)	2.5 (.41)
5	3.0%	12.0%	-	.79 (.06)	2.7 -	.80 (.06)	2.6 (.31)

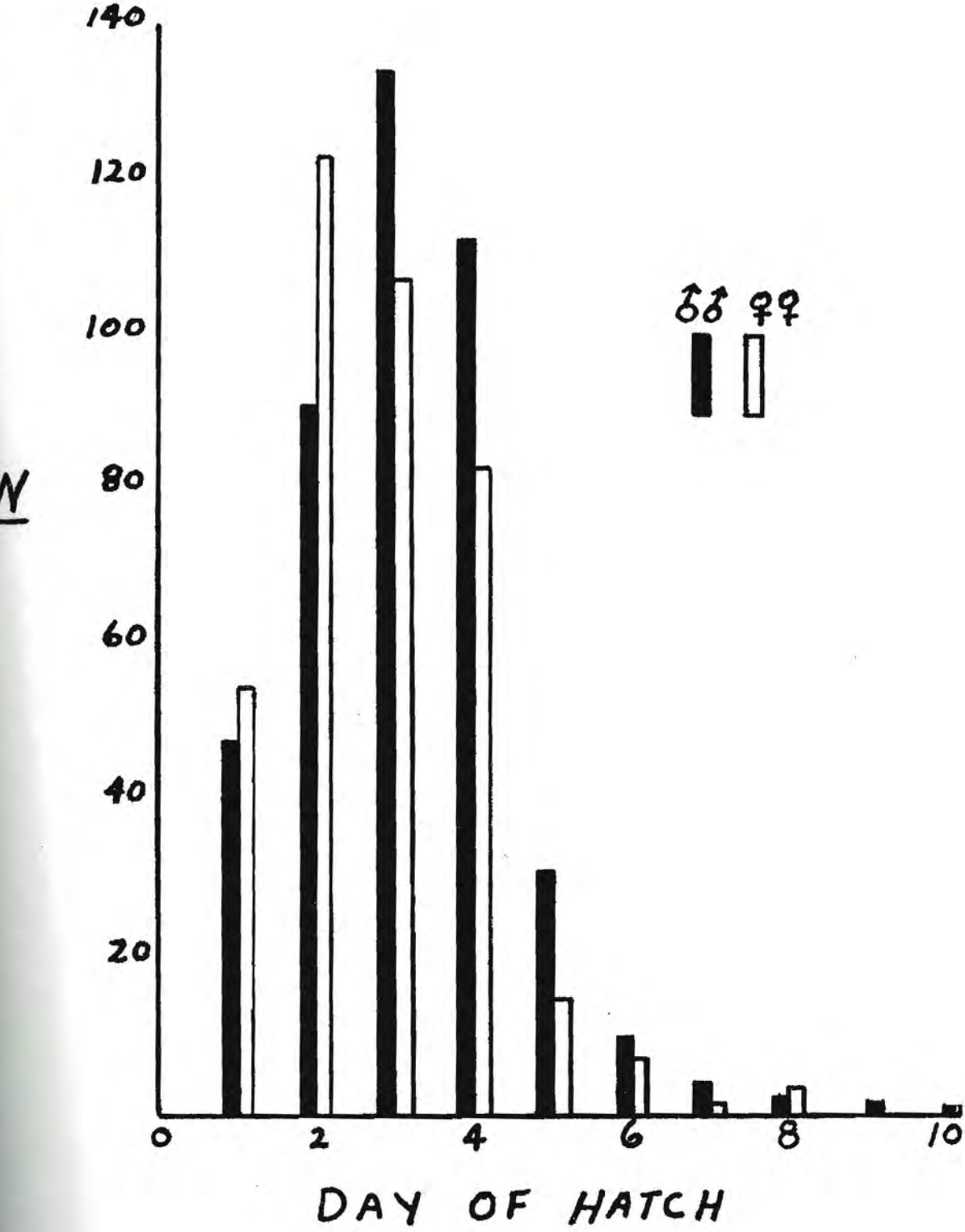
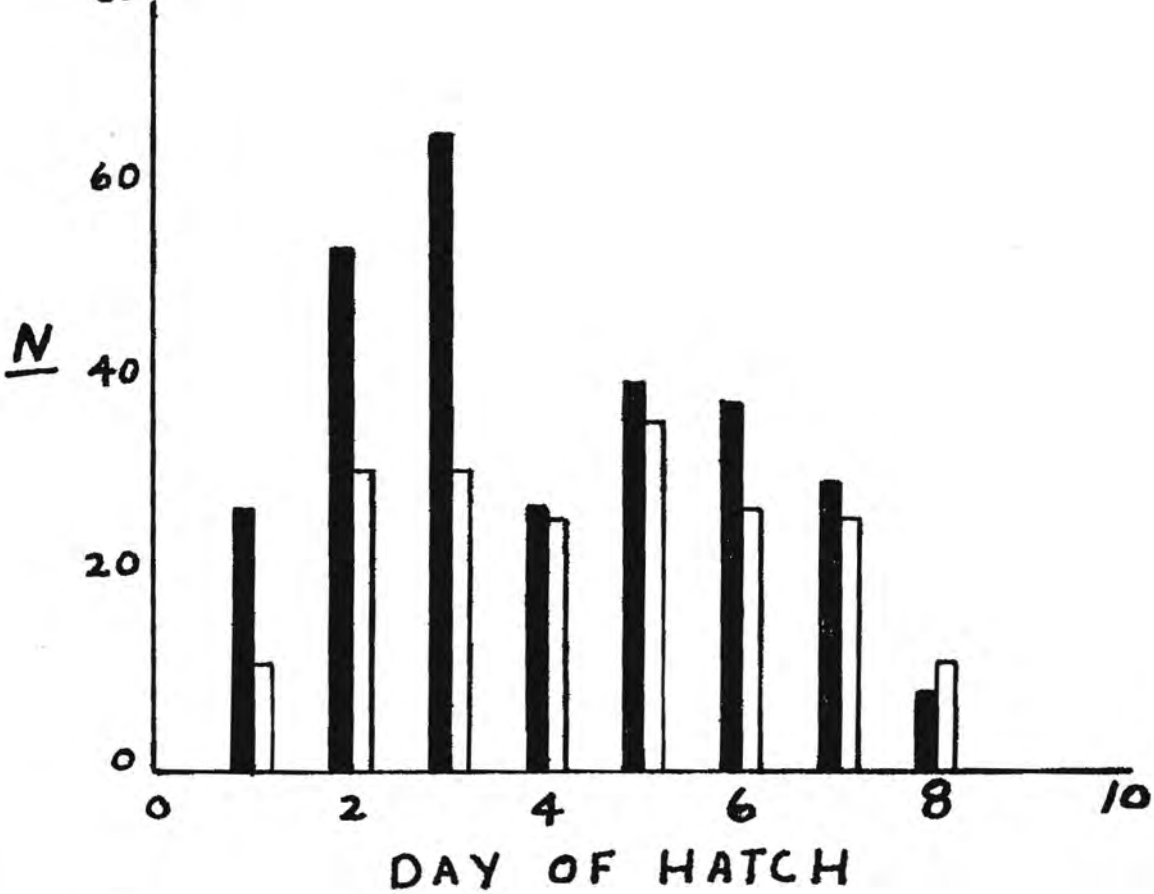


Figure 1. Proportions of males and females in relation to time of hatch (NJF_{14} colonized strain; eggs chilled 5 months at 5°C).



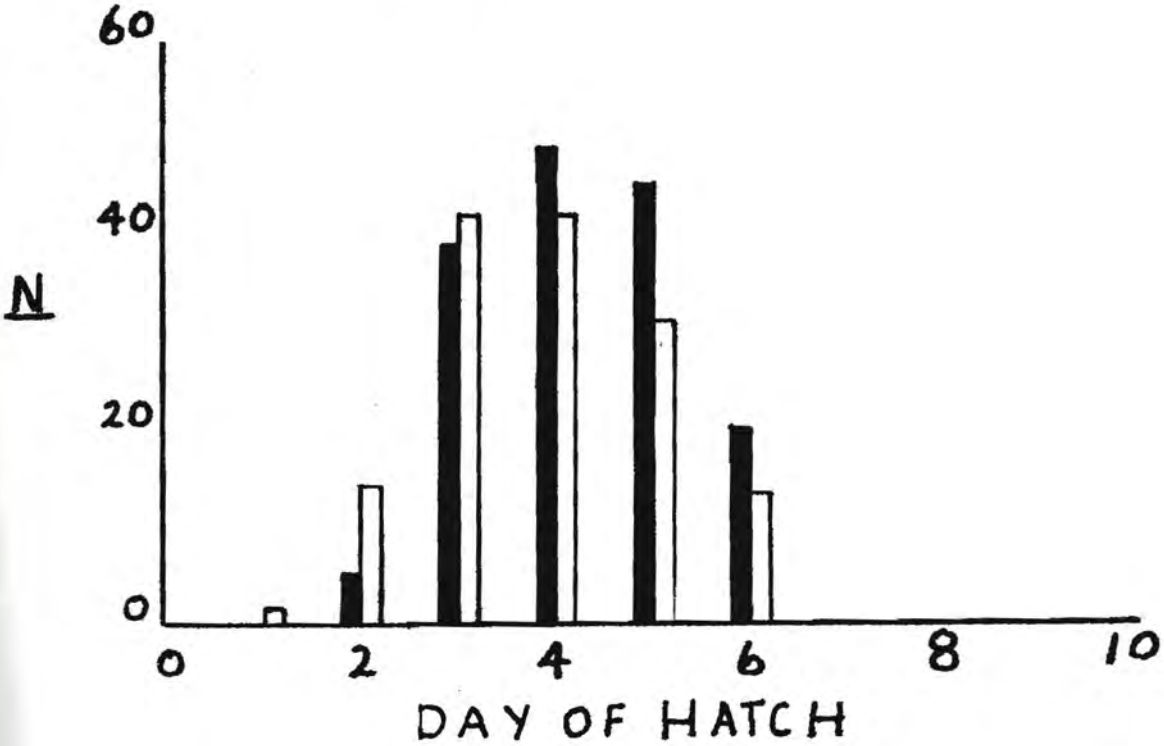


Figure 2. Proportions of males and females in relation to time of hatch (a) Dighton, MA field collected eggs (b) Stroudsburg, PA field collected eggs.

