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October 1, 1977 - March 31, 1978

Laboratory Report
Otis Methods Development Center
Animal and Plant Health Inspection Service
United States Department of Agriculture
Otis Air Force Base
Massachusetts
02542

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Animal and Plant Health Inspection Service

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PESTICIDE TESTING SECTION

Project Number	Project Title	Report Type	Page
GM 6.1.5	Regulatory Treatments (Laboratory - Field)	Interim	4
GM 7.1.0	Contact Toxicity of Registered Insecticides Against Gypsy Moth Larvae	Interim	5
GM 7.1.2	Delayed Effects of Sub-lethal Dosages of Insecticides on Fecundity and Fertility of Gypsy Moth	Final	6
GM 7.1.3	The Effects of pH and Temperature on the Chemical Stability of Carbaryl and Orthene	Final	22
GM 7.1.7	1977 Field Studies of Dimilin W-25	Final	25
GM 8.1.1	Evaluation of Ultraviolet Screening Agents for Microbials	Interim	28
GM 8.1.2	1978 Field Studies of Dimilin W-25 and Sevin - 4 Oil	Preliminary	39
GM 8.1.3	Laboratory Screening of Candidate Pesticides Against the Gypsy Moth	Interim	45

PHEROMONE, BEHAVIOR AND ECOLOGY SECTION

Project Number	Project Title	Report Type	Date
GM 6.2.3	Periodicity of Pheromone Release from Female Gypsy Moths	Interim	52
GM 6.2.8	Field Evaluation of Controlled Release Formulations of Disparlure for Gypsy Moth Mating Disruption	Final	53
GM 7.2.1	Efficiency of (+) Disparlure-Baited Traps in Arrays for Estimating Male Moth Density and Delimitation of Infestation	Interim	54
GM 7.2.4	Development and Assessment of Techniques for Comparing the Competitiveness of Populations of Male Gypsy Moths	Interim	55
GM 7.2.7	Evaluation of Commercial Controlled Release Dispensers of (+) Disparlure	Final	62
GM 8.2.1	Release Characteristics of Polyvinyl Chloride Disparlure Wicks	Final	64
GM 8.2.2	Radiological Sterilization of Male Gypsy Moths	Interim	70
GM 8.2.3	Gypsy Moth Mating Behavior	Interim	77

PHYSIOLOGY AND MASS REARING SECTION

Project Number	Project Title	Report Type	Page
GM 6.3.5	Development of Diet and Efficient Rearing Techniques	Interim	79
GM 6.3.6	Establishing Standards	Interim	80
GM 6.3.8	Development of Methods for Avoiding Contact With Insect Scales and Urticating Hairs Associated with Adults, Eggs and Larvae of Gypsy Moth	Interim	82
GM 6.3.9	Evaluation and Development of Automated Procedures for Rearing Gypsy Moths	Interim	83
GM 6.3.15	Development of Efficient Methods for Gypsy Moth Virus Production and a Pilot Plant Facility	Interim	85
GM 7.3.3	Weight Changes in Gypsy Moth Egg Masses During Pre-Diapause Development	Final	86
GM 7.3.4	Insect Production and Distribution	Interim	92
GM 7.3.5	Evaluation and Colonization of Gypsy Moth from the San Jose, California Infestion	Final	93
GM 8.3.2	Rearing Techniques and Production Costs	Interim	95

Project Number: GM 6.1.5
Project Title: Regulatory Treatments (Laboratory - Field)
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leader: Winfred H. McLane, J. A. Finney

Although work was not continued during this reporting period, work conducted from April 1, 1977 - September 30, 1977 was promising enough to indicate that it should continue. Detergents such as light water, Top Job and Pine Scent should be further investigated as ovicides.

Emulsifiers such as Sponto AK 16 - 95 prevent hatch when applied to egg masses.

Materials such as Orthene, Sevin, Resmethrin, FMC - 45498, FMC - 33297 and Bioethanomethrin have prevented hatch when applied to egg masses in a liquid formulation.

Dust applications of Dimilin, Dylox, Gardona, Orthene, Diazinon and Folpet have also reduced hatch when applied to egg masses.

A number of regulatory personnel within the field have been contacted to get their feelings as to what kind of treatment could best help them. The general response was that they needed a treatment for house trailers, recreational vehicles, yard equipment and furniture. They are very interested in a detergent and/or dust treatments that could be used either when the larvae are active or after egg masses have been deposited on the surface of the regulated article. It was felt that log treatment was lower on the list of priorities.

The insecticide section has recently discussed regulatory treatments at a number of section meetings. The feeling is that we may already have the right materials, we only lack the proper technique to apply them. A micronized DDT gun has been acquired to start work in application techniques. A number of chemical companies have been contacted to acquire dust formulations to be laboratory tested for possible use in regulatory treatment.

Project Number:	GM 7.1.0
Project Title:	Contact Toxicity of Registered Insecticides Against Gypsy Moth Larvae
Report Period:	October 1, 1977 - March 31, 1978
Report Type:	Interim
Project Leaders:	Winfred H. McLane, J. A. Finney

No work was conducted as all available time was used conducting irradiation test with various formulations of nuclear polyhedroris virus.

This work will be completed and a final report proposed during period April 1, 1978 - September 30, 1978.

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, 1977 - March 31, 1978
Report Type: Final
Project Leaders: R. G. Reeves, P. Yelton

Abstract:

Laboratory strains of gypsy moth larvae were reared on artificial diets treated with Sevin 80S, Dylox 80S, Orthene 75S, and Dimilin 25W. Sub-lethal dosages of each pesticide was established for 1st, 2nd, and 3rd instar larvae. Larval mortality, pupal and egg mass weights, egg hatchability and larval development of the next generation is reported in this paper.

Methods and Materials:

Diet: The artificial diet used in these tests was Yamamotos' Tobacco Hornworm diet modified for rearing gypsy moth (1). Batches of diet from 2 to 3.5 were mixed in a waring blender and poured into 2 or 3 oz. solo cups. These cups were put into 16 oz. (paper can) cup with 15 larvae infested on each cup of diet. At least sixty insects were infested on untreated diet for controls to monitor with each test. All test cups were held in an environmental chamber at 27^{0} C and 60% humidity.

Insecticide Solution: Stock solutions of insecticides were prepared by weighing 25g of each one into a 1l Erlenmeyer flask and diluting to 600 ml with H_2O. A teflon stirring bar was put in each flask and the solution was mixed vigorously while pipeting aliquots for dilutions. Stock solutions were stored in a refrigerator, and they were made fresh weekly.

Diet Incorporation and Analysis: Aliquots from the stock solution were diluted with water and a small amount of acetone to yield the desired treatment level. Concentration levels (ppm or ppb) of insecticides were based on the total weight of the diet, and the appropriate dilutions were added to the diet with a Burette during the mixing process. Diets were blended for 3 minutes at high speed after the addition of the insecticides.

Diet samples containing Orthene , Carbaryl , and Dylox were extracted and analyzed by G.L.C. to check accuracy of the technique. Orthene and Dylox was extracted basically like biological samples described by Reeves et al. (2) and purified by partitioning between 2 immiscible soluents, Hexane and Acetonitrile, Wiersma et al. (3) A GLC method developed by Chevron Chemical Company was used for Orthene, and a modification of this method was used for Dylox. Carbaryl was extracted by blending 25 gm diet samplers in a 1l blender jar with Acetone and analyzed by methods described by Sevins et al. (4) All diets were fortified with 10ppm of pesticide for analysis and the results proved the technique accurate within + 0.15 ppm. The absence of a reproducable GLC procedure for Dimilin prevented its being analyzed for accuracy.

Egg masses from adults which were reared on treated and untreated diets were monitored for egg hatchability, and 500 larvae from each test were reared to 3rd and 4th instar on untreated hornworm diet to study larval development as compared to controls.

1. Sevin 80S - Table #1 presents pupal weights and mortality, larvae mortality and egg mass weights for all insects on treated and untreated diet. Fig. 1 shows graph of larvae mortality vs. concentration of insecticide in the diet. Generally, pupae and egg masses weighed less on treated diet than those from untreated diet. Pupal mortality was sometimes considerably higher on untreated than treated diet, therefore these results are not significant. The control larval mortality was negligible. Larvae mortality of LD 10 and L-I + II was scattered evenly from stage II through V, while L III's mortality occurred mostly in stage V. At LD 50 and L-I, most larvae died in stage III, L-II in stage IV, and L-III in the V instar. Nearly all instars of LD 90 diet before reaching the next molt.

At concentrations causing >70% mortality, surviving larvae attempting to molt from L-II to L-V could not shed their head capsules or exuvia from posterior sections of their bodies. Insects on Sevin treated diet had a high incidence of virus contamination while the controls were virus free. Tests which became virus infected were repeated.

Dylox 80S - Dylox test results are found in table #2 and figure 2. As with the Sevin test, many control pupae never emerged. LD 10 with L-I, most larval mortality occurred at IV stage, and L-II & III in V & VI stage. LD 50 produced most mortality during the second molt after being infested on contaminated diet, and LD 90 mortality occurred almost exclusively during the next stage of development. After injesting Dylox, the larvae was significantly softer than normal setae, and concentration causing >75% mortality, the larvae which molted from 4th to 5th instar were incomplete with the preceding head capsule remaining Many pupae were deformed, male and female pupae were virtually identical in size and few adults emerged.

Orthene 75S - Table 3 & figure 3 presents the data from the Orthene 75S tests. Larval mortality of the LD 10, 50 & 90 was nearly identical to those of the Dylox tests. Orthene levels causing >75% mortality resulted in rampant cannibalism. Surviving larvae devoured the dead as quickly as they died. Dying larvae had incomplete molts from 4th to 5th, and 5th to 6th instars by retaining their head capsules and exuvia from the posterior section of their bodies. Larvae were much smaller, and they retained more setae than the control insects. Resulting pupae were smaller than controls and many would pupate with the head capsule and prolegs from the larvae stage undisposed. Male and female pupae were virtually the same size.

Dimilin 25W - As is indicated in table #4 and figure #4 it takes a much lower concentration of Dimilin to achieve sub-lethal dosages of insects than any of the other insecticides tested. Mortality results were much more erratic than the other pesticides. Also the same concentration of Dimilin mixed at different times produced 4 highly variable mortality results. Inaccuracy of the diet incorporation could have contributed to the erratic mortality. We did not have a reproducable G.C. method to monitor Dimilin levels in the diets.

Many larvae that survived LD 80 and above would prey upon those that had pupated. Very few adult females emerged from these treatment levels. The female larvae would reach 6th instar and live several days past time to pupate and then die attempting to spin. Surviving pupae from the Dimilin experiment compared to controls disclosed little difference in weight.

Pesticide effect on the subsequent generation: Ten egg masses from each test were monitored for egg hatchability, and the hatch averaged ca. 72% for insects reared on treated or untreated diets. In some cases the controlled hatch was lower than the treated hatch, but there was no significant difference in the overall average.

Five hundred larvae from each test were reared to 3rd and 4th instar on untreated diet. Larval development of those from parents reared on treated diet was no different from the controls.

It is our conclusion that sub-lethal dosages of the 4 insecticides in artificial diet of laboratory strains of larvae has little or no effect on the fertility and fecundity of the gypsy moth.

Table #1.