Project Number: GM 6.3.6
Project Title: Establishment of Standards for Monitoring Performance and Quality of Mass Reared Gypsy Moths
Report Period: October 1, 1976 - March 31, 1977
Report Type: Preliminary
Project Leaders: Robert A. Bell, M. Shapiro, C. Schwalbe, V. Mastro

This work is still in the early stages of evaluation. Quality standards are being established from observations on the performance of a healthy wild strain from Stroudsborg, Pa. Also, background information will be developed this summer on pheromone production and response in field collected moths as well as F_1 lab-reared and long term colonized strains. In addition, diapause characteristics and mating competitiveness of wild vs lab-reared stock will be compared. Quality assessment of strains for virus yield and produced efficacy are in progress.

Project Number: GM 6.3.7
Project Title: Monitoring for Pathogens and Adventitious Microbes Associated with Gypsy Moth Rearing and Development of Effective Sanitation Procedures
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leaders: Martin Shapiro, R.A. Bell, C.D. Owens

Simple procedures are being developed to monitor and maintain quality of (1) diet ingredients with respect to nutritive value and microbial contaminants. Nutritive value can be determined by observing developmental rate and uniformity in addition to larval weight at 25 days (when they can be easily sexed). However, progress in this area is dependent upon present tests to determine the minimal nutritional requirements particularly with regard to protein, lipid and vitamin components. Deviations in quality of gelling agents can be determined by rate of water loss in a standardized environment.

Microbial contaminants have been isolated from various dietary ingredients and are being cultured for positive identification. Major sources of contamination ranked from highest to lowest are (1) raw wheat germ, (2) torula yeast, (3) casein and soybean protein, (4) vitamin mix and (5) agar. Trace amounts in sucrose and salt mix are considered insignificant.

The incidence of microbial contamination on prepared diet is a seasonal phenomenon; the occurrence is lowest during the winter and highest in the summer. We will be monitoring microbial levels throughout the seasons of the current year. Observations to date indicate that microbial contamination within the rearing facility originates primarily from (1) egg masses, (2) diet ingredients and (3) personnel. The contamination can be controlled by disinfection of egg masses, use of appropriate microbial inhibitors in the diet, control of humidity or moisture and ventilation in the rearing containers and by proper care on the part of personnel to observe certain sanitation procedures.

Upon completion of the monitoring work now in progress, we will issue a set of recommendations or procedures for monitoring and controlling microbial contamination in the mass rearing facility.

Project Number: GM 6.3.8
Project Title: Development of Methods for Avoiding Contact with Insect Scales and Urticating Hairs Associated with Adults, Eggs and Larvae of the Gypsy Moth
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Investigators: Charles D. Owens, J. G. R. Tardif, R. A. Bell

To reduce the incidence of airborne insect scales and hairs, the prototype rearing complex was equipped with clean air (HEPA) filters. The diet preparation and larval infesting area was equipped with HEPA filters in addition to the two rearing units. The filters are rated at 95% efficiency in removal of airborne particles down to 0.3 μ in diameter. After 3 months of operation, with fairly heavy traffic at times, no particular problems with insect setae have been encountered.

The most troublesome area with regard to insect scales is the room in which moths are harvested and mated for egg production. Several hundred moths are handled in the area each day and special precautions were taken to protect the workers. The dust collecting equipment involved was described in the previous report (April - September, 1976, p. 64). The cyclone separator is effective in removal of intact moths and most of the dislodged scales are drawn in and collected by the bag filter unit. Some of the fine particles, however, escaped through the bag filter and were returned into the moth room in the recirculating air stream. Attempts to remove all of these particles with an electrostatic precipitator mounted in the return duct were unsuccessful . Placement of a HEPA filter (99.97% efficient) in the return duct, however, appears to have taken care of the problem. Currently, all moths, upon emergence are transferred to oviposition containers in the confinement of special hoods which are ducted directly to the cyclone separator and dust collector. The hoods are constructed so that the area through which the air stream is drawn decreases from the front to the rear.

Thus the high suction force at the rear of the hood (up to 1500 ft/min) enables the worker to quickly remove moths from the oviposition containers as well as the fine insect scales. The containers in which the moths are held for mating and oviposition are placed in a wall mounted cabinet with plexiglass doors. Ducts connecting the bottom of the cabinet with the ductwork to the dust collecting equipment, convey moth scales from the cabinet facility. At present the moth room seems to be working satisfactorily.

A portable sampler for monitoring non-viable airborne contaminants was recently purchased. The sampler consists of several stages with each stage consisting of filters for trapping increasingly smaller particles. The sampler is designed to simulate the distribution of airborne particles that enter the human respiratory tract. When this unit was placed in the moth room and allowed to run for up to 4 hours, the amount of collected contaminants was insufficient for determination by gravimetric means.

The monitoring of airborne contaminants will be carried out over the the next several months and the amount of contaminants determined by a more sensitive method involving direct microscope counts.

Two work stations equipped with pre- and HEPA filters were constructed for use in transferring or handling insects. These were designed to draw air from the work bench area (away from the worker) and up through the filters thus discharging the clean air back into the room. These units are inexpensive to build and permit the worker to handle all stages of the insect without being exposed to urticating hairs and scales.

Project Number: GM 6.3.9
Project Title: Evaluation and Development of Automated Procedures for Rearing Gypsy Moths
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Investigators: Charles D. Owens, J. G. R. Tardif, R. A. Bell

Previously used equipment for preparing and dispensing diet at the APHIS mass rearing facility involved (1) a 30 gallon steam jacketed kettle (2) a transfer pump and an automatic filling machine for dispensing diet into 1 oz. creamers and (3) a capper to automatically place plastic closures on the cup. The cups were filled with diet, capped and placed in a refrigerated room until needed.

There were several disadvantages inherent with this type of equipment; (1) the kettle was designed mainly for bakery use and, therefore, had limited mixing capability (low rpm stirrer and scraper), (2) batch sizes under 20 liters could not be mixed, (3) the filling machine could only handle 1 container size and had limited flexibility in regard to volume dispensed. Thus when new lower cost methods, diet and containers were developed, most of the old diet preparation equipment could not be utilized without considerable modification.

It was envisioned quite early that the type of equipment best suited for processing and handling diet must have several features: (1) capability of accomodating a wide variety of batch sizes depending upon need, (2) rapid and thorough mixing and homogenization of the product (3) precise control of temperature during processing (4) wide flexibility in dispensing the product with regard to rate and volume and (5) ease of service, simplicity of operation and easy to clean and sanitize.

Keeping the above criteria in mind, several components have been tested on a pilot scale basis. A 10 gallon steam jacketed kettle (Hamilton) enabled us to prepare batch sizes ranging from 10 to 40 L. Water was piped into the kettle at 180°F and heated by steam to boiling within 5-6 minutes. Upon reaching a boil, the steam was automatically cut off and the gelling agents (agar of Gelcarin) were quickly blended in with the aid of a 1 HP Homo-mixer (Speco, Inc.) with variable speed control up to 10,000 rpm. The mixer can be raised or lowered to different depths within the kettle and can be adjusted to attain differential vortex action.

The water-agar mixture is quickly cooled to 80°C by (1) flushing out of the steam existing in the jacket, (2) running cold water through the jacket until the desired temperature is reached and then replacing it with steam. The steam held within the jacket permits holding of the diet with the desired temperature range for dispensing. This procedure only requires a few minutes. At 80°C, the dry mix is blended in using the homo-mixer at high speed. Diet is then dispensed with a piston-action filling machine (Filamatic) into containers and loaded on carts to cool before larval infestation. By using 2 piston fillers, 1 worker can fill 40 cups/minute at volumes ranging from 10 to 130 ml per cup. This, in 1 hour a worker can fill 2400 6 oz. cups with 90 ml diet which is sufficient to rear 24,000 gypsy moths. By adding larger pistons and

2 more filling lines the capacity can be doubled. The cost of the filling machine is about $3000.