Pupal, Larval and Egg Mass Weights; Pupal and Larval Mortality of Insects on Sevin 80S

Treated and Untreated Diet

ppm + Instar	TREATED DIET						UNTREATED DIET					
	Pupae 1/				Larvae	Egg Mass	Pupae 1/				Larvae	Egg Mass 3/
L-I	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)
.75	1.2788	0	.3650	0	1.0	.5015	1.5633	0	.6447	0	0	.7529
1.5	1.1007	0	.3871	0	9	.3283	1.4596	0	.5729	0	0	.4570
3.0	.9802	0	.4239	0	50	.4807	1.4440	0	.6654	0	0	.4205
4.0	.6748	42.4	.3027	0	39	.3792	1.3271	9.0	.5763	0	0	.6550
5.0	1.2870	19.0	.3480	11.76	61	.5677	1.3786	50	.6359	0	0	.6731
6.0	1.3981	7.9	.3849	3.70	40	.4600	1.4321	0	.6877	0	0	.5932
7.0	.9876	24	.3722	0	88	.5692	1.3780	13	.5603	2.6	0	.7548
8.0	.9763	13	.3461	0	90	.6033	1.4256	0	.6331	0	0	.6602
Overall average	1.2623		.3623			.4862	1.4260		.6220		0	.6208
L-II												
4.0	-	-	-	-	4.0	-	-	-	-	-	0	-
8.0	1.0953	6.45	.4628	2.98	18.0	5502	1.6643	0	.5781	0	0	.6842
10.0	.6656	12.82	.3445	9.37	60	3051	1.5773	0	.5432	0	0	.7371
12.0	.8660	47.7	.3743	11.10	36	5137	1.2987	0	.6732	0	0	.7101
20.0	1.2731	48.07	.3365	30.90	90	4876	1.4865	22.2	.5466	12	0	.5436
Overall Average	.9750		.3795			.4641	1.5076		.5852			.6687

Table #1 (Cont'd)

		Pupae 1/			Larvae	Egg Mass		Pupae 1/			Larvae	Egg Mass 3/
L-III	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)
3.0	.8993	13.4	.4102	0	24	.6032	1.4588	0	.6732	21	0	.6463
7.0	1.1663	8.3	.3041	11.76	20	.4774	1.3974	86	.6653	33.3	0	.6804
10.0	1.3476	6.5	.3701	27.4	10	.4927	1.3685	0	.6102	0	0	.4939
11.0	.8681	39.6	.3989	7.9	70	.5831	1.5473	83	.5783	33.0	0	.7469
12.0	1.2009	30.0	.3097	23.5	80	.5642	1.5666	0	.5572	0	0	.7098
15.0	1.0667	31.86	.3956	6.17	40	.4636	1.5582	75	.6701	37.5	0	.7642
20	.7630	32.55	.4430	18.5	80	.4602	1.6739	87	.5136	14.28	0	.5562
	1.0045		.3759			.5206	1.5108		.6097			.6568

[illegible]	[illegible]		[illegible]		Larvae	Egg Mass 3/		UNTREATED DIET Pupae				Larvae	Egg Mass 3/
L-I	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)	Weight (gm)	% Mortality	Weight (gm)	% Mortality		% Mortality	Weight (gm)
.75	1.5810	11	.5464	0	28	.4647	1.4437	0	.6407	0		0	.4016
1.5	1.3664	0	.4873	8	8	.5076	1.3768	21	.6981	1		0	.4467
3.0	1.0112	28	.3744	12	55	.5990	1.3420	0	.5535	10		0	.5730
4.5	1.5163	5	.5302	0	12	.5733	1.5201	20	.5032	0		0	.6542
5.0	1.4629	0	.6191	0	30	.3291	1.4454	17	.6224	0		0	.6331
7.0	1.5440	41	.5766	0	90	.4006	1.7032	0	.5298	0		0	.5968
Overall average													
	1.4138		.5223	10		.4790	1.4718		.5962				.5508
L-II													
1.0	-	-	-	-	4.0	-	-	-	-	-		0	-
2.0	1.5361	32	.5521	18	15	.6496	1.6602	0	.5887	0		0	.6444
6.0	1.0362	9	.4787	4	54	.5007	1.6899	0	.6532	0		0	.6991
7.0	.7172	38	.2960	26	60	.4734	1.4643	51	.6411	13		0	.6492
9.0	1.3261	44	.2654	22	98	.5206	1.4569	21	.6224	0		0	.5873
10.0	.9416	0	.4144	44	72	.3027	1.3882	0	.6130	14		0	.5920
Overall average													
	1.1114		.4013			.4892	1.5519		.6236				.6344

Table #2. (Cont'd)

L-III	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)
3.0	-	-	-	-	2	-	-	-	-	-	0	-
4.0	-	-	-	-	5	-	-	-	-	-	0	-
5.0	1.4736	12	.5846	0	50	.5655	1.5469	11	.5900	0	0	.4993
6.0	1.3375	50	.5618	20	78	.4202	1.4601	25	.5973	33	0	.6742
7.0	1.2132	50	.3392	22	81	.6797	1.4233	0	6482	57	0	.6736
9.0	.9886	24	.5459	85	98	.5017	1.5632	0	.6329	0	0	.6582
10.0	1.2006	40	.5074	40	99	.3968	1.5076	44	.6894	0	0	.5460
overall average												
	1.2427		.5077			.5127	1.5002		.6315			.6102

Table #3.

Pupal, Larval, and Egg Mass Weights, Pupal and Larval Mortality of Insects on Orthene 75S

Treated and Untreated Diet

ppm + Instar	TREATED DIET							UNTREATED DIET				
	Pupae 1/				Larvae	Egg Mass 3/		Pupae			Larvae	Egg Mass 3/
L-I	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)
.25	1.4984	27	.5337	7.8	12	.5713	1.3790	0	.6462	0	0	.5368
.5					10						0	
1.0	1.5776	36	.5331	0	15	.6504	1.2172	21.7	.5894	8	0	.6692
2.0	1.3752	0	.5165	0	19	5990	1.3098	0	.5510	0	0	.6526
3.0	.6836	50	.4121	18.75	55	.6067	2.1647	0	5623	0	0	.6864
4.0	1.3987	19.6	.4569	7.0	70	.6329	1.6536	0	.5466	10	0	.6645
8.0	1.4871	42.37	.4002	23.91	45	.3892	1.3689	25	.6531	0	0	.4901
12.0	.9834	65.8	3637	37.5	70	.4076	1.6650	16	.6117	0	0	5679
16.0	-	-	-	-	100	-	-	-	-		0	
Overall average												
	1.2862		.4594			.6414	1.5370		.5940			.6096
L-II												
3.0	-	-	-	-	2.0	-	-	-	-	-	0	-
4.0	.6932	27	.3220	10	59.0	.2040	1.8830	0	.5350	0	0	.5179
5.0	.9358	33.3	.2971	15	60.0	.5565	1.4454	22	.7098	20	0	.6868
6.0	.5461	19	.3552	33	90	.5787	1.6983	0	.6431	0	0	.4900
7.0	.4993	50	.2987	27	95	.5320	1.6521	8	.6606	0	0	.6097
12	.4912	80	.3638	0	88	.3689	1.6742	11	.5989	0	0	.5912
16	-	-	-	-	95	-	-					
Overall average												
	.6331	-	.3273	-		.4480	1.6606		.6294			.5191

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.6021	[illegible]	50	.6301	0	0	.6884
6.0	-				4.0	-					0	-
6.0	-				91						0	
7.0	.9224	18	.3098	0	90	.4002	1.7780	1.0	.6424	0	0	.6392
20	-				100						0	
Overall average												
	.9752		.3085			.4355	1.5678		.6316			.6600

Table #4.

Pupal, Larval, and Egg Mass Weights, Pupal and Larval mortality of Insects on Dimilin 25WP Treated and Untreated Diet.

ppm + Instar	TREATED DIET						UNTREATED DIET					
	Pupae 1/				Larvae	Egg Mass 3/	Pupae				Larvae	Egg Mass 3/
L-I	Weight (gm)	% Mortality	Weight (gm)	Mortality	Mortality	Weight (gm)	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)
0.10	-	-	-	-	9	-	-	-	-		0	
0.15	1.6877	3	.7989	0	17	.4891	1.5781	13	.4984	6	0	.6460
0.25	1.7099	4	.7633	0	22	.5619	1.1998	0	.4142	0	0	.4266
1.5	1.7237	15	.6994	1	12	.6230	1.5983	8	.6012	0	0	.6489
3.0	1.7112	18	.8505	0	10	.6599	1.6448	75	.4990	33	0	.4802
5.0	1.7343	28	.6734	0	12	.5553	1.2789	20	.6002	4	0	.6375
7.0	1.6944	47	.7620	31	14	.6764	1.6692	10	.5144	11	0	.5981
10.0	1.6582	67	.7357	82	35	.5372	1.5933	60	.6156	20	0	.5436
12.0	1.6302	78	.5999	75	50	.3814	1.6746	0	.5505	30	0	.4619
15	1.4300	84	.5664	52	86	.4611	1.6989	0	.6431	0	0	.6200
Overall average												
	1.6634		.7166			.5494	1.5484		.5484			.5636
L-II												
.25	-	-	-	-	6		-	-	-	-	0	-
4.0	1.7321	0	.6984	0	15	.5425	1.6990	83	.6272	0	0	.6073
7.0	-	-	-	-	18	-	-	-	-	-	0	-
10	1.6471	79	.6214	78	10	.6002	1.6563	0	.5833	0	0	.6212
12	1.4296	26	.6988	11	75	.6519	1.6325	8	.6401	29	0	.5166
15	1.5647	19	.5963	1	95	.3276	1.6634	33	.5862	1	0	.4134
Average												
	1.5933		.6469			.5305	1.6628		.6401			.5546

Table #4 (Cont'd)

ppm + Instar L-III	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)	Weight (gm)	% Mortality	Weight (gm)	% Mortality	% Mortality	Weight (gm)
.25	1.7123	15	.7351	28	12	.5295	1.4891	0	.6451	32	0	.3821
12	1.6899	88	.7116	83	50	.6174	1.6848	20	.6174	16	0	.5673
15	1.6944	16	.7518	29	90	.4244	1.5006	51	.5437	11	0	.5812
18	1.5543	0	.5941	7	98	.5861	1.5431	0	.6361	0	0	.4371
Overall average												
	1.6627					.5143	1.5544		.6105		0	.4919