The Speco homo-mixer does a thorough job of mixing if permitted to run for several minutes; a holding kettle with a more cylindrical shape should permit the mixer to operate more efficiently. The diameter of the present experimental kettle is about 8 inches too large. The least expensive type of operation we can envision at present would involve a steam jacketed 20 gallon kettle (cost =$700) which has about 14" diameter. The diet could then be mixed using the homo-mixer and pumped into a colloidal mill which homogenized the product at the rate of 2-10 gallons/minute depending on desired degree of homogenization and discharges the product into a temporary holding tank for dispensing. This system would assure a product that was uniformly mixed and homogenized. If the timing of each step is standardized the quality of the product should be fairly uniform. The entire cost of the equipment is about $7500, which should permit 1-2 people to prepare enough diet in 4 hours to rear 100,000 insects.

Two additional diet processing systems will be tested in the next few months. One is a Speco mixer-blender built directly into a pipeline and is referred to as "a pipeline mixer". Dry ingredients are introduced into a funnel and fed into a stream of recirculating water agar. By adjustment of the flow rate of the dry and liquid components, mixing (at up to 9000 rpm) occurs almost instantly. We will be testing a unit that mixes 10 gallons/minute which is more than sufficient for our needs. The other mixer to be tested is known as "liquifier" and is designed somewhat like a huge Waring Blendor, i.e., the mixing blades are located in the bottom of the tank. The mixing and shearing head revolve at 5000 rpm and, according to technical information, 50 gallons can be uniformly mixed in 5 minutes. This unit would appear to be nearly ideal for large scale diet processing.

Conveying and holding of diet and insects during rearing.

Several carts (2x2x6 and 2x4x6') have been constructed in the shop for holding and moving diet and insects within the rearing facility. These were constructed of aluminum and hold from 6000-10,000 insects/cart. Trays for holding insect containers were constructed from polyethylene louvers. The tray units are of 2 sizes (24"x24" and 24"x16") and are equipped with rollers. The larval, pupal and adult holding rooms are equipped with several tiers of aluminum tracks or rails which, together with the trays, serve as shelving units. Trays of infested neonates are placed on rails at one end and are sequentially rotated on a daily basis along the railing in an assembly line fashion. Pupae and adults are removed at the end of the line. This rotational procedure ensures developmental uniformity since all insects are moved through the same environmental conditions. Insects of a given age always occupy the same position along the track which enables workers to set up check points along the route at appropriate positions to check on development. Generally speaking, the procedure allows a more orderly and uniform production schedule.

Project Number: GM 6.3.15
Project Title: Develop Efficient Methods for Gypsy Moth Virus Production and a Pilot Plant Facility
Report Period: October 1, 1976- March 31, 1977
Report Type: Interim
Project Leaders: Martin Shapiro, R. A. Bell, J. A. Tanner

A cost analysis based on larval equivalents (LEQ) of virus (assuming that 1 LEQ=$1x10^9$ polyhedral inclusion bodies or PIB'S) shows that the present cost of producing 1000 LEQ is in the order of $60-80. Therefore, field applications run from $12-40 when applied at the currently used dosages of $1x10^{11}$ to $5x10^{11}$ (200-500 LEQ) per acre. Thus the cost of conducting large scale field trials is considerable and commercial use of the product would seem far away indeed.

A major part of the overall problem occurs because of the limited efficiency of techniques for rearing the host caterpillars. Partly because of limitations of mass rearing technology, limited effort has been made toward development of efficient methods of virus production.

Due to recent improvements in mass rearing of gypsy moths by ARS-APHIS personnel here at Otis, the impetus was provided for considering the initiation of a program to improve efficiency and develop technology for mass production of the virus. Therefore, in the latter part of 1976, an agreement between the gypsy moth program managers, FS and ARS-APHIS personnel was made and a research and development program was initiated. Subsequently, FS solicited the help of the ARS-APHIS mass rearing unit in producing the projected $5x10^{14}$ viral inclusions needed for the 1977 field trials.

A brief outline of the content of the research program, observations of preliminary experiments and a summary of the results from the NPV production for FS field tests are reported here.

Basically, the research program involves optimizing in vivo NPV production with respect to the following factors (a) method and timing of inoculation (b) environmental factors (c) dosage (d) time and method of harvest (e) processing and storage and (f) quality control procedures. A prototype facility for efficient production of 30,000 LEQ/day will also be developed and demonstrated.

A. Method and timing of inoculation- A suspension of the virus can either be incorprated into the artificial diet or simply placed on the diet surface. The latter method has more advantages in that less inoculum is required to attain infection and at Otis the diet can be prepared in the regular diet processing area and transfered to the virus production area for inoculation, thus avoiding duplication of diet preparation equipment. Also, by introducing virus on the surface of the diet after cooling, as opposed to incorporation into hot diet, there is less chance of inactivation of the virus by heat. The efficiency of the twomethods have been investigated by Forest Service per-

sonnel (Frank Lewis and W. Rollinson, 1976 personal communication) and significant differences were not observed. We have experiments in progress in which the two methods are being intensively investigated. Because of the previously mentioned advantages, we used the technique of surface inoculation of NPV for FS field trials. With regard to timing of inoculation, preliminary tests have indicated that the highest yields of NPV are obtained when larvae are infected shortly after entry into the fourth instar. One also has to take into account the rate of larval growth after inoculation and when the infected should be harvested. Thus the aim here was to select a larval stage which when exposed to a minimum virus dosage would produce the highest yield of NPV/unit of time. Thus a series of dosage mortality curves generated by challenging larvae at different growth stages with the virus suggested that infection of 4th instar larvae was most satisfactory. Prior to inoculation, the NJF_{14} strain reared at 25.5°C on modified hornworm diet was held in plastic 3 ounce cups with paper lids @ 10-20 L/cup. After entry into the 4th instar, (14 days after infestation) larvae were transferred to 6oz plastic cups (10/cup) with 90 ml of diet (see below).

B. Effect of environmental factors- A series of tests were run in which groups of larvae were reared at 23, 26, 29, and 32°C after inoculation. Rate of growth was greatest and time to larvae mortality was optimal at 29°C; above or below this temperature, rate and insect growth and, therefore, virus replication and maturation, were decreased. Humidity, diet, and host density factors are also being evaluated (see later discussion).

C. Optimal inoculum dosage- After having selected 4th instar larvae as the optimal stage for inoculation, and 29°C for post-inoculation incubation, the desired inoculum dose was investigated. In addition to producing a high yield of NPV, we wanted to induce a uniform high mortality response in minimal time.

In production, a uniform response to virus is required e.g. 100% infection and/or 100% mortality. We must be assured that all insects are infected in order to maximize production. Although different virus concentrations may lead to 100% infection, it is important to minimize the time from virus challenge to harvest. The shorter this period, the greater the amount of insects and NPV produced/unit of time. In these and in all other tests, 10 larvae/6 ounce cup were used and 1 ml of virus suspension was added to the diet surface. The results of several tests indicated that $5x10^6$ PIB/ml/container was near the optimal inoculum dose.

D. Harvesting and Processing- Larvae may be harvested at any time after mortality occurs in the infected populations. Although the virus yield/insect increases during the infection period, it becomes more difficult to collect virus when all insects have died. After the larvae die, they frequently rupture resulting in a leaking of virus into the diet and feces which is difficult to collect.

At 29°C, the slope of the mortality curve was quite steep, especially

between day 10 and day 12. thus, the time difference between 29% mortality and 76% mortality is only 2 days, and even by day 10, the infected larvae have nearly ceased feeding. Moreover, the yield of PIBs/larva is similar at days 10 and 12 (see Table 1). In addition, at day 10, most virus would be recoverable as mortality was still low (29%). For these reasons, we chose to harvest at day 10. Both living-infected and virus-killed insects were frozen at day 10. Freezing prior to harvest was an advantageous and convenient method for (1) inhibiting bacterial growth within insects (2) removing the larvae from the containers and (3) harvesting virus from wilted insects.

Larvae and containers were placed in a large freezer (-20°F) and left overnight. The next day, the frozen larvae (including those wilted) were easily removed by spoon or by forceps. Ten thousand cadavers could be harvested by 4 persons in 1 hour.

Following harvest, the larvae were placed in plastic bags, returned to the freezer, and held for subsequent processing. Once a week these larvae were sent to Boyce Thompson Institute for processing. In toto, 502,500 infected and virus killed larvae were produced in 47 days (=10,670/day). The cost for this production was 2 cents/larvae.

Table 1. Virus induced mortality and virus yield of gypsy moth larvae in relation to time[1]

Days After Incubation	Percent Mortality	Virus Yield PIB/Larva
7	0	2.468×10^8
8	2.1	1.065×10^9
9	12.5	1.239×10^9
10	29.4	1.889×10^9
11	56.8	1.695×10^9
12	76.0	1.859×10^9
13	88.1	-
14	93.3	-
15	95.9	-
16	100.0	-

[1] 2000 4th instar larvae were treated; 10 larvae/6 ounce cup; 5×10^6 PIB/cup.

The experience afforded as a result of the virus production was invaluable. It forced us to answer practical questions within a short period of time. Moreover, it demonstrated our capability of producing 500,000 infected and/or virus killed insects on a daily production schedule under less than optimal conditions.

More research must be done to increase the efficiency of NPV production. However, it was demonstrated that, with the current state of knowledge, NPV can be produced for at least $20/1000 LEQ or $10/1x10^{12} PIBs.

Summary of Production Run

The total production consisted of 502,500 larvae over a 47 day period. During 37 days of this period, samples of 30 larvae/day were taken and frozen. Weights of each group were taken and larvae were processed, i.e. blending and clarification, in order to obtain counts of polyhedral inclusion bodies (PIBs).

The insects were inoculated with 5x10^{6} PIB/10 larva, to give an expected LD_{30} 10 days later. The average mortality obtained for the run was 27%, well within our expectations.

Data from the entire run are summarized in Table 1. Two billion PIBs were obtained per insect for the entire run. These results were pleasantly surprising since we expected only 1x10^{9} / larva.

Table 1. Yield of L. dispar NPV during the production run.

No. of Insects Processed	$\bar{x}$ wgt/larvae (mgm)	$\bar{x}$ PIB/larvae x 10^{9}	Range x 10^{9}
1050	891	2.04	1.29-2.90

Additional Virus Research (Preliminary observations)

For the past several months work has been conducted on the biology of the gypsy moth NPV, in order to better understand the pathogen-host relationship. With a better understanding of this relationship, we will be in a better position to optimize in vivo virus production. Preliminary observations are summarized below:

1. Susceptibility of larvae at different instars.

Dosage-mortality studies of first-fifth instar larvae have been initiated. Both virus mortality and yield of PIBs/larva will be evaluated.

2. Sex of larvae.

Females appear to be as susceptible to NPV as males, but produce more virus. On a PIB/gram of larva basis, however, no differences appear to occur.

3. Humidity.

Initial data indicate that virus mortality was similar at a low (35% RH) and high ambient humidity (62% RH).

4. Harvest time vs yield and activity.

As infection progresses, the yield of virus/larva also increases. When virus from 7-day to 12-day old infected larvae were compared, little difference in inherent activity was found.

5. pH of diet.

The pH of the standard hornworm diet (pH 5.8) was adjusted to pH 4, 5, 7, 8. Little difference in virus mortality was noted when larvae were inoculated with high virus concentrations. At lower concentrations ($5x10^3$ - $5x10^4$) more virus mortality was obtained at a pH of 4.5 than at 6, 7, or 8.

6. Diet.

The standard hornworm diet was compared with simpler diets. In addition, gelcarins were substituted for agar in several diets. Preliminary results indicate cheaper diets may be utilized for virus production.

Project Number: GM 7.3.1
Project Title: Gypsy Moth Mass Rearing: Hatch Rates of Laboratory and Wild Strains of Gypsy Moth Eggs as Affected by Duration and Temperature of Chilling
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leaders: John Allen Tanner, R. A. Bell, C. Owens, M. Shapiro, O. T. Forrester, J. J. Baker

The Gypsy Moth Methods Development Laboratory maintains three gypsy moth strains; Pennsylvania, Massachusetts and New Jersey. The Pennsylvania strain has been maintained in the laboratory for 6 generations, the Massachusetts and New Jersey strains for 15 and 16 generations respectively.

Goldschmidt (1932) observed geographical variations in the duration of diapause among local gypsy moth populations. A similar situation may exist in our strains especially when comparing an F_{15} or F_{16} laboratory strain to a wild strain. Conditions favorable for the elimination of diapause in one strain may not be as favorable for another strain.

OBJECTIVES:

To determine differences in response to chilling temperatures between the laboratory strains and a wild strain.

METHODS AND MATERIALS:

One hundred egg masses were selected from the Pennsylvania, Massachusetts and New Jersey laboratory strains and the Dighton, MA wild strain. The masses from each strain were randomly divided into 20 groups of five masses each and placed into plastic petri dishes. Each grouping was assigned to one of the following chilling temperatures and periods:

Temperature (°C)	Period (Days)
4	
6	
8	30, 60, 90, 120, 150
10	

The appropriate temperatures were maintained within a refrigerator modified into four chambers. Precondition air (-4°C) was forced into the refrigerator and warmed to the appropriate temperature by a series of heating elements.

After the prescribed chilling period, the egg masses were individually dehaired and four 25 egg samples drawn from each mass. Each sample was placed into individual, tight fitting, petri dishes and incubated 30 days. The field collected masses were shabby and broke easily during handling making it difficult to determine five masses. Because of this, the five masses within each group were collectively dehaired and five samples of 100 eggs drawn and incubated. Three replications were conducted over a period of time.

RESULTS AND DISCUSSION:

Eggs chilled up to and including 120 days have completed incubation. Longer chilling periods increased the hatch rates of Dighton wild eggs. Percent hatch was highest (72%) at 4°C followed by 6°C. Percent hatch was highest at 8°C for all chilling periods less than the 120 day period.

Hatch rates have been low for the laboratory strains. New Jersey eggs chilled 120 days at 4°C produced only 14.5% hatch. This is considerably lower than the 72% hatch observed with Dighton wild eggs. It is also considerably lower than the 80%+ hatch observed by Tanner and Kennedy (1976a) utilizing the same strain, temperature and chilling period. The reduced hatch rate may be due to egg density, toxic material from anti-freeze used to prevent freezing in the conditioning system or microenvironmental conditions in the petri dishes. This experiment will be repeated using more reliable testing equipment.

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Tanner, J. A. and L. F. Kennedy, 1976b. Effect on the Hatch Rate of Dehairing L. dispar Egg Masses Prior to Incubation, Ibid: 73.

Project Number: GM 7.3.2
Project Title: Gypsy Moth Mass Rearing: Development of an Efficient Production System of Fourth Instar Gypsy Moth Larvae for Nuclear Polyhedrosis Virus Production
Report Period: October 1, 1976 - March 31, 1977
Report Type: Final
Project Leaders: O. T. Forrester, R. A. Bell, L. F. Kennedy

On November 29, representatives from APHIS, ARS, USFS and GMP met at the Gypsy Moth Methods Development Laboratory to discuss the production of NPV for the scheduled 1977 field tests and subsequent EPA registration. This was planned as a precautionary production in the event that production via the current commercial contract was unsuccessful. It was decided to produce 500,000 larval equivalents and to begin production in mid-February. This schedule provided time to obtain materials and make any necessary production preparations and still complete production in time for May field testing. Many of the techniques of rearing and virus production to maximize efficiency had not been developed. The facility in which the virus production work was performed was inefficient in design and lacked adequate environmental control. The short production time (6 weeks) necessitated a seven day work week and the use of professional personnel which greatly increased the labor component production costs.

Rearing Procedure: Several different containers and insect densities were used during the six week production run. Modified hornworm diet (current cost - $.60/liter) was used. Insects were infested as neonates and held 14 days (4th instar) at 26°C, 60% RH and 16:8 L:D photoperiod and transferred to the virus production unit in a separate building. From February 14 through February 27, 30 neonates were infested onto 45 ml of diet in 3 ounce plastic cups (Sweetheart Plastics ME-3 and TL-3 paper lids). This system was changed to 10 neonates per 1-3/4 ounce waxed paper cup (Dixie ST23) on 20 ml of diet. This change was an attempt to be more compatible with the procedures of the virus unit. The 1-3/4 ounce cup proved to be a very inefficient system. The diet dried excessively, lids were difficult to seat, 2 liters of diet were required per 1000 insects as compared to 1.5 liter and only 1140 insects could be held per tray compared to 3360 per tray at 30 per cup. A second change was made to eliminate the diet drying problem. A 1-1/2 ounce Thunderbird Plastics cup with 15 ml of diet and 10 insects was tried for five days. The drying problem was eliminated but labor requirements for dispensing diet and infesting neonates was restrictive. The third and final changed reverted to the 3 ounce plastic cup with 20 neonates on 45 ml of diet. This system seemed to be a reasonable compromise to increase the compatibility between insect production and virus production.

Results and Discussion: Production costs for 501,400 cadavers was $18850.42 or $37.59/1000 cadavers (Table 1). This does not include processing of the cadavers to the finished product which was handled on a separate contract with Boyce Thompson Institute. However, this is not a true picture of costs that could be expected in the future production. If GS-1 labor and a recently developed less expensive high wheat germ diet were used, the cost

would have been $19.70/1000 cadavers. Increased personnel proficiency developed by the end of the production run would further reduce the cost to $18.44/1000. If high wheat germ diet were used, the cost could be reduced to $14.27/1000 (Table 2).

Further cost reduction can be obtained when the compatibility of containers and insect density is improved between insect production and virus production.

Table 1. Production Costs

PRODUCTION COST FOR 647,920 NEONATES TO 4TH INSTAR

A. Diet Preparation

32,396 cups ME-3 and lids TL-3 @ $18.98/M	$ 612.04*
1457.8 liters hornworm (HW) diet @ $.60/liter	874.68*
CONTAINER AND DIET COST	$1486.72

B. Infestation

	Overtime		Regular Time		
GS-3/10	27 hours	$ 219.99			
GS-2/10	30 hours	326.85	136 hours	$ 558.96	
GS-1/1	43 hours	217.70	136 hours	379.44	
GS-1/1	35 hours	181.40	136 hours	379.44	
GS-9/3	23 hours	285.59	68 hours	491.64	
GS-11/3	-	-	176 hours	1540.00	
TOTAL	158 hours	$1231.53	652 hours	$3349.48	
LABOR COST					$4581.01

C. Diet Preparation and Dispensing

	Overtime		Regular Time		
GS-3/10	22 hours	$ 189.76	172 hours	$ 796.36	
GS-3/1	22 hours	145.96	172 hours	612.32	
TOTAL	44 hours	$ 335.72	344 hours	$1408.68	
DIET PREPARATION LABOR COST					$1744.40*

Material	$1486.72
Labor	6325.41
TOTAL	$7812.13

Table 1 cont'd.