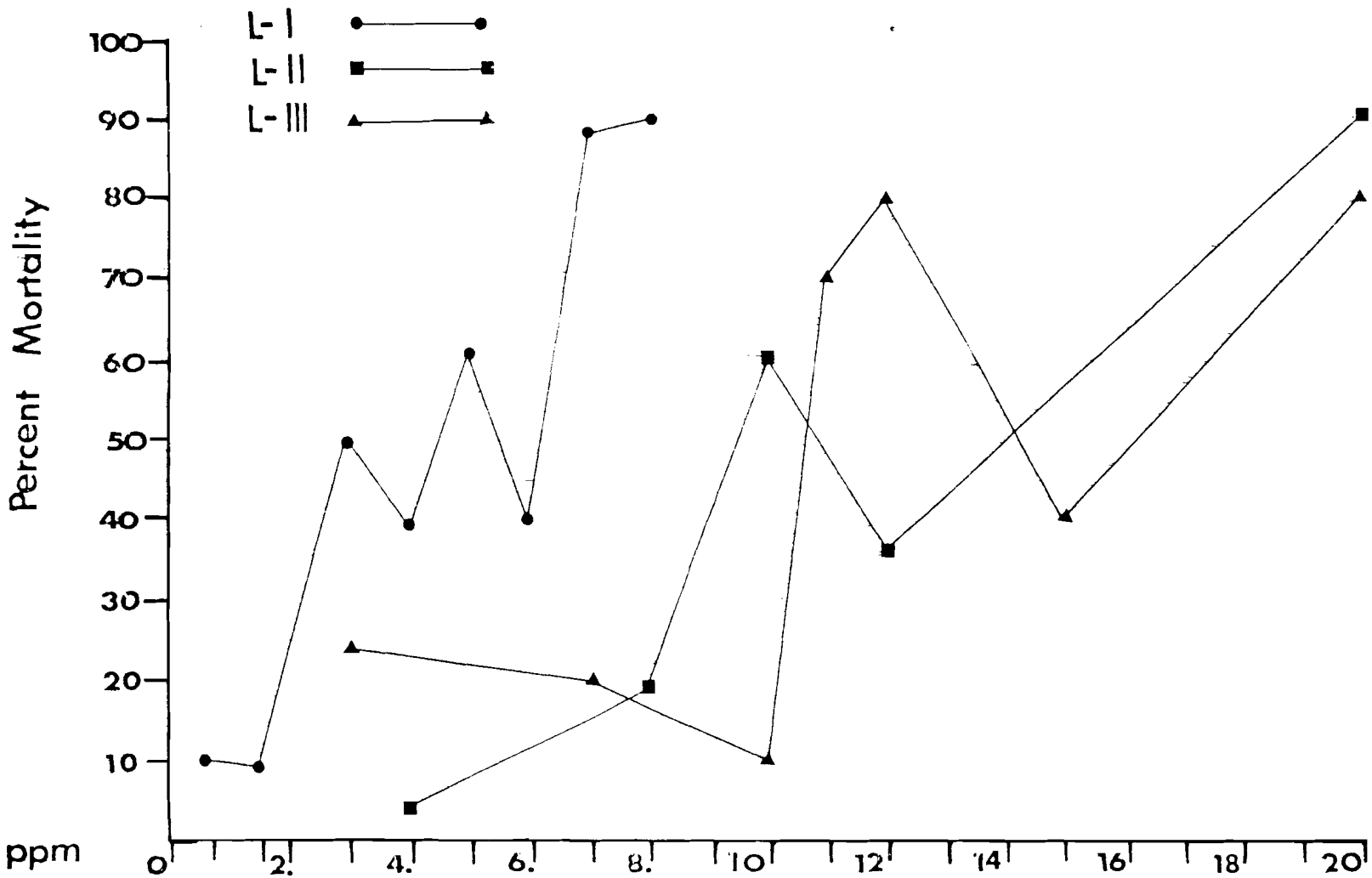

Figure 1. Gypsy Moth Larval Mortality [illegible] Treated Diet

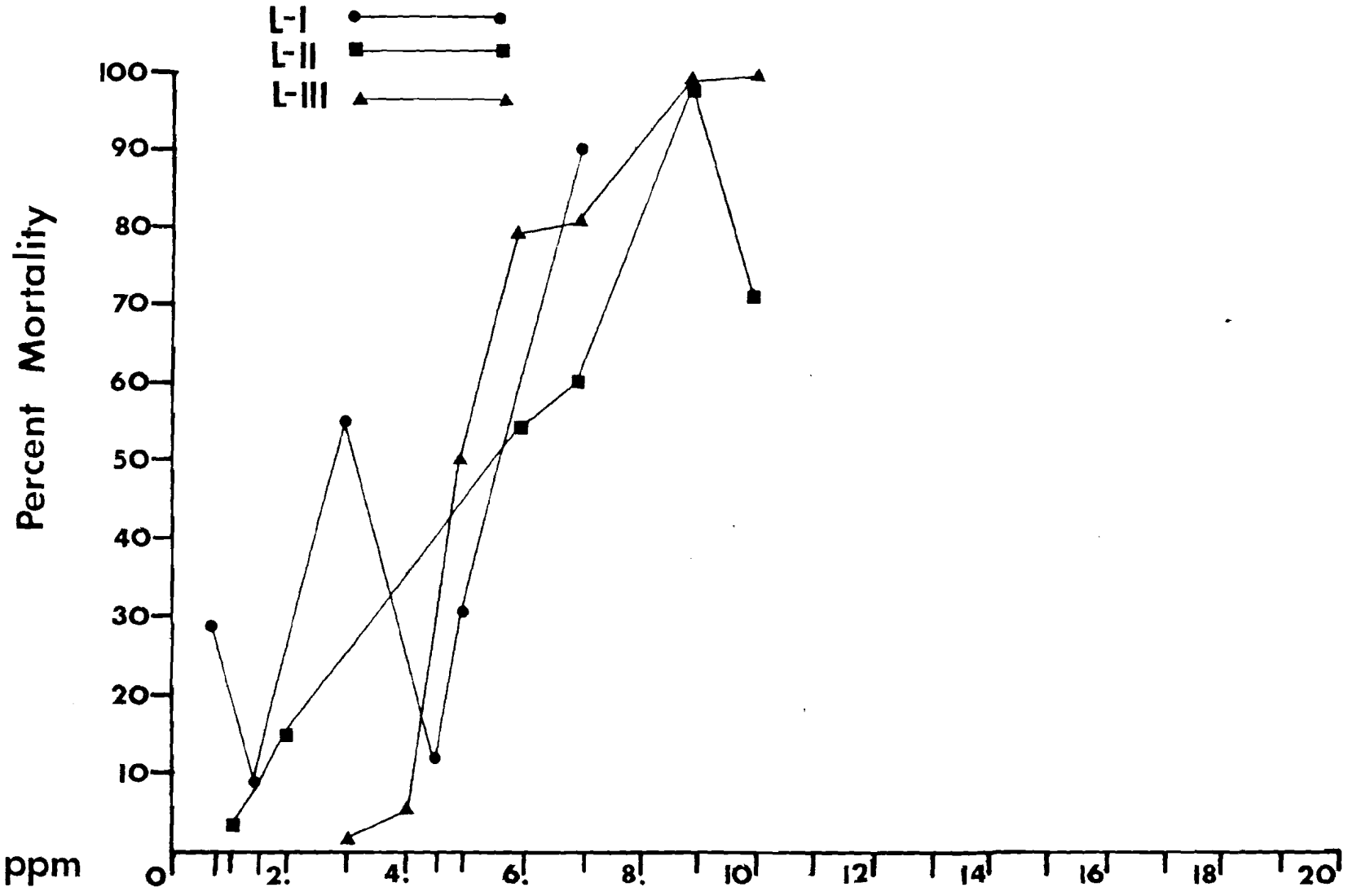

Figure 2. Gypsy Moth Larval Mortality vs. ppm of Dylox 80S Treated Diet

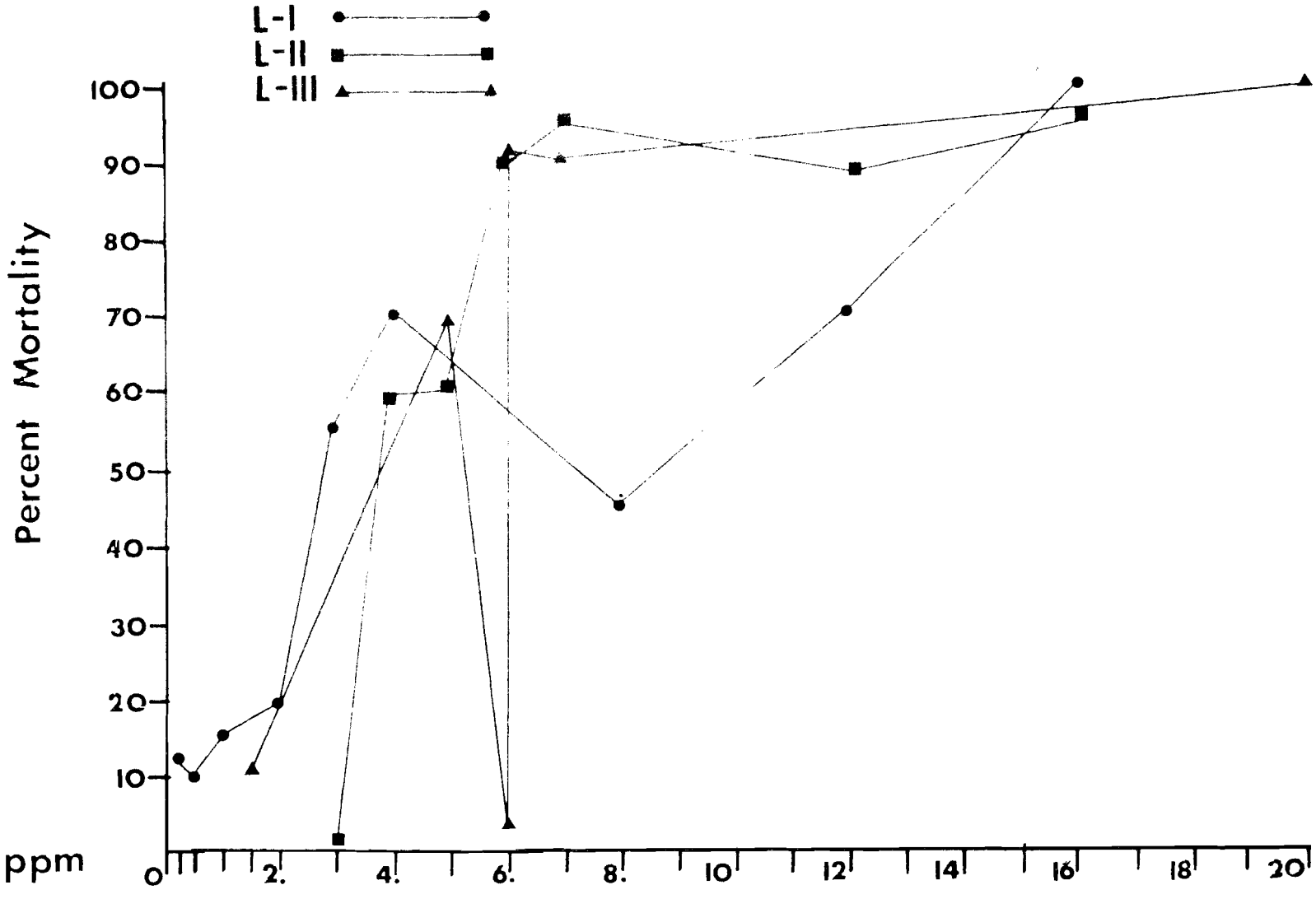

FIG #3 GYPSY MOTH LARVAL MORTALITY VS. ppm OF ORTHENE 75S TREATED DIET

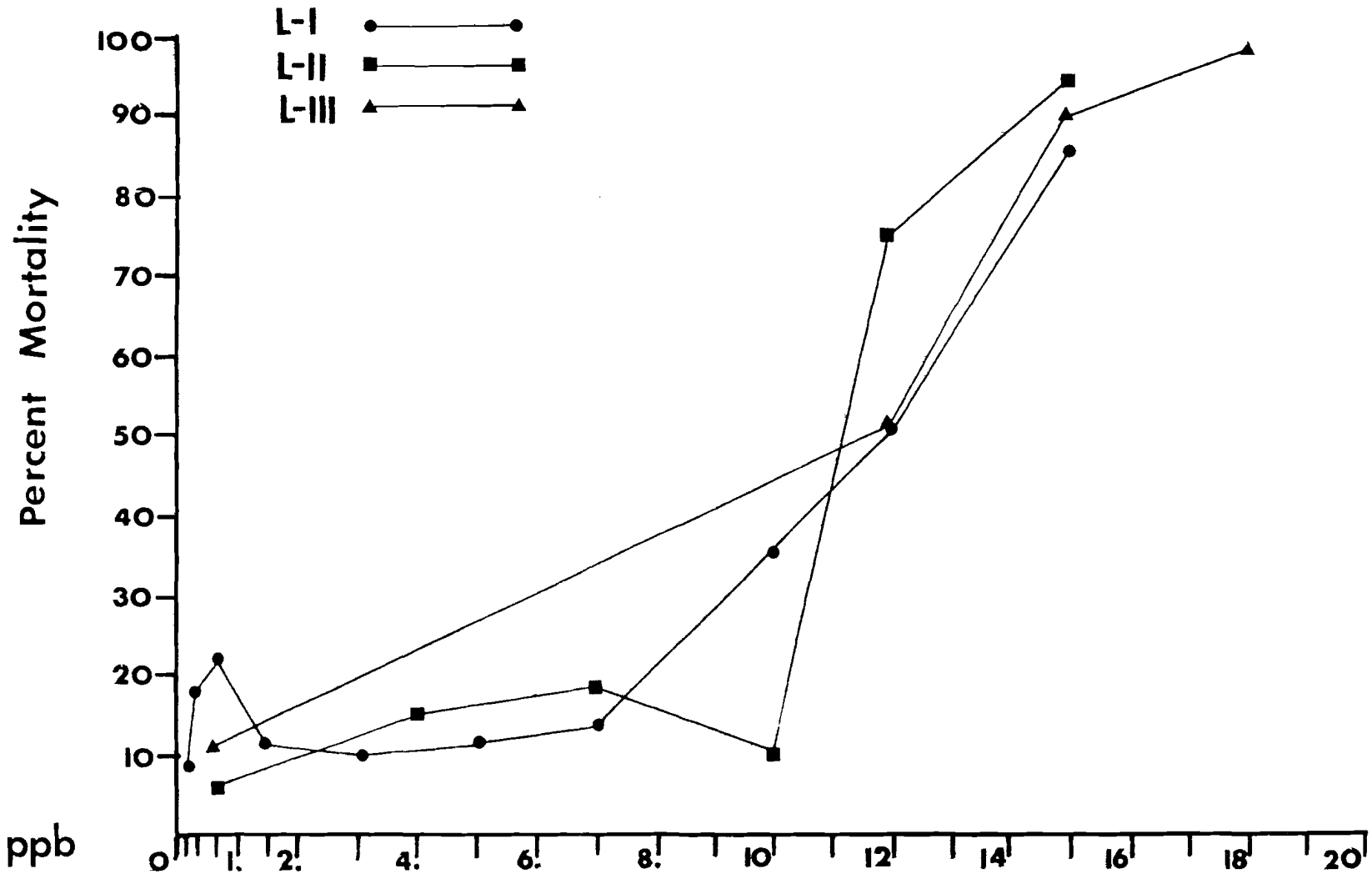

Figure 4. Gypsy Moth Larval Mortality vs. ppb of Dimilin 25WP

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Project Number: GM 7.1.3
Project Title: The Effects of pH and Temperature on the Chemical Stability of Carbaryl and Orthene .
Report Period: October 1, 1977 - March 31, 1978
Report Type: Final
Project Leaders: R. Reeves, J. Yarko

ABSTRACT

The hydrolysis rates of Sevin 80S and Orthene 75S were established at pH values ranging from 5 to 12, and temperatures of 25° and 35° C. Quantitative analyses were accomplished using gas-liquid chromatography.

Introduction:

Many pest control failures are attributed to the acidity and alkalinity of the water used in preparing the pesticide spray solution (1) Carbamates and organophosphates in particular, have short half-lives (due to hydrolysis) in pH medium above 7.0 (2).

This study investigates the rate of hydrolysis of Sevin 80S and Orthene 75S at varying pH valves and temperatures in order to estimate the maximum holding time of these pesticide mixtures prior to application.

Methods and Materials

Sevin and Orthene was mixed according to label instructions except the water pH was adjusted prior to adding the pesticide - in a 1000 ml erlenmeyer flask. A teflon stirring bar was put into the flask and the mixture was agitated during the entire time period.

Orthene 75S was tested at pH of 8.0, 9.0, 10.0, and 12.0. All mixtures were held at 25° and 35° C in a constant temp water bath. The pH values used for Sevin 80S was 5.0, 7.0, 8.0, 9.0 and 11.0 at 25° C, and 35° C.

The mixtures were sampled and extracted immediately after adding the pesticide. These extracts were used for standards to measure the degradation rates of the mixtures at 24, 48, and 72 hours. At termination of a time period five - 25 ml aliquots were pipeted into a 500 ml. separatory funnel, and the pH of the solution was immediately adjusted to 6.0 to prevent further hydrolization. The insecticides were extracted with three - 25 ml portions of CH_2 CL_2 and dried by filtering through Na_2 SO_4.

An instrumental procedure developed by Cook et al. (3) was used to analyze Sevin, and a modification of Chevrons Method (4) was used for Orthene.

Table No. 1 - Hydrolysis rates of Sevin 80S

pH	25° C % Hydrolysis			35° C % Hydrolysis		
	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.
5	3	6	7	4	2	5
7	5	23	25	8	22	29
8	45	53	61	59	70	81
9	62	64	94	73	97	100
11	93	98	100	99	100	100

Table No. 2 - Hydrolysis rates of Orthene 75S

pH	25° C % Hydrolysis			35° C % Hydrolysis		
	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.
8	6	7	[illegible]	4	14	20
9	28	41	45	26	31	24
10	56	80	96	77	92	96
12	98	100	100	99	100	100

Results and Conclusions:

Table No. 1 presents the hydrolysis rates of Sevin 80S. At 25° C, it is stable to hydrolysis at the acidic pH of 5.0. However, at pH 7.0 quick rise in the hydrolysis rate was observed and increased with the increase in pH. The half-life time is about 6 days at pH 7.0, about 40 hours at pH 8.0, less than 24 hours at pH 9.0, and less than 1 hour at pH 11.0. At 35° C Sevin remains stable at pH 5.0. But the half-life time decreases to about 5 days at pH 7.0, and less than 24 hours at pH 8.0. At pH 11.0 the half-life time is probably 4 to 10 minutes.

Table No. 2 presents results from the Orthene 75S tests. Orthene is more stable at a pH medium over 7.0 than parathion and some other organophosphates (4). Orthene 75S is stable at pH 8.0 for up to 72 hours at 25° C. But it is over 25% hydrolized at pH 9.0 and the half-life time is less than 24 hours at pH 10.0. The hydrolysis rates of Carbaryl and Orthene is dependent upon both the pH of the water and the temperature at which the solution is stored.

Sevin 80S mixed with water at pH 3-5 and stored at a temperature between 25° and 35° C should maintain its stability for up to 72 hours. However, the pesticide mixed with water at pH 7 or above should not be held more than 4 hours.