PRODUCTION COSTS FOR 501,400 4TH INSTARS THROUGH CADAVER HARVEST

A. Diet Preparation

50,140 cups and lids, XE-6 and AS3061:1 @ $24.60/M	$1233.44*
4512.6 liters hornworm (HW) diet @ $.60/liter	2707.56
CONTAINER AND DIET COST	$3941.00

B. Infestation and Harvest

	Overtime		Regular Time	
GS-2/5	6 hours	$ 37.59	152 hours	$ 544.16
GS-2/10	52 hours	376.19	144 hours	591.84
GS-2/10	52 hours	376.19	144 hours	591.84
GS-3/1	43 hours	270.60	144 hours	512.64
GS-4/1	33 hours	238.00		
GS-9/3			188 hours	1359.24
GS-13/2			188 hours	2271.04
TOTAL	186 hours	$1298.57	960 hours	$5978.72

Labor	$ 7097.29
Materials	3941.00
TOTAL	$11038.29

PRODUCTION COSTS FOR NEONATES THROUGH CADAVER HARVEST

Labor	$13422.70
Materials	5427.72
TOTAL	$18850.42

Material Cost $10.83/1000
Labor Cost $26.81/1000

Estimated Cost Saving

Production costs for 501,400 if all labor at GS-1 rate, no overtime (or $23.87/1000 or a savings of $13.72/1000)	$11967.48
Estimated cost savings using high wheat germ (B-4) diet and GS-1/1 labor, no overtime (or $19.70/1000 or a savings of $17.89/1000 cadavers)	$ 9877.84

Table 2. Projected Production Cost Breakdown for 10,000 LEQs.

COST OF REARING 12,000 NEONATES TO 4TH INSTAR

A. Diet Preparation	
20 neonates/cup 600 cups and lids @ $18.98/M	$ 11.39
27 liters HW diet @ $.60/liter	16.20
27 liters high wheat germ (B-4) @ $.25/liter	6.75
B. Neonate Infestation	
GS-1 4 hours	$ 11.16
GS-1 4 hours	11.16
TOTAL 8 hours	$ 22.32

COST OF REARING 10,000 4TH INSTARS THROUGH CADAVER HARVEST

A. Diet Preparation	
10 larvae/cup 1000 cups @ $24.60/M	$ 24.60
90 liters HW diet @ $.60/liter	54.20
90 liters high wheat germ (B-4) @ $.25/liter	22.50
B. Inoculation and Larval Transfer	
1 GS-3 1.5 hours	$ 5.43
4 GS-1 1.5 hours each	16.76
TOTAL 3.0 hours	$ 22.19
C. Cadaver Harvest	
4 GS-1 1 hour each	$ 11.16

PROJECTED COSTS OF REARING NEONATES TO CADAVER

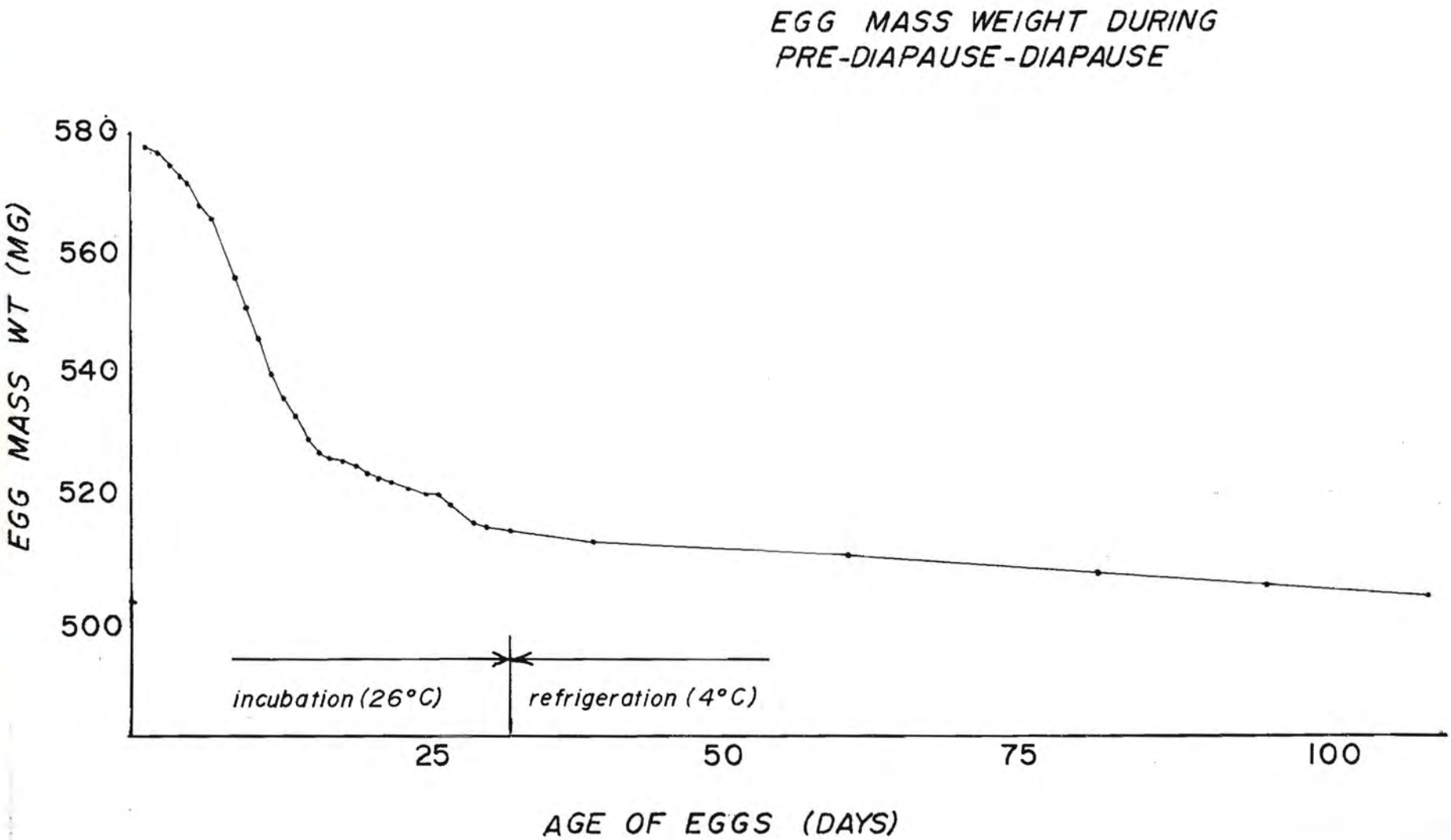
Cost/1000 using HW diet	$ 18.44
Cost/1000 using high wheat germ (B-4)	$ 14.27

Project Number: GM 7.3.3
Project Title: Weight Changes in Gypsy Moth Egg Masses During Pre-Diapause, Diapause and Post-Diapause Development
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leaders: O. T. Forrester and R. A. Bell

Weight losses in gypsy moth egg masses during the pre-diapause developmental period can be used to estimate the time required to enter diapause. This study was designed to quantify weight changes in newly deposited egg masses.

Ten female gypsy moths were mated and allowed to oviposit on brown craft paper. The egg masses were collected 24 hours after mating. The egg masses were left on the paper to facilitate weighing and keep the egg masses intact. The excess paper was trimmed to about 5 mm from the edge of each egg mass. The egg masses were weighed daily at 1300 hours for 30 days and stored at 4°C after 32 days. Weights were taken at 32, 39, 81, 94 and 108 days during storage. Mean weight changes are given in Figure 1.

The most rapid weight loss occurred during the first 15 days after oviposition. The weight loss slowed during the next 15 days indicating a completion of embryonation and diapause began at ca. 30 days. Further studies will be carried out to determine the weight changes during pre-diapause, diapause and post-diapause development in a wild and laboratory strain. Also, incidence and duration of hatch chilled after 14, 21 and 28 days of pre-diapause development will be determined.


EGG MASS WEIGHT DURING
PRE-DIAPAUSE-DIAPAUSE
EGG MASS WT (MG)
580
560
540
520
500
incubation (26°C)
refrigeration (4°C)
25
50
75
100
AGE OF EGGS (DAYS)

Project Number: GM 7.3.4
Project Title: Insect Production and Distribution
Report Period: October 1, 1976 - March 31, 1977
Report Type: Interim
Project Leaders: Leonard F. Kennedy, J. J. Baker, O. T. Forrester

The primary object of the rearing facility of this laboratory is to produce sufficient quantities of all stages of the gypsy moth to support research projects at this laboratory, several universities and experiment stations in the United States and foreign countries. The following is a summary of production and distribution of this material.