The holding time of Orthene 75S is not as critical as Sevin 80S in water at pH above 7.0. It could be held at pH 8.0 and 25° C for up to 72 hours. But at 35° C it should not be held for more than a few hours.

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Project Number: GM 7.1.7
Project Title: 1977 Field Studies of Dimilin W-25
Report Period: October 1, 1977 - March 31, 1978
Report Type: Final
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 were 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.

Objectives:

1. Determine the activity and compare the efficiency of Dimilin W-25 applied at a single application for use in regulatory treatment of hazardous areas.

2. Determine the relative utility for eradication of infestations using several different treatment schedules of Dimilin W-25.

Objectives and methods are explained in detail in Project Number GM 7.1 7 dated October 1, 1976 - March 31, 1977.

Results and Discussion:

Each treatment plot evaluated for eradication had 200 burlap bands distributed in the center 10 acres (total of 600 bands per treatment). Table 1 gives the totals for each treatment. It should be noted that the 16 pupae found in plots treated with Dimilin at 0.06 lb/gal/A were all under two burlap bands and may be due to migration or wind spread. Table 1 also shows a trend toward multiple applications being more effective against gypsy moth larvae. For example, the single application of Dimilin at 0.06 lb/gal/A yielded 448 larvae under 600 bands compared with 0 larvae using the same dosage with two applications. Dimilin at 0.03 lb/.5 gal/A at two applications yielded 17 larvae under 600 bands compared with 0 larvae at three applications.

Table 1. Larval, Pupal and Egg Mass Counts from Burlap Band Monitoring, Pike County, PA 1977. (Numbers are totals from 600 bands distributed in 3 plots).

Dosage	# of Applications	Number Larvae	Number Pupae	Number of Egg Masses
0.06 lb/gal/A	1	448	26	0
0.06 lb/gal/A	2	0	0	0
0.06 lb/.5 gal/A	2	59	0	0
0.03 lb/gal/A	2	12	0	0
0.03 lb/.5 gal/A	2	17	0	0
0.03 lb/.5 gal/A	3	0	0	0
0.03 lb/gal/A	3	0	0	0
Check	None	25,157	12,060	1,121

Each center 10 acre plot was surveyed both pre-and post spray for egg masses. This egg mass survey was accomplished using the diagnostic prism method developed by the USFS. Table 2 reflects the average egg mass populations for 1977 treatments.

Table 2. 1977 Prism Point Egg Mass Counts, Pike County, PA (average of 3 plots)

Dimilin Dosage	# of Applications	# Spring Egg Masses/A	# Fall Egg Masses/A
0.06 lb/gal/A	1	711	0
0.06 lb/gal/A	2	1788	0
0.03 lb/gal/A	2	1883	0
0.03 lb/gal/A	3	636	0
0.06 lb/.5 gal/A	2	1630	0
0.03 lb/.5 gal/A	2	841	0
0.03 lb/.5 gal/A	3	1592	0
0.06 lb/.5 gal/A	1	1063	0
0.03 lb/.5 gal/A	1	1509	0
Checks (average of 10 plots)	None	1994	910

Visual defoliation estimations were made at pre-spray and at peak defoliation in increments of 20 . In all cases the pre-spray estimates fell within the 0 - 20% defoliation range. Post spray estimates also fell within 0 - 20% for the treated areas whereas the checks fell in a range from 60 - 100% defoliation.

All rates and dosages of Dimilin W-25 applied by air in 1977 to forested areas gave excellent foliage protection. These same formulations caused egg mass populations to be reduced to a "non-detectable" level using current survey expertise.

Project Number: GM 8.1.1
Project Title: Evaluation of Ultraviolet Screening Agents for Microbials
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leaders: Winfred H. McLane, J. A. Finney

Abstract:

Laboratory tests demonstrated that spray deposits of gypsy moth (*Lymantria dispar* (L.)) nucleopolyhedrosis virus (NPV) on tender oak foliage are readily inactivated by ultraviolet light. NPV in water was inactivated after an exposure of 15 minutes of Westinghouse sunlamp FS 40, and Westinghouse blacklight blue, F 40 BLB, side by side, 7.62 cm above treated foliage. Three Pro Tec® 100 formulations were tested along with the standard Shade® formulation. The Shade formulation was superior to all other formulations tested including Pro Tec® 100 plus green pigment. A 12 hour exposure to Westinghouse blacklight blue lamps, used side by side 20.3 cm above treated foliage, had little if any effect on NPV. Higher percentage of mortality with Pro Tec® 100 formulations, not exposed to ultraviolet radiation, compared with NPV + water indicates that the material acts as a feeding stimulant.

Introduction:

During the past two decades a considerable amount of research has been directed to the development of gypsy moth nucleopolyhedrosis virus as a biological treatment to surpress gypsy moth populations. Until recently, this effort has been severely restrained by the inability to produce sufficient quantities of virus in the laboratory. With only limited amounts available, it was not possible to conduct meaningful field tests to establish efficacy. This has all changed as a result of the Expanded Gypsy Moth Program. With new artificial diets and efficient rearing procedures it is not possible for rear 20,000 insects/day. As the average female gypsy moth larva will produce $5x10^9$ PIBs, extensive amounts of virus can be stockpiled for experimental and operational projects.

Aerial field tests have been conducted in Pennsylvania for the past few years by the U.S. Forest Service. Experimental plots ranging in size from 14 ha (35 A) to 60 ha (150 A) have been treated with fixed wing aircraft. Some treatments have been applied in two applications 10 days apart; others were single applications. Most spraying is normally conducted in the morning. However, some were designed to receive only evening treatments. Various spray dispensing equipment has been used to disseminate virus, Beecomist® Spray nozzles being the most commonly used.

The standard formulation that has been used for most virus treatments consists of virus, molasses, Chevron spray sticker, Shade® and water. Although compatible, components of this formulation are messy to work with and require considerable mixing in a nurse tank before application can be made.

While foliage coverage has been good with most treatments, insect mortality has often been less than expected. It is possible that the lack of activity is due to the effects of ultraviolet irradiation on the virus. A number of investigators have reported on effects of ultraviolet radiation on insecticides, Bacillus thuringiensis and insect viruses (David, 1968, Krieg, 1975, Cantwell, 1966, and Bullock, 1970.) It has been determined that wavelengths near 307.5 nm undoubtedly play an important role in the breakdown of virus in the field (Bullock, 1970).

The stability of gypsy moth NPV was first studied by the U.S. Forest Service, Hamden, Connecticut. The standard molasses formulation and a Pro Tec® 100 (ultraviolet absorber) mix were evaluated in natural sunlight. Those tests indicated that ultraviolet radiation is critical in determining efficacy of NPV. Therefore, it was determined that work should be done to establish a suitable formulation to minimize the effects of ultraviolet light.

In tests by the U.S. Forest Service, Pro Tec 100 had exhibited some potential as a good screening agent. However, all tests were conducted outdoors in early fall. As the Hamden laboratory had no indoor ultraviolet simulator, the U.S. Department of Agriculture, Gypsy Moth Methods Development Laboratory at Otis Air Force Base, Massachusetts was asked to do a series of ultraviolet tests comparing the standard formulation and Pro Tec 100.

Methods and Materials:

A Westinghouse sunlamp FS 40 and a blacklight blue F 40 BLB lamp were used as a source of ultraviolet radiation. Sunlamp FS 40 covers a wavelength of 275 - 375 nm and peaks at 310 nm. The blacklight blue peaks at 365 nm and covers a range from 250 - 500 nm. This lamp combination was recommended by Westinghouse to simulate uv radiation on a clear June day. This was as close to actual field conditions as we could get in the laboratory without spending large sums of money for sophisticated equipment.

Lamps were mounted in a plywood made box 44 h x 96 w x 137 l cm. In initial tests all surfaces of the chamber were lined with aluminum foil. After the first series of tests were completed, foil was removed from all surfaces except directly over the lamps. Lighting was extended directly over the center line of the chamber floor on adjustable chains. Lighting was 8 cm above foliage in some tests and 20 cm in others.

Tender northern red oak seedlings were treated in a spray chamber with the experimental formulations (McLane, 1973). Five seedlings were used for each formulation. Treated plants were then exposed to the ultraviolet radiation for various periods of time. A similar group of plants, treated with the same formulations, were not exposed. After treatment, newly moulted second instar, laboratory reared, gypsy moth larvae were exposed to treated foliage for a 48 hour period. Twenty larvae were exposed to each plant; the foliage was nearly all consumed at the end of the 48 hour period. Test insects were then transferred to rearing containers (5 x 9.5 cm) with 55 ml of artificial diet. Ten insects were reared in each container in an environmental chamber at 23° C and 12 hour photoperiod.

Mortality readings were made daily for at least 16 days in all tests. In some tests readings were extended to 30 days. Mortality appeared to peak in 16 days, then level out and peak again at about 25 to 30 days. It was decided to terminate tests after 16 days because we felt mortality after that time could be due to horizontal transmission.

Results and Discussions:

Initial tests were conducted with a blacklight blue and sunlamp in combination, 7.6 cm. above the foliage. Tests were conducted using Hamden, CT virus lot #35. Dosage was 1.54 g/ac applied in 2 gal (2.64×10^7 PIBs/ml). A series of formulations and exposure times were compared (Table #1).

Table 1. Effect of exposure to Westinghouse FS 40 Sunlamp and F 40 BL Blacklight used in combination, 7.62 cm above treated foliage.

	Time of exposure of plants to uv									
	5 Min.		15 Min.		30 Min.		60 Min.		120 Min.	
	Percent Mortality After 16 Days									
Formulation	NO	UV	NO	UV	NO	UV	NO	UV	NO	UV
Check	4	2	1	4	1	1	4	4	3	1
NPV+H_2O	46	59	57	3	30	6	59	0	63	5
NPV+PT 1 1/	64	35	66	23	60	7	76	11	69	16
NPV+PT 2 2/	72	45	69	41	70	32	78	44	79	39
NPV+PT 3 3/	50	61	87	42	90	45	78	40	75	22
Std. (Shade) 4/	70	60	74	74	59	61	55	70	51	53

1/ Standard Pro Tec® 100 formulation used at 8 parts water to 1 part Pro Tec® 100.

2/ Standard Pro Tec® 100 formulation used at 8-1 with double the ultraviolet absorber.

3/ Standard Pro Tec® 100 formulation used at 8-1 with triple the ultraviolet absorber.

4/ One pound of Shade/acre, 3 percent chevron spray sticker, 25 percent molasses and water.

Three formulations were exposed to blacklight blue and sunlamps separetely and in combination. Plants were treated with virus lot #35. Dosage was 1.54g/ac applied in 2 gal. (2.64×10^7 PIBs/ml). (Table 2).

Table 2. Effects on activity of NPV when exposed to Sunlamp and Blacklight singly and in combination, 7.62 cm above treated foliage.

	Percent Mortality After 16 Days			
		30 min. Exposure		
Formulation	NO UV	Sunlamp	Blacklight	Combination
Check	3	3	2	5
NPV + H_2O	81	3	72	5
NPV + PT 2	72	60	87	70
Std. (Shade)	78	72	72	68

It was felt by Dr. Mason, Professor of Physics and Meteorology at the University of Michigan that we were over exposing the formulations with the sunlamp and blacklight blue lamp in combination, 7.6 cm above the plants. He, therefore, suggested that we remove all aluminum foil from the chamber except directly over the lamps and use 2 blacklight blue lamps in combination 20 cm above the treated foliage.

Tests were conducted using 5 formulations and various time exposures. Treatments were the same as those in test 1. (Table 3).

Table 3. Effect on activity of NPV exposed to two blacklights 20 cm above treated foliage.

	Percent Mortality After 16 Days							
	4 Hours		8 Hours		8 Hours		12 Hours	
Formulation	NO	UV	NO	UV	NO	UV	NO	UV
Check	1	2	3	1	0	1	3	0
NPV + H_2O	31	30	13	8	32	29	34	28
NPV + PT 1	53	57	49	31	47	45	45	34
NPV + PT 2	64	47	44	17	55	48	33	40
NPV + PT 3	62	54			50	50	41	45
Std. (Shade)	59	35	22	27	50	47	22	22

A test was conducted using a combination sunlamp and blacklight blue, 7.6 cm above treated foliage, to determine the stability of the standard Shade® formulation. Application dosage and rates were as before. (Table 4).

Table 4. Effect of 3-6 hour exposure to UV (sunlamp and blacklight blue) on standard Shade formulation.

	Percent Mortality After 16 Days				
Formulation	NO UV	3 HR	4 HR	5 HR	6 HR
Check	0				9
Std. (Shade)	71	65	66	69	54

A test was conducted using 3 experimental formulations of charcoal, coppertone and sunscreen gel. The formulations were poor as the ingredients did not blend well and efficacy was poor compared to the standard formulation (Table 5).

Table 5. Ultraviolet test with NPV in three trial formulations.

Formulation	Percent Mortality After 16 Days	
	15 Min. Exposure to Sunlamp and Blacklight	
	NO UV	UV
Check	0	0
NPV + H_2O	70	2
Std. (Shade)	74	59
NPV + Charcoal 1/	75	13
NPV + Coppertone 2/	60	21
NPV + Sunscreen Gel 3/	52	7

1/ NPV, .1 lb charcoal, 1 ml of Triton X-190 and water

2/ NPV, 1 ml of coppertone, 1 ml of Triton X-190 and water

3/ NPV, 1 lb sunscreen gel, 1 ml Triton X-190 and water

In all prior tests, it was standard practice to expose test insects to treated foliage for a period of 48 hours, after which time they were transferred to artificial diet. We felt that mortality after 16 days was unusually low in our standard, and therefore, set up a test to see if we could increase mortality by increasing the amount of time larvae were exposed to treated foliage (Table 6). Dosage and rate of application were the same as in test 1.

Table 6. Test results when larvae were exposed to treated foliage for various amounts of time.

Formulation	Days on Foliage	Percent Mortality After 16 Days	
		NO UV	30 Min. UV (Combo)
Check	2	0	2
	3	2	1
	4	3	2
	6	1	2
NPV - H_2O	2	79	2
	3	66	1
	4	62	16
	6	93	9
NPV + PT 2	2	61	31
	3	87	53
	4	68	42
	6	93	75
Std. (Shade)	2	71	72
	3	53	57
	4	63	71
	6	89	91

A test conducted to determine if the addition of a green pigment to the Pro Tec® formulation would give extra protection (Table 7).

Table 7. Effect of addition of green pigment to Pro Tec® formulations.

Formulation	Percent Mortality After 16 Days	
	NO UV	30 Min. UV 1/
Check	0	1
NPV + H_2O	87	86
NPV + 2 + 1% green dye	58	38
NPV + 2 + 2% green dye	68	32
NPV + 3 + 2% green dye	64	44
Std. (Shade)	[illegible]	46

1/ Sunlamp and blacklight were used side by side, 7.6 cm above treated foliage.

A test was conducted to determine what component in the standard formulation was acting as the ultraviolet screen. Earlier testing with pyrethrins indicated that molasses was an effective ultraviolet absorber. This test would confirm earlier findings (Table 8).

Table 8. Ultraviolet screening test using various components of standard NPV formulation.

	Percent Mortality After 16 Days	
Formulation	NO UV	30 Min. 1/
Check	2	2
Std. (Shade - molasses)	35	29
Std. (Molasses only)	40	49
Std. (Shade only)	45	29

1/ Sunlamp and blacklight were used side by side, 3" (7.6 cm) above treated foliage. Test was conducted using Hamden, CT virus, lot #35. Dosage and rate of application was 1.45 g/2 gal/ac. (2.54×10^7 PIBs/ml)

At a virus formulation meeting held in Chicago, Illinois, March 9-10th, 1978, Dr. Mason suggested that we should not expose treated plants to radiation source below 300 nm since wavelengths less than 300 nm rarely penetrate to the earth surface.