Production:

Larvae infested - 983,895**
Eggs refrigerated - 16,323,000

** 660,000 for virus production

Distribution:

Methods Development and ARS, Otis
Insecticide screening
2nd instar - 12720
3rd instar - 6360

Pheromone - Adults ♂ - 3586

Diet tests - 7700 newly hatched

Container tests - 10000

Parasite maintenance - 2nd instar - 1060
6th instar - 690

Virus tests - 1st instar - 2400
2nd instar - 2700
3rd instar - 1800
4th instar - 700
5th instar - 300

Egg storage tests - Tanner and Kennedy - 330 masses

Cooperators:

University of Massachusetts - Barbosa

3rd and 4th instar	2300
♀ pupae	2300

Franklin Institute - Goodman

♂ adults	245

NY Agricultural Experiment Station - Miller

♂ pupae	2300

Penn State University - Mastro

♂ pupae	400
♀ pupae	50

Forest Service, Hamden, CT

Egg masses	850
Virus infected	502500

Michigan State University - Carde'

♂ pupae	2850
♀ pupae	2500

Syracuse University - Galleger

Egg masses	15

Brown University, RI

Egg masses	30

Penn State University - Cameron

♀ pupae	10
♂ pupae	40

Bio-Serv Corporation, NJ

Egg masses	10000

California Department of Food and Agriculture, Dr. George Okumura (Life cycle specimens of the gypsy moth - not viable).

Egg masses	500
Each instar	500
♂ pupae	500
♀ pupae	500
♂ adult	500
♀ adult	500

Project Number: GM 7.3.5
Project Title: Evaluation and Colonization of Gypsy Moth from the San Jose, CA Infestation
Report Period: October 1, 1976 - March 31, 1977
Report Type: Preliminary
Project Leaders: O. T. Forrester, R. A. Bell

An infestation of gypsy moths was detected near San Jose, CA in early October of 1976. Egg masses from this infestation were requested to determine developmental rates, diapause requirements, incidence of virus, parasitism, general quality of the population and for colonization.

Three egg masses were received on October 18 and stored at 4°C. Hatch was reported in the field in California in mid-November. On November 19, one-third of each collected egg mass was incubated at 26°C, 60% RH. Hatch began December 15 and ended January 23.

A second shipment of 13 gypsy moth egg masses was received from California on December 1. Hatch had occurred in 3 of the egg masses during shipment. One-half of each egg mass was stored at 4°C, the remaining halves were incubated at 26°C, 60% RH. Egg hatch was completed on January 12.

Methods and Materials

The portion of the egg masses used were not surface sterilized. This was omitted to enable virus to be detected. Modified hornworm diet was used for rearing the insects. Two rearing methods were used. Neonates from the first 3 egg masses were reared individually in 1-1/2 ounce Thunderbird styrene plastic cups with paper lids for 14 days and transferred to 3 ounce ME-3 Sweetheart Plastic cups with paper lids on 45 ml of modified hornworm diet. Neonates from the second shipment of egg masses were aggregately reared on 45 ml of diet at 10 insects per 3 ounce ME-3 plastic cups with paper lids. After 14 days of development, the insects were transferred to fresh diet at 5 insects per cup.

Results and Discussion

The time required to complete hatch was considerable. The mean time to complete hatch was 30.6 days. The range was 25 days, from a high of 43 days to 18 days (2 egg masses with no hatch and 1 hatch were not considered). The deviation of hatch is not unusual in unchilled or eggs chilled for a short period of time (2 months or less). The syncrony of hatch should increase in the remaining portion of these egg masses after diapause has been completed. The remaining eggs will be incubated in early May.

The establishment of the California insects on artificial diet was exceptional. The 95% establishment obtained is 5% higher than that generally expected in our standard laboratory strain. Six percent of the insects did not reestablish after the transfer to fresh diet at 14 days of development.

The incidence of virus was low, 0.7% of the insects lost as larvae showed symptoms of virus. All of the insects that died in larval stages were collected and frozen. These will be checked for disease.

The quality of the insects from the egg masses received were good. Developmental rate of the insects was normal when compared with other field collected insects reared on artificial diet. One egg mass in the second shipment of egg masses was not viable; all of the eggs in the mass were collapsed. Percent hatch in the egg masses ranged from 0 to 99.1 percent and the mean hatch for all masses was 53%. The sex ratio was determined at pupation; 57.6% were male and 42.4% were female.

A total of 1062 insects hatched from the 16 partial egg masses and yielded 225 egg masses. The egg mass yield is somewhat low due to poor syncrony in male-female eclosion and losses during pupation. The egg mass yield is expected to be better in the egg masses that have completed diapause.

For: Environ. Entomol.

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Contrasting Effectiveness of (+) and (-) Enantiomers of Disparlure for Trapping Native Populations of the Gypsy Moth [1/] in Massachusetts [2/]

J. R. Plimmer [3/], C. P. Schwalbe [4/], E. C. Paszek [4/], B. A. Bierl [3/], R. E. Webb [5/], S. Marumo [6/], and S. Iwaki [6/]

Running head: Trapping gypsy moths with (+)-disparlure

Indexing phrases: chirality, effect on pheromone activity
(+)-disparlure for gypsy moth trapping
gypsy moth, trapping with (+)-disparlure
Lymantria dispar, trapping with (+)-disparlure
pheromone of gypsy moth, trapping with

ABSTRACT

Trapping experiments were conducted in Massachusetts to compare the attractiveness of the optically active enantiomers of disparlure (*cis*-7,8-epoxy-2-methyloctadecane), the sex attractant pheromone of the gypsy moth, *Lymantria dispar* (L.). The (+) enantiomer of disparlure showed much greater activity than the (-) enantiomer or an equivalent amount of racemic disparlure.

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Specificity of pheromonal communication in insects has been demonstrated by numerous descriptions of the precise, and often complex, combinations of chemical signals required to elicit a given behavioral response. In several instances, chirality of receptor sites on antennal membranes appears to be a factor in this specificity (Riley et al. 1974 , Kafka et al. 1973, Wood et al. 1976). Experiments in Japan showed that the optically active enantiomers of disparlure, cis-7,8-epoxy-2-methyloctadecane, the sex attractant pheromone of the gypsy moth, *Lymantria dispar* (L.), differ considerably in their biological activity (Iwaki et al. 1974; Yamada et al. 1976). Trapping experiments were conducted in a natural population of gypsy moths in Massachusetts to make further comparison of the attractiveness of the enantiomers of disparlure with that of the racemic [7] material.

Methods and Materials

The optically active isomers of disparlure were synthesized at the Dept. of Agricultural Chemistry, Nagoya University, Nagoya, Japan. The synthetic (+)-disparlure contained 6.6% of the (-) enantiomer and synthetic (-)-disparlure contained 6.6% of the (+) enantiomer [8]. Throughout this paper, the terms (+)-disparlure and (-)-disparlure refer to these synthetic materials of 93.4% optical purity.

Disparlure obtained commercially (ChemSamp Co., Columbus, Ohio) was the racemic mixture of the 2 enantiomers; in addition, it contained 4.7% of the geometrical isomer trans-7,8-epoxy-2-methyloctadecane. This material is referred to as commercial disparlure in this paper.

Hercon® dispensers (Herculite Protective Fabrics Corp., New York, N.Y.) contained 5% of the commercial disparlure (ca. 6 mg disparlure/dispenser) (Beroza et al. 1975).

Test materials were applied to cotton wicks (1.0 x 0.6 cm) at rates indicated in the tables and exposed in Graham (Holbrook et al. 1960) or USDA gypsy moth detection and survey traps (delta traps similar to those described by Beroza et al. (1975)) with the wick located centrally. In some experiments, trioctanoin (2 mg) was added to each wick as a "keeper" to retard evaporation. Traps were suspended at about 1.3 m on trees of 15-20 cm diameter at breast height. At the termination of each test, the cotton wicks were retrieved and the residual disparlure content was analyzed by gas-liquid chromatography.

Field tests were conducted from July 19 to Aug. 11, 1976 at Dighton Rock State Park and Rehoboth, Mass., in woodland areas separated by several miles. Egg mass surveys and trap data indicated a light infestation in the state park (less than 25 egg masses/hectare) and a heavy infestation at Rehoboth (ca. 600 egg masses/hectare). Traps were placed on lines in a completely randomized arrangement. Distance between lines was at least equal to the distance between individual traps. Spacings between traps, types of traps used and other experimental details are shown in Table 1.

In the experiments described, the attractiveness of (+)-disparlure and (-)-disparlure, alone and in combination, was determined. In the first experiment a Hercon dispenser and a virgin feral female were also included for comparison. Female pupae were collected from natural populations in Pennsylvania and New Jersey and were held in groups of 20 in 500-ml containers at 20 - 22°C. Emerged virgin female moths were collected daily and used the day following emergence; they were discarded after they were 4 days old.

The major constraint on the experimental design was the small amount of each disparlure enantiomer available (ca. 5 mg).