As the lamp gives out considerable power below 300 nm, we might eliminate some potentially good ultraviolet screening materials.

Mylar type "A" (10 mil; 0.25 mm) polyester film (DuPont) will cut out all radiation emitted by the FS 40 sunlamp below 315 nanometers (nm) (W.B. Sisson and Caldwell M.M. 1975). Therefore, tests were set up using filters compared to the standard no filter technique (Table 9).

Table 9. Test results with 3 formulations of virus exposed to ultraviolet radiation, with and without polyester film filters.

	Percent Mortality After 16 Days							
	15 Min. Exposure				30 Min. Exposure			
Formulation	NO UV	UV	1F 1/	2F 2/	NO UV	UV	1F 1/	2F 2/
Check	1				2			
NPV + H_2O	45	2	25	28	11	12	3	5
NPV + PT 2	46	38	43	63	36	51	41	41
Std. (Shade)	44	34	55	53	55	55	49	43

1/ One filter used 1.2 cm below lamps.

2/ Two filters used, one on top of the other, 1.2 cm below lamps.

3/ Sunlamp and blacklight blue were used 7.6 cm above foliage.

Test results indicate that a Westinghouse Sunlamp FS 40 and Blacklight blue F 40 BLB, used in combination, 7.6 cm above treated foliage, is a suitable way to test ultraviolet radiation effects on virus formulations. Using this technique favorable formulations can easily be identified from mediocre mixes.

Preliminary tests with filters to eliminate radiation below 315 nm indicate that the filters are having an effect on the reaction of the active materials. However, additional testing must be conducted before a conclusion can be made.

Little if any positive results were obtained by incorporating color pigment into the Pro Tec formulations.

It was found that blacklight blue lamps, 20 cm above the treated foliage had little effect on NPV. This indicates, as Westinghouse engineers stated, that one needs to use the sunlamp and blacklight in combination.

In all tests, the standard (Shade) formulation appeared to be superior to all other formulations tested. Until an improved formulation is developed, it is recommended the standard be used. The formulation seems to be stable as it withstood 6 hours exposure to ultraviolet radiation.

Since shade and molasses are not particularly easy to mix and work with, and little formulation work has been conducted with gypsy moth NPV to date, it is very important that formulation work get started with this material as soon as possible. If ultraviolet radiation is a detrimental factor altering the efficacy of microbials, then an active screening program should be initiated to ascertain the best possible radiation absorbers.

Project Number: GM 8.1,2
Project Title: 1978 Field Studies of Dimilin W-25 and Sevin - 4 - Oil
Report Period: October 1, 1977 - March 31, 1978
Report Type: Preliminary
Project Leader: Larry L. Herbaugh, W. H. McLane, J. A. Finney

The gypsy moth is a major pest in large tracts of forested land in the northeast and threatens invasion of susceptible forest in other parts of the U. S. Recently, there have been outbreaks in isolated areas such as California, Michigan, Maryland and Virginia. With these conditions occurring, it is necessary to develop efficacious control methods to retard the spread and/or control populations of gypsy moth. Because of escalating control costs and acute public interest in protecting the environment, economical and efficacious treatment that deliver control with minimal dosages must be established. The following tests are designed to establish lower effective dosages of Dimilin W-25 and Sevin-4-oil than those currently registered and used for gypsy moth control.

Dimilin W-25 was applied in Pike County, PA in 1977 with 0.03 lb ai/A at .5 gal/A and the results showed no detectable egg masses in the evaluation area during the fall egg mass survey.

Objectives:

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

Material	Dosage-lb AI/A	Rate/A	No. Applications	No. Plots
Dimilin W-25	0.03 + oil	1 qt.	1	6
Dimilin W-25	0.03	1 qt.	1	6
Dimilin W-25	0.02	1 qt.	1	3
Dimilin W-25	0.02	1 qt.	1	3
Dimilin W-25	0.03	1 pt.	1	3
Sevin - 4-oil	1.0	4 oz.	1	3
Sevin - 4-oil	0.75	4 oz.	1	3

These treatments will be applied when the majority of gypsy moth larvae are in the 2nd instar and oak leaf expansion is 80% (normal timing).

2. Determine the effectiveness of the following treatment schedules in eradicating gypsy moth populations.

Material	Dosage-lb AI/A	Rate/A	No. Applications	No. Plots
Dimilin W-25	0.02	0.5 gal.	2 1/	3
Dimilin W-25	0.02	1 qt.	2	3
Dimilin W-25	0.01	0.5 gal.	2	3
Dimilin W-25	0.01	1 qt.	2	3
Checks	0	0	0	6 2/

1/ 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 treatment.

2/ These six untreated checks will serve a dual purpose and be used for comparison and evaluation for both objectives 1 and 2.

Methods:

An area in Clinton County, PA has been tentatively selected as an area that meets the following criteria for establishing study plots: (1) a healthy field infestation of gypsy moth in which there has 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).

Forty-five 50 acre (20 ha) square plots will be laid out by establishing an accessible base corner and plotting boundary lines using compass headings (see Figure 1). Within each of these spray plots a centrally located 10 acre (4 ha) unit will be established where all sampling data will be collected throughout the study period.

The corners of each 50 acre (20 ha) plot will be marked with double ribbon and small 2"x3" (5 cm x 8 cm) paper tags to identify the plot and corners. A single orange line comprised of 12' (30 cm) or less streamers will mark 2 opposite boundaries of a plot to facilitate location of the inaccessible corners. A single pink line will lead from an accessible point and connect all prism sampling points located on 10 acres (4 ha) within each 50 acre (20 ha) plot.

All material will be applied aerially using the USDA, APHIS airplane. When feasible, the second application will be flown perpendicular to the first on those plots receiving 2 applications. Communications will be maintained between a ground spotter man and the spraying aircraft by two-way radio. If available, guidance will be facilitated by using the Loran-C navigation system.

Evaluation:

Each 10 acre (4 ha) plot will be surveyed both pre- and post-spray for egg masses. This egg mass survey will be accomplished using, with modification,

the diagnostic prism method. Each study unit will have 20 prism points established in it. The egg mass data will be recorded on the form shown in Figure 2.

In addition to the egg mass survey, each plot will have 200 burlap bands (approximately 9" or 23 cm in size) attached to host trees 4" (10 cm) and larger in diameter and to monitor late larval and pupal numbers and egg laying activity within the sampling units. Ten of these bands will be recorded 3 times; once during late larval activity, once during pupation and the last time after egg laying has occurred. Each band will be numbered and its identity carried throughout the study as shown in Figure 3.

Each plot will be appraised at peak defoliation by making an estimate of foliage protection.

Treatment efficacy will be expressed in terms of larval and pupal population reduction and egg mass reduction.

Duration of Project and Report Schedule:

The project will run from January 1, 1978 to December 31, 1978. Progress reports will be provided monthly with a preliminary progress report included in the September 1978 Laboratory Report. A final recommendation will be published shortly after termination of the report.

Cooperators and Participants:

Pennsylvania Department of Environmental Resources

Bureau of Forestry, District No. 10

Pennsylvania Game Commission

Pennsylvania Fish Commission

Clinton County GM Coordinator

Thompson-Hayward Chemical Company

Environmental Protection Agency

USDA-FS

USDA-APHIS-Pilot, Jack Henderson

USDA-APHIS-Methods Development Staff

FIGURE 2

REGION	STUDY	UNIT	LINE	POINT

TREE	SPEC	DBH	LARVAE	PUPAE	EGG

Remarks: _____

FIGURE 2

REGION	STUDY	UNIT	LINE	POINT

TREE	SPEC	DBH	LARVAE	PUPAE	EGG

Remarks: _____

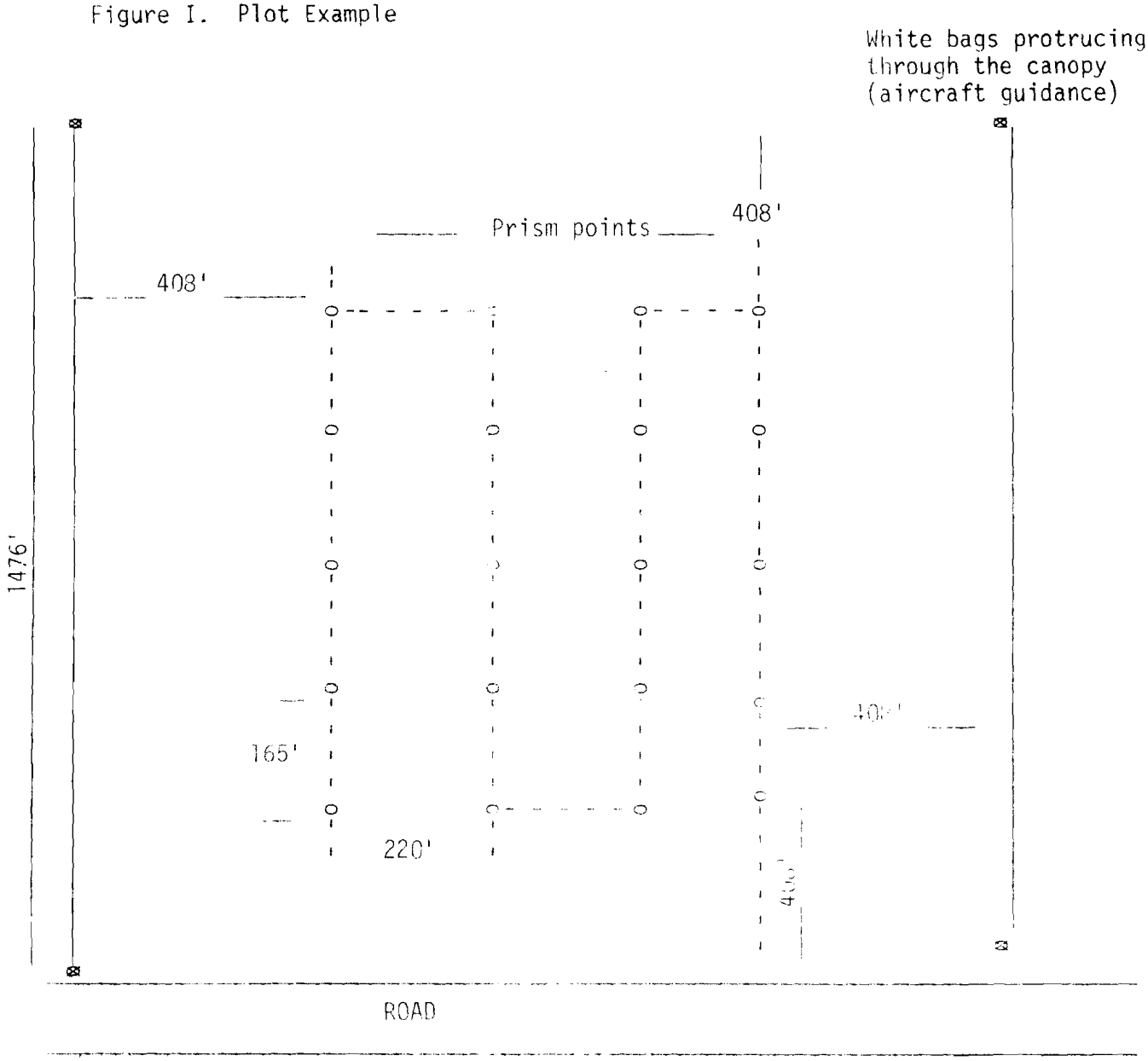
Region	Study	Unit	Line	Point	Mo./Day/Yr.