Results

Experiment "a" was conducted in the area of heavy infestation and results indicated that (+)-disparlure was a very effective attractant when 10 μg was used (caught 94 moths). (-)-Disparlure showed little, if any, attractant properties (10 μg trapped 9 moths). The activity of the 10 μg of commercial disparlure (caught 35 moths) appeared to fall midway between that of equivalent amounts of (+)- and (-)-disparlure and to be about as active as a Hercon dispenser (32 moths). A single virgin feral female was less attractive (trapped 16 moths) than a Hercon dispenser or 10 μg of racemic disparlure on a wick. At the 1.0 μg level, (+)-disparlure was only slightly more attractive than racemic disparlure.

In experiment "b" (-)-disparlure again caught few moths (average 0.4/trap), as did the blank control (1.1). Moreover, the addition of (-)-disparlure to the (+)-disparlure depressed the attractiveness of the latter. Traps baited with 5 μg of the nearly pure (+)-disparlure (actual 4.7 μg (+) enantiomer; 0.3 μg (-)) caught an average of 13.0 moths/trap, whereas the combination of 5 μg (+) enantiomer with 5 μg (-) caught only 1.8 moths/trap; 6.4 μg (+) enantiomer mixed with 23.6 μg (-) caught only 0.4 moths/trap.

Experiment "c" confirmed the previous findings and clearly demonstrated the superior attractancy of the (+) enantiomer as compared to various mixtures of (+) and (-) enantiomers. A straight-line relationship between dosage of the (+) enantiomer and response was shown by a plot of the logarithm of the applied amount versus trap catch. The depressive effect of the (-) enantiomer is shown by the three samples (Table 2) containing 23-35 μg (+) enantiomer, with varying amounts of (-) enantiomer; the average trap catch in the low density area dropped from 135.2 for 1.6 μg (-) to 117.6 for 6.3 μg (-) and to 84.8 for 25 μg (-). Analysis of the lure content of the wicks after ca. 1 wk in the traps showed that the residual lure content ranged from 6 to 23% of the amount originally applied.

(+)-Disparlure was compared (experiment "d") with a similar amount of (+) enantiomer in commercial disparlure. (+)-Disparlure caught 5-6 times as many moths as did the commercial lure at the 5 and 25 μg (+) enantiomer level in the area of light infestation.

Trap catch for (+)-disparlure was then compared with that for commercial disparlure at several concentrations in the lightly-infested area (experiment "e"). Fig. 1 shows the increase of catch with the amount of (+)-enantiomer. Approximately 10 times as many male moths were caught by 10 μg and 100 μg of the (+)-disparlure as by the same amounts of (+) enantiomer in commercial disparlure. At the 1 μg level, this difference dropped to a factor of 2.5. The average catch per trap using 1 μg (+)-disparlure (0.9 μg of the (+) enantiomer) was 4.3 moths. This increased to 28.4 and 54.0 moths when 10 and 100 μg (+)-disparlure (9.3 and 93 μg (+) enantiomer) were used, respectively. By contrast 1 μg of commercial disparlure caught 1.4 moths, 10 μg caught 3.3 moths and 100 μg caught only 5.2 moths. An increase in the amount of commercial disparlure from 100 to 1000 μg did not increase the catch. The residual lure content of the wicks after 13 days in the traps was approximately 4% of the original quantity at the 1 μg level and ranged from 22 to 60% at the higher dosages.

A further experiment "f" again showed the depression of trap catch caused by addition of varying amounts of (-)-disparlure (93.4% (-) enantiomer) to 10 μg of (+)-disparlure (93.4% (+) enantiomer). A plot of trap catch vs. actual quantity in μg of (-) enantiomer per 10 μg of the (+) enantiomer is shown in Fig. 2. The average number of moths trapped was normalized to a base of 10 μg (+) enantiomer/trap to account for the slightly differing amounts of (+) enantiomer/trap. There was a decrease in trap catch in proportion to the logarithm of the amount

of (-) enantiomer until a 1:1 ratio (racemic) was achieved. At still higher amounts of the (-) enantiomer, the magnitude of this decrease became less. Extrapolation of this plot suggests that if 99% (+) enantiomer were available, it would probably trap 15-20 times as many insects as racemic disparlure at the 10 μg level. The 10 μg (+)-disparlure with no added (-)-disparlure caught 8 times as many insects as the racemic mixture. Analysis of the wicks after 1 wk of exposure in the traps showed that the residual lure content was 10% or less of the original amount.

Discussion

Disparlure, with chiral centers at C_7 and C_8, can have two optically active isomers. The (+) and (-) enantiomers of cis-7,8-epoxy-2-methyloctadecane were synthesized by Iwaki and his co-workers (Iwaki et al. 1974) who found (+)-disparlure to be more active in preliminary tests. These experiments and subsequent studies (Yamada et al. 1976), utilized the so-called Japanese gypsy moth, *Lymantria dispar japonica* (Motschulsky) which closely resembles the North American strain.

Yamada et al. (1976) subjected enantiomers of both the cis and trans isomers of 7,8-epoxy-2-methyloctadecane to bioassay by electro-antennogram (EAG) and behavioral techniques. The largest EAG response was elicited by (+)-disparlure and it was concluded that this could correspond to a natural sex pheromone of the moth. Thus the antennae of the gypsy moth would appear to possess receptor sites capable of distinguishing chirality. The EAG experiments indicated an increase of response with increased concentration of (+)-disparlure. At the lower levels of

concentration (10^{-9} to 10^{-7} g/ml), the responses to (+)- and (-)-disparlure were similar; however at 10^{-6} g/ml and higher levels, the EAG response to (+)-disparlure was about 3 times greater than that to (-)-disparlure. When the EAG was measured with the racemic mixture at 10^{-3} g/ml, the amplitude of response was 6.4 mv vs 7.3 mv for (+)-disparlure. The (+) and (-) enantiomers of trans-7,8-epoxy-2-methyloctadecane produced only very weak responses.

In their behavioral studies Yamada et al. (1976) found that responses to a puff of air containing a solution of (+)-disparlure were similar to those elicited by the racemic mixture at the lower end of the concentration scale (between 10^{-5} g/ml and 10^{-11} g/ml); at the 10^{-3} g/ml level, (+)-disparlure was clearly more active than the racemate.

Our own experiments were conducted with the North American gypsy moth and were not designed to provide behavioral information. Nevertheless our observations provide a useful supplement to those of Yamada et al. (1976) and confirm the superior attractive power of the (+) enantiomer. This material was much more effective than the racemic disparlure and the disparity between the catch with (+)-disparlure and with an equal amount of the (+) enantiomer as racemic disparlure increased with higher quantities of material.

At low concentrations (+)-disparlure and racemic disparlure approached one another in their ability to trap moths. As the amount of (+)-disparlure/trap was increased there was a corresponding increase in trap catch (Fig. 1). This increase was not as great with 1-100 μg of the commercial disparlure and an increase from 100 to 1000 μg failed to increase trap catch. Since no traps containing more than 100 μg

of (+)-disparlure were tested, it is not known whether trap catch would show a similar leveling off with this material.

The effect of adding (-)-disparlure to (+)-disparlure was to reduce trap catch to a level below that anticipated for the same amount of (+)-disparlure alone. Thus it appears that the role of (-) disparlure is not merely that of an inert diluent but that it plays some role in influencing communication between male and female insects. A competitive role may be envisaged, but Schneider et al. (1974) consider it unlikely that the antennal receptor sites would be fully saturated in a field situation and their EAG data do not support the hypothesis of competition between disparlure and very closely related structural analogues.

These experiments form part of a program that also includes field studies in which air permeation with the pheromone is used in attempts to disrupt mating. Commercial disparlure has been used in these experiments as a broadcast formulation (Beroza et al. 1974) and the field data indicate that if atmospheric concentrations of the lure can be maintained at a sufficiently high level, males will have difficulty in locating a calling female. Since the (-) enantiomer clearly influences pheromonal communication, possible applications of (-)-disparlure in disruption of mating should be investigated. We cannot now predict whether the (+) or (-) enantiomer would be more effective than racemic disparlure Furthermore, practical applications of the enantiomers of disparlure will not be possible until an economical method

of synthesis has been developed.

During the preparation of this paper we learned of the work of Vite et al. (1976) that also demonstrated the superior effectiveness of the (+) enantiomer of disparlure in trapping male gypsy moths.

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Footnotes

1/ Lepidoptera: Lymantriidae

2/ Received for publication . Mention of a proprietary product does not constitute an endorsement by the USDA.

3/ Agricultural Environmental Quality Institute, ARS, USDA, Beltsville, MD

4/ Gypsy Moth Methods Development Laboratory, APHIS, USDA, Otis Air Force Base, MASS

5/ Plant Genetics and Germplasm Institute, ARS, USDA, Beltsville, MD

6/ Dept. of Agricultural Chemistry, Nagoya University, Nagoya JAPAN

7/ Racemic disparlure (or (±)-disparlure) contains equal amounts of the (+) and (-) enantiomers and is therefore optically inactive.

8/ In terms of absolute configuration, the (+) enantiomer of disparlure is 7R, 8S-epoxy-2-methyloctadecane and the (-) enantiomer of disparlure is 7S, 8R-epoxy-2-methyloctadecane (Iwaki et al. 1974).

Table 1.--Experimental design.