Card	No. Trees	Crew	EM Visibility					New Egg Masses			
			Excel	Fair	Poor	Prec	Wet	0-1	1-3	3-6	6+

Tree	Spe.	DBH	New Egg Masses				Crwn
			0-1	1-3	3-6	6+	
01							
02							
03							
04							
05							
06							
07							
08							
09							
10							

NOTES:

Figure I. Plot Example



Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides Against the Gypsy Moth
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leaders: Winfred H. McLane, J. A. Finney

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

The following tests were conducted using the standard oak seedling technique.

Table 1. Comparing an oil formulation of Dimilin® to a water formulation using 25 w/p in both mixes.

Dosage	Percent Mortality		Days after treatment
	Orchex oil	Water	
0.0001 lb. AI/gal/AC	75	49	8
0.0004 lb. AI/gal/AC	90	93	8
0.0009 lb. AI/gal/AC	100	100	8
0.0039 lb. AI/gal/AC	100	100	8
0.0156 lb. AI/gal/AC	100	100	8
0.0625 lb. AI/gal/AC	100	100	8
Check	3	3	8

There appeared to be no substantial difference between the two mixes when tested under laboratory conditions using second instar gypsy moth larvae. However, at the lowest dosage the oil formulation did give superior results.

A number of tests were conducted using Knoplex® and Target® NL stickers. The tests were conducted to determine the effectiveness of Target® NL when used with Imidan® and Sumithion .

Table 2. Test results when comparing two stickers in a formulation of Imidan® 1E.

Material	Dosage	Weather	Percent Mortality Phoplex	Percent Mortality Target NL	Days after Treatment
Imidan 1E	1.0 lb. AI/gal/AC	7.6 cm	79 1/	67 1/	5
"	"	7.6 cm	94 2/	75 2/	5
"	"	7.6 cm	93 3/	60 3/	5
Check	"	-	0	0	5

1/ One percent sticker

2/ Two percent sticker

3/ Three percent sticker

When tested with Imidan® 1E, Target® NL gave fair results after 7.6 cm of rain. However, Rhoplex gave much better results.

The two stickers were then tested using a formulation of Imidan® 50 w/p.

Table 3. Test results when comparing two stickers in a formulation of Imidan 50 w/p.

Material	Dosage	Weather	Percent Mortality Rhoplex	Percent Mortality Target NL	Days after Treatment
Imidan 50 W/P	1.0 lb. AI/gal/AC	7.6 cm	86 1/	22 1/	4
"	"	7.6 cm	91 2/	8 2/	4
"	"	7.6 cm	86 3/	13 3/	4
Check	-	-	0	0	4

1/ One percent sticker

2/ Three percent sticker

3/ Five percent sticker

When tested with the Imidan 50 W/P formulation Rhoplex looked far superior to the Target® NL formulation.

The two stickers were then tested with Sumithion® 8E.

Table 4. Test results when comparing two stickers in a formulation of Sumithion® 8E.

Material	Dosage	Weather	Percent Mortality Rhoplex	Percent Mortality Target NL	Days after Treatment
Sumithion 8E	1.0 lb.AI/gal/AC	2.5 cm	91 1/	9 1/	5
"	"	"	99 2/	31 2/	5
"	"	"	99 3/	20 3/	5
"	"	7.6 cm	97 1/	19 1/	5
"	"	"	100 2/	17 2/	5
"	"	"	100 3/	6 3/	5
"	"	"	100 4/	12 4/	5
"	"	"	100 5/	3 5/	5
Check					

1/ One percent sticker

2/ Two percent sticker

3/ Three percent sticker

4/ Five percent sticker

5/ Ten percent sticker

In general Target® NL performed very poorly and would not be recommended for use as a sticking agent.

Additional testing was conducted using Dimilin® 25 W/P with water, oil, oil and emulsifiers.

Table 5 Test results using two experimental formulations of Dimilin®

Material	Dosage	Percent Mortality		Days after Treatment
		Emul oil+H_2O 1/	Savol oil+H_2O 2/	
Dimilin	0.25 lb.AI/gal/AC	100	100	11
"	0.06 lb.AI/gal/AC	100	100	11
Check	-	0	0	11

1/ 49% H_2O - 49% Orchex oil - 2 Triton X-190

2/ 50% Savol - 50% H_2O

A bioassay was made on a DDT sample for V. LaFleur.

Table 6. Results of bioassay on DDT sample.

Material	Dosage	Percent Mortality	Days After Treatment
DDT	2.0 lb.AI/gal/AC	100	5
"	1.0 lb.AI/gal/AC	98	5
"	0.5 lb.AI/gal/AC	100	5
"	0.2 lb.AI/gal/AC	100	5
Check	-	0	5

A series of tests were conducted with Pro Tec® and Plantgard® to determine if they acted as feeding stimulants or deterrents.

Twenty 3rd instar larvae were deposited on the bottom of gallon containers and allowed to select treated or untreated foliage to feed upon. Frass was then collected under each set of foliage disc over a period of 24 hours. Each treatment was repeated 5 times. Weighings were then made of the frass collected.

Material	Replicate	Ratio Protec:H_2O	Volume Applied	Frass Weight Check	Frass Weight Treated
Pro Tec 2	1	2 - 1	Painted On	0.0065g	0.0080g
Pro Tec 2	2	2 - 1	Painted On	0.0148g	0.0177g
Pro Tec 2	3	2 - 1	Painted On	0.0124g	0.0136g
Pro Tec 2	4	2 - 1	Painted On	0.0095g	0.0098g
Pro Tec 2	5	2 - 1	Painted On	0.0094g	0.0056g
			Average	0.0105g	0.0109g
Pro Tec 2	1	4 - 1	Painted On	0.0133g	0.0152g
Pro Tec 2	2	4 - 1	Painted On	0.0145g	0.0098g
Pro Tec 2	3	4 - 1	Painted On	0.0112g	0.0081g
Pro Tec 2	4	4 - 1	Painted On	0.0124g	0.0090g
Pro Tec 2	5	4 - 1	Painted On	0.0144g	0.0099g
			Average	0.0131g	0.0104g
Plantgard	1	1 - 2	2 gal/ac	0.0067g	0.0091g
Plantgard	2			0.0061g	0.0067g
Plantgard	3			0.0155g	0.0089g
Plantgard	4			0.0061g	0.0074g
Plantgard	5			0.0064g	0.0095g
Plantgard			Average	0.0081g	0.0083g

Material	Replica	Ratio to H_2O	Volume Applied	Grass Weight Check	Treated
Plantgard	1	1 - 4	2 gal/ac	0.0046g	0.0052g
Plantgard	2	1 - 4		0.0068g	0.0079g
Plantgard	3	1 - 4		0.0100g	0.0141g
Plantgard	4	1 - 4		0.0033g	0.0050g
Plantgard	5	1 - 4		0.0063g	0.0069g
			Average	0.0062g	0.0078g

A series of tests were conducted testing the viscosity of formulations containing various amounts of Shade.

Formulation	Temperature	Spindle	Viscosity
1 lb. Shade/gal	21° C	2	110 CPS
2 lb. Shade/gal	21° C	2	160 CPS
3 lb. Shade/gal	21° C	2	310 CPS
4 lb. Shade/gal	21° C	2	460 CPS
5 lb. Shade/gal	21° C	2	530 CPS
H_2O only	21° C	2	80 CPS
1 lb. Shade/gal	8° C	2	140 CPS
2 lb. Shade/gal	8° C	2	190 CPS
3 lb. Shade/gal	8° C	2	310 CPS
4 lb. Shade/gal	8° C	2	460 CPS
5 lb. Shade/gal	8° C	2	1000 CPS
H_2O Only	8° C	2	90 CPS

Formulation	Temperature	Spindle	Viscosity
Pro Tec (8-1) 1/	21° C	2	110 CPS
Pro Tec (4-1)	21° C	2	110 CPS
Pro Tec2 (8-1)	21° C	2	110 CPS
Pro Tec2 (4-1)	21° C	2	110 CPS
Pro Tec3 (8-1)	21° C	2	110 CPS
Pro Tec3 (4-1)	21° C	2	110 CPS

1/ Ratio of H_2O to Pro Tec .

Project Number: GM 6.2.3
Project Title: Periodicity of Pheromone Release from Female Gypsy Moths
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leader: R. G. Reeves

A detailed report covering work conducted in this project will be included in the next laboratory report.

Project Number: GM 6.2.8
Project Title: Field Evaluation of Controlled Release Formulations of Disparlure for Gypsy Moth Mating Disruption
Report Period: October 1, 1977 - March 31, 1978
Report Type: Final
Project Leaders: C. P. Schwalbe and E. C. Paszek

Appendix 1 is a manuscript covering this project which has been submitted for publication in the Annals of the Entomological Society of America.

Project Number:	GM 7.2.1
Project Title:	Efficiency of (+) Disparlure-Baited Traps in Arrays for Estimating Male Moth Density and Delimitation of Infestation
Report Period:	October 1, 1977 - March 31, 1978
Report Type:	Interim
Project Leaders:	Charles P. Schwalbe, E. C. Paszek

A detailed report covering work conducted in this project will be included in the next laboratory report.

Project Number: GM 7.2.4
Project Title: Development and Assessment of Techniques for Comparing the Competitiveness of Populations of Male Gypsy Moths
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leader: V. C. Mastro

Introduction:

The objectives of this project were:

(1). to evaluate monitoring tools for comparing the competitiveness of strains of male gypsy moths.

(2). to compare characteristics which may affect the competitiveness (ecolosion, dispersal, longevity, male searching behavior and response to pheromone sources) of laboratory-reared and wild strains of male gypsy moths.

This report contains results of these studies. However, because of the large number of variables involved, analysis of the data is not complete.

Strain Comparisons

A preliminary comparison of the competitiveness of several test strains was presented in the last report. Males from various strains displayed similar ecolosion and dispersal patterns. When comparing the proportions of males recovered, laboratory reared (NJ_{15}) and wild male were nearly the same. Stated differently, laboratory males located pheromone sources as well as feral males. However, some differences between strains were noted. Table 1 presents the average age of the laboratory-reared and wild males when captured. When comparing the proportions of total capture with age, a larger proportion of feral males are captured on day 0 (the day they ecolosed). It appears that NJ_{15} males require either a greater length of time to mature and become sexually active and/or secondary dispersal from intermediate resting sites is delayed. It is unlikely that this difference in activity alone would be critical to a sterile male program because the continuous release of males required, would offset any difference in age and activity. Although laboratory males are able to search out and locate pheromone sources as well as feral males, the periodicity of sexual activity is not the same. Peak capture of NJ_{15} males generally occurs later in the day than peak capture of feral males (Table 2 - 5). From 7/18 to 7/23 (Table 4) peak capture of NJ_{15} males averaged 3 hours later than peak capture of feral males, this was the largest difference noted throughout the test. During other periods activity peaks of wild and laboratory strains were not as widely separated (Table 2, 3 and 5). The reasons for this observed asynchronous behavior are unknown. Environmental variables (temperature, R. H., and solar radiation) are being analyzed for possible relationships to male sexual activity periodicity. Periodicity of capture in traps appears to be mediated by temperature. Shifts of peak trap capture occur later in the day with cooler temperatures and earlier in the day with warmer temperatures. A complete analysis of the data and complementary laboratory studies, however, will have to be completed before the exact relationships can be explored.