Expt.	Insect infestation level	Trap spacing(m)	Trioctanoin/ wick (mg)	Trap type	Replicates per sample	Duration of experiment
a	high	50	2	Delta	4	1 day
b	low	25	2	Delta	10	6 days
c	high-low	25	0	Graham	5	5 days
d	low	25	0	Delta	10	6 days
e	low	25	2	Delta	10	15 days
f	low	25	0	Delta	10	9 days

Table 2.--Variation in trap catch vs. enantiomer composition.

Enantiomer/trap µg		Ave. catch/trap [a]	
(+)	(-)	High density area	Low density area
0	0	0.5	4.8
0.93	0.07	2.5	39.8
1.6	23.4		9.9
9.3	0.7	13.3	104.4
12.7	47.3	6.5	28.6
23.4	1.6	51.8	135.2
23.7	6.3		117.6
25	25	13.3	84.8
93.4	6.6		149.0

a/ Trap catch in high density area was determined after 3 h of exposure; that in the low density area was subsequently obtained from totals of three readings made over a 4-day period, using the same wicks and three additional ones.

Figure Captions

Fig. 1.--Effect of lure amount on trap catch with (+)-disparlure (93.4% (+) enantiomer) and commercial (racemic) disparlure.

Fig. 2.--Effect of added (-)enantiomer of disparlure on average trap catch with 10 μg (+) enantiomer of disparlure.

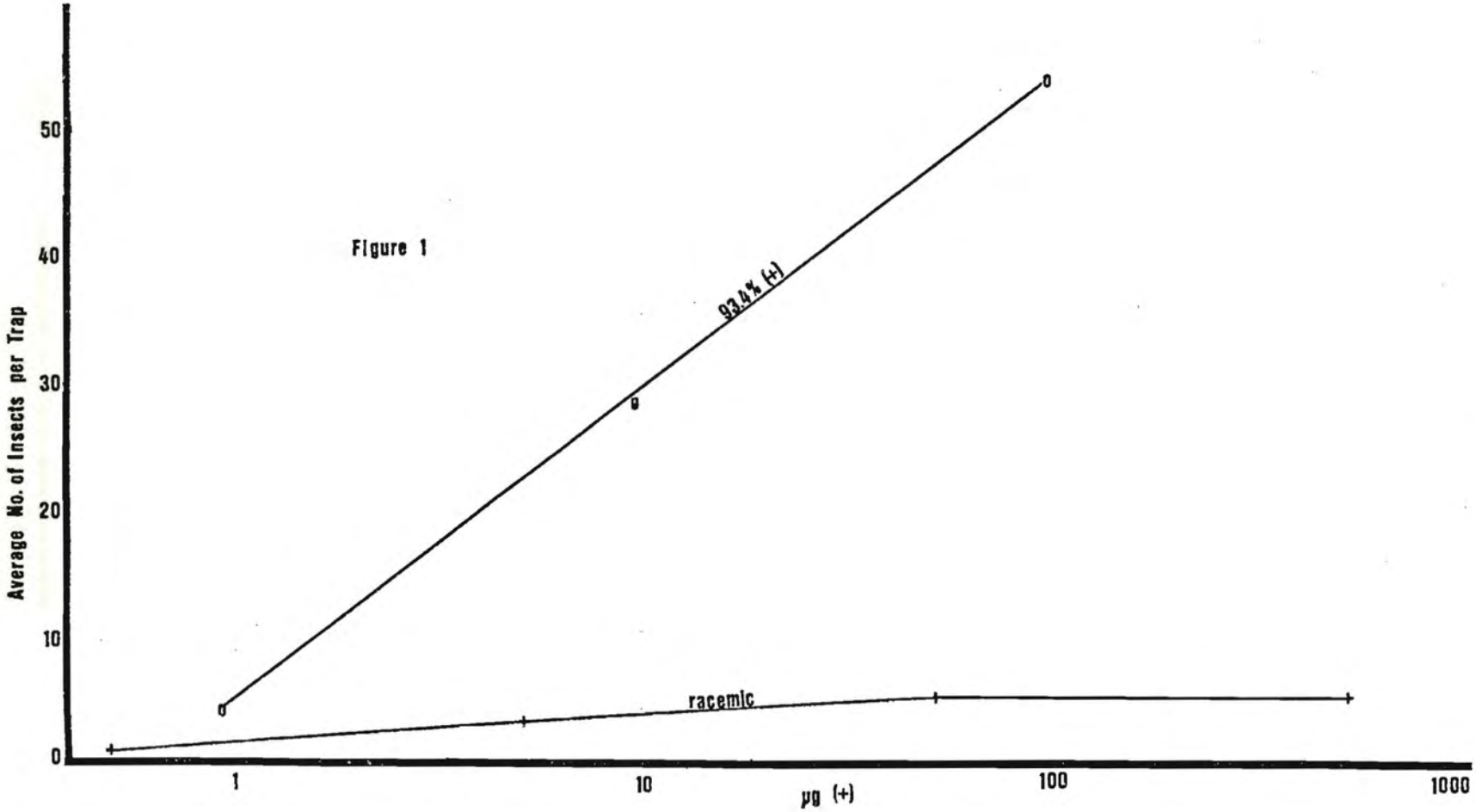
Average No. of Insects per Trap
0
10
20
30
40
50
93.4% (+)
racemic
1
10
100
1000
µg (+)

Figure 1
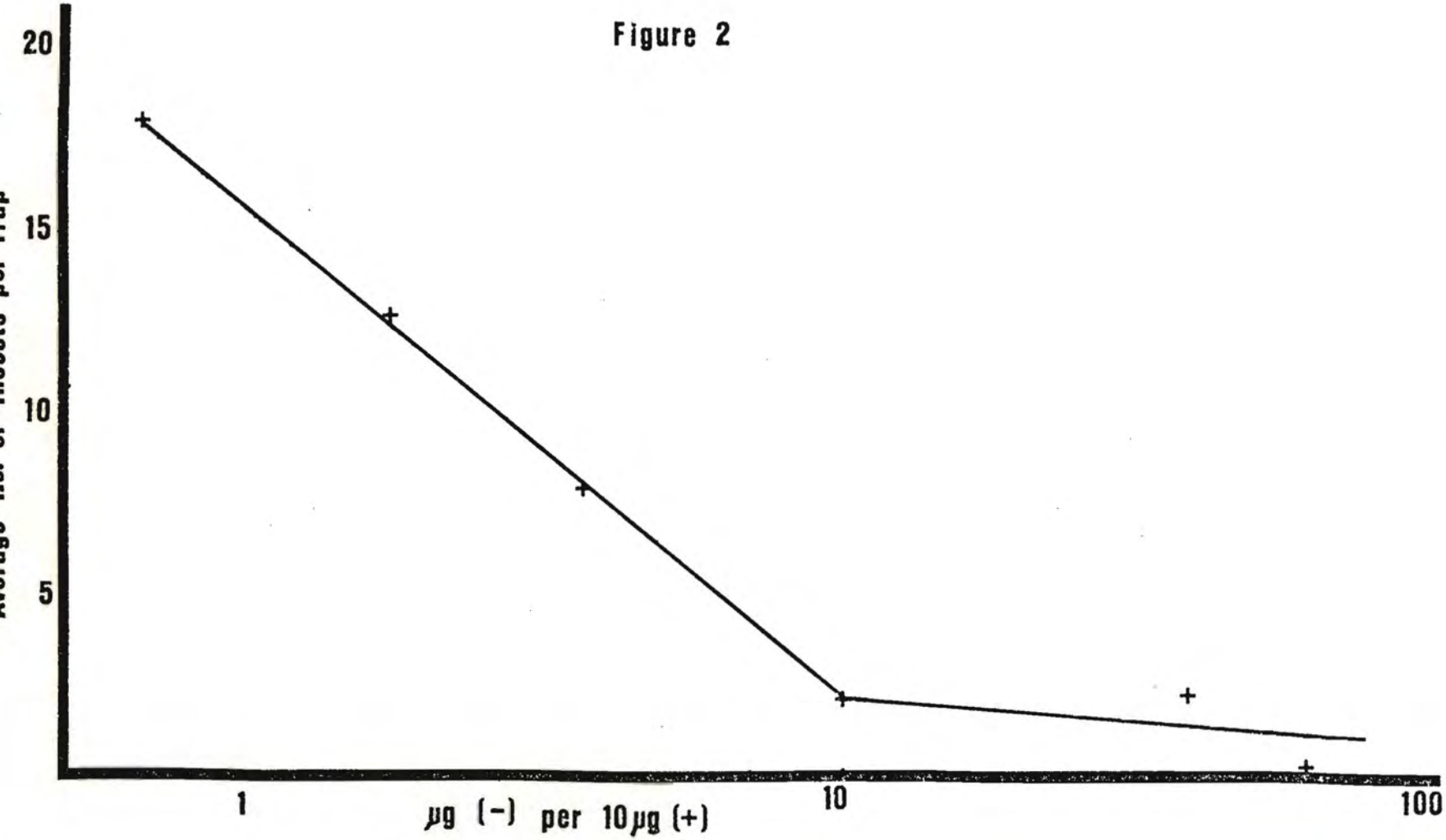
Figure 2