Trap Comparison:

To develop a tool for monitoring male sexual activity, six treatments were compared. The standard for comparison was a one day old virgin feral female placed on the bole of a tree. Other treatments included traps baited with laboratory reared (NJ_{15} or P_1) females or feral females. As a fifth treatment, traps were baited 100ug of (+) disparlure. Blank traps were also included to serve as controls. Preliminary analysis of the data indicates there were no differences in the age or the strain of males captured between baited trap treatments. For example, traps baited with NJ_{15} females caught equivilent proportions of wild and laboratory reared (NJ_{15}) males. When comparing the total number of males captured by each trap treatment, (+) disparlure baited traps outcaught all other treatments; approximately 31% of the total moths caught were captured in disparlure-baited traps. Each of the four female-baited traps captured ca. 16% of the total males. Blank traps accounted for ca. 4% of the total capture. It appears that pheromone baited traps will be useful for monitoring male sexual activity. The only significant difference noted between (+) disparlure baited traps and any of the female treatments was a higher capture rate during periods when females normally are not calling i.e. early morning and late evening. Additionally, the asynchrony between strains in periodicity of capture described earlier was also apparent in the results from the (+) disparlure-baited traps and the female-baited traps.

Conclusions:

Preliminary analysis indicates that male moths from the NJ_{15} strain are competitive. They displayed behavioral patterns similar to the feral strains. Also, the laboratory strain searched out the located pheromones sources as well as wild males. The significance of the shift in the periodicity of activity of laboratory males is still undetermined. Periodicity of feral female mating behaviors will have to be characterized to describe its importance. Also, the factors regulating activity remain to be explored.

Table 1. Mean capture age and % of Total Capture by day of capture of Laboratory reared (NJ_{15}) and Feral strains of male gypsy moths.

PLOT	STRAIN	$\bar{x}$ AGE	0 DAY	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY
1	NJ_{F15}	1.212	4.029	73.993	18.681	3.297	0.000	0.000	0.000
1	FL-wld	.954	14.943	78.161	4.598	1.724	0.000	0.575	0.000
2	NJ_{F15}	1.233	3.137	79.943	14.259	1.901	.380	.095	.285
2	AB-wld	1.029	14.000	71.556	12.444	1.556	.444	0.000	0.000
3	NJ_{F15}	1.271	7.762	67.870	17.690	3.610	2.347	.722	0.000
3	Di-wld	1.064	15.525	67.123	13.927	2.740	.228	.457	0.000
4	NJ_{F15}	1.383	15.789	43.609	31.579	6.015	1.504	1.504	0.000
4	PA-wld	1.303	13.147	57.769	20.717	3.187	4.382	0.792	0.000

TABLE 2 PERIODICITY OF CAPTURE OF DIFFERENT STRAINS OF MALE GYPSY MOTHS 7/5 - 7/9

NN=NJ_{F15} LABORATORY REARED

WW= BUSHKILL, PA. WILD

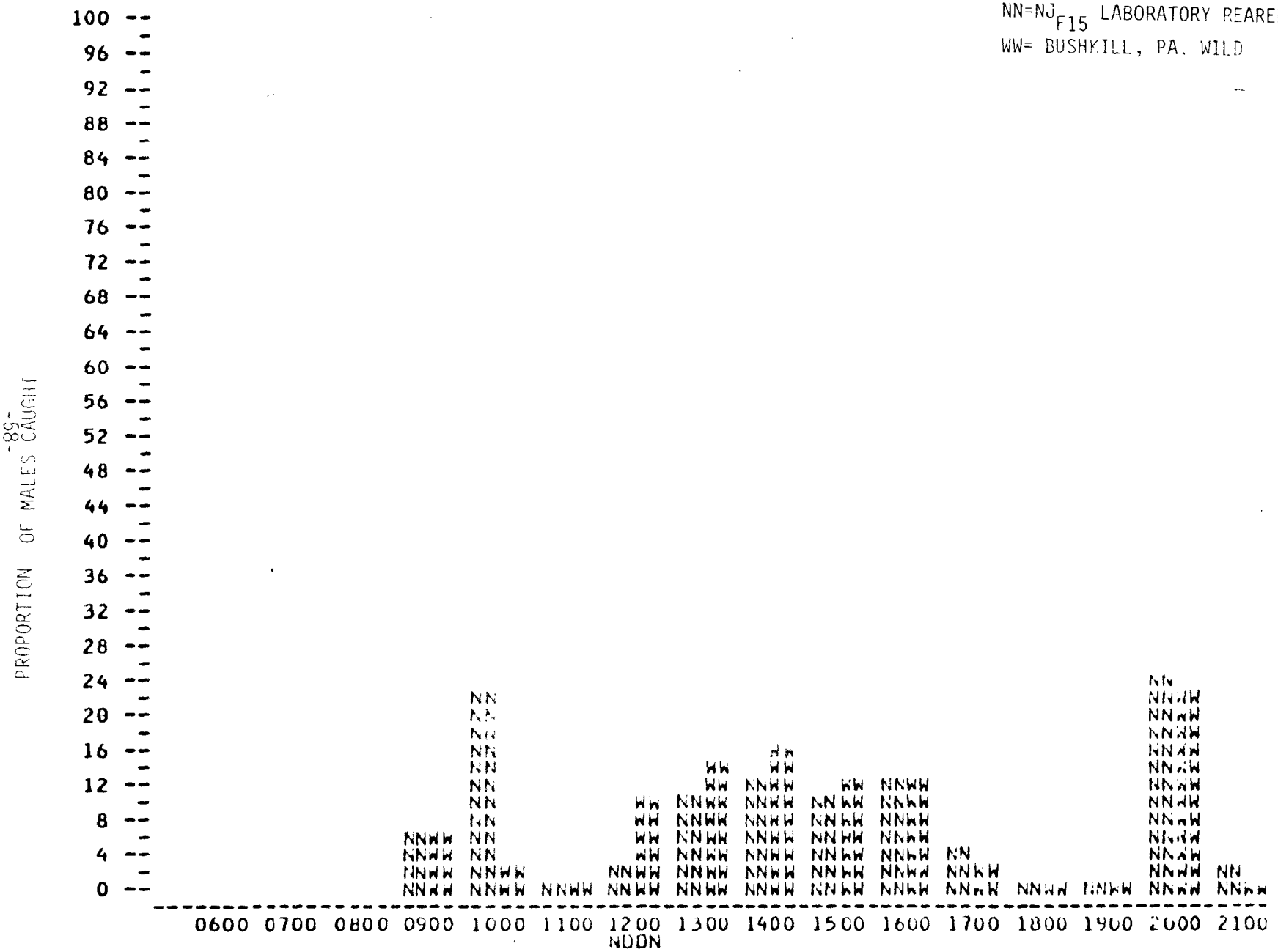


TABLE 3 PERIODICITY OF CAPTURE OF DIFFERENT STRAINS OF MALE GYPSY MOTHS 7/11 - 7/14

NN=NJ_{F15} LABORATORY REARED

WW= DIGHTON, MASS. WILD

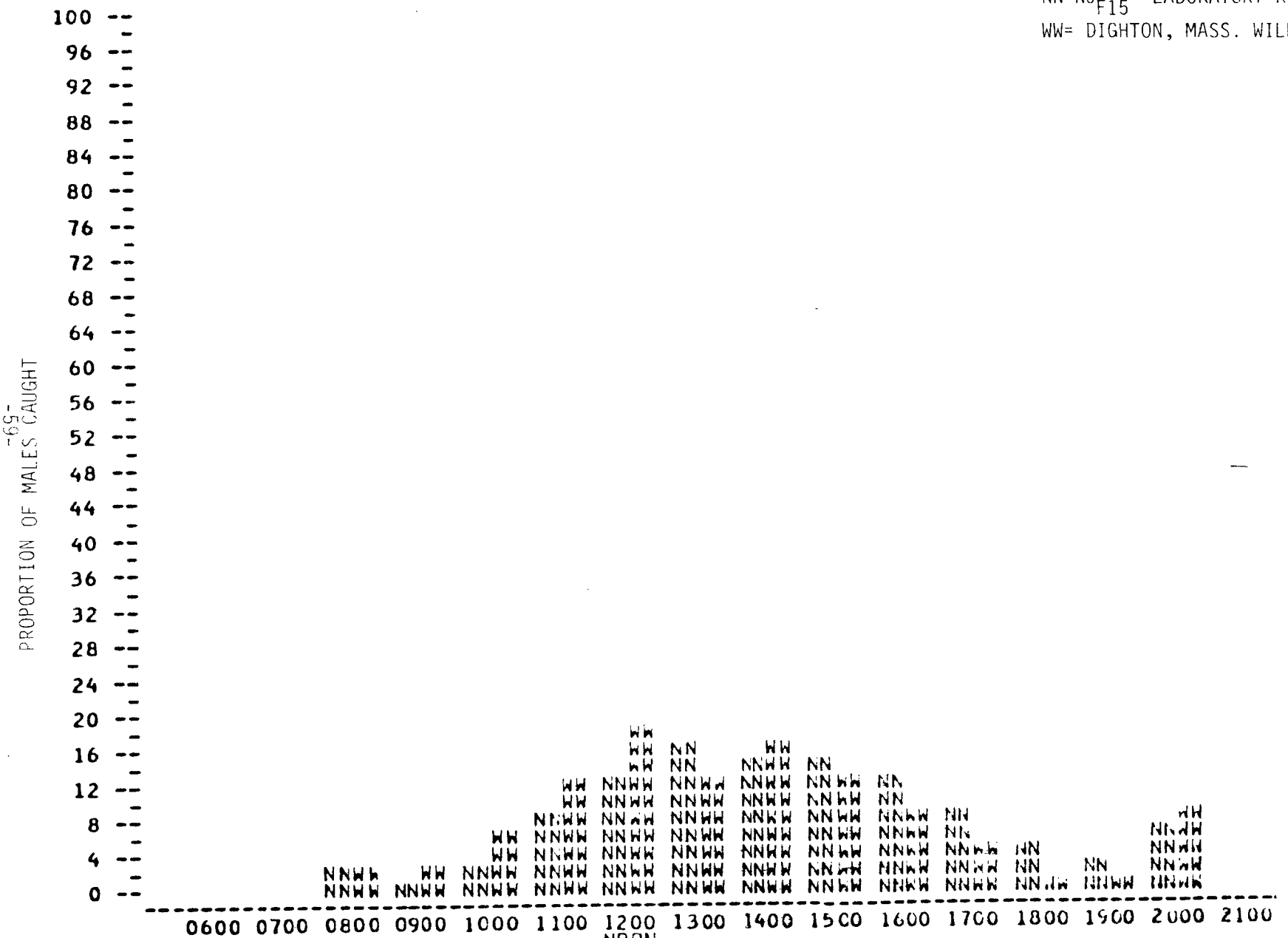


TABLE 3

PERIODICITY OF CAPTURE OF DIFFERENT STRAINS OF MALE GYPSY MOTHS 7/11 - 7/14

NN=NJ_{F15} LABORATORY REARED

WW= DIGHTON, MASS. WILD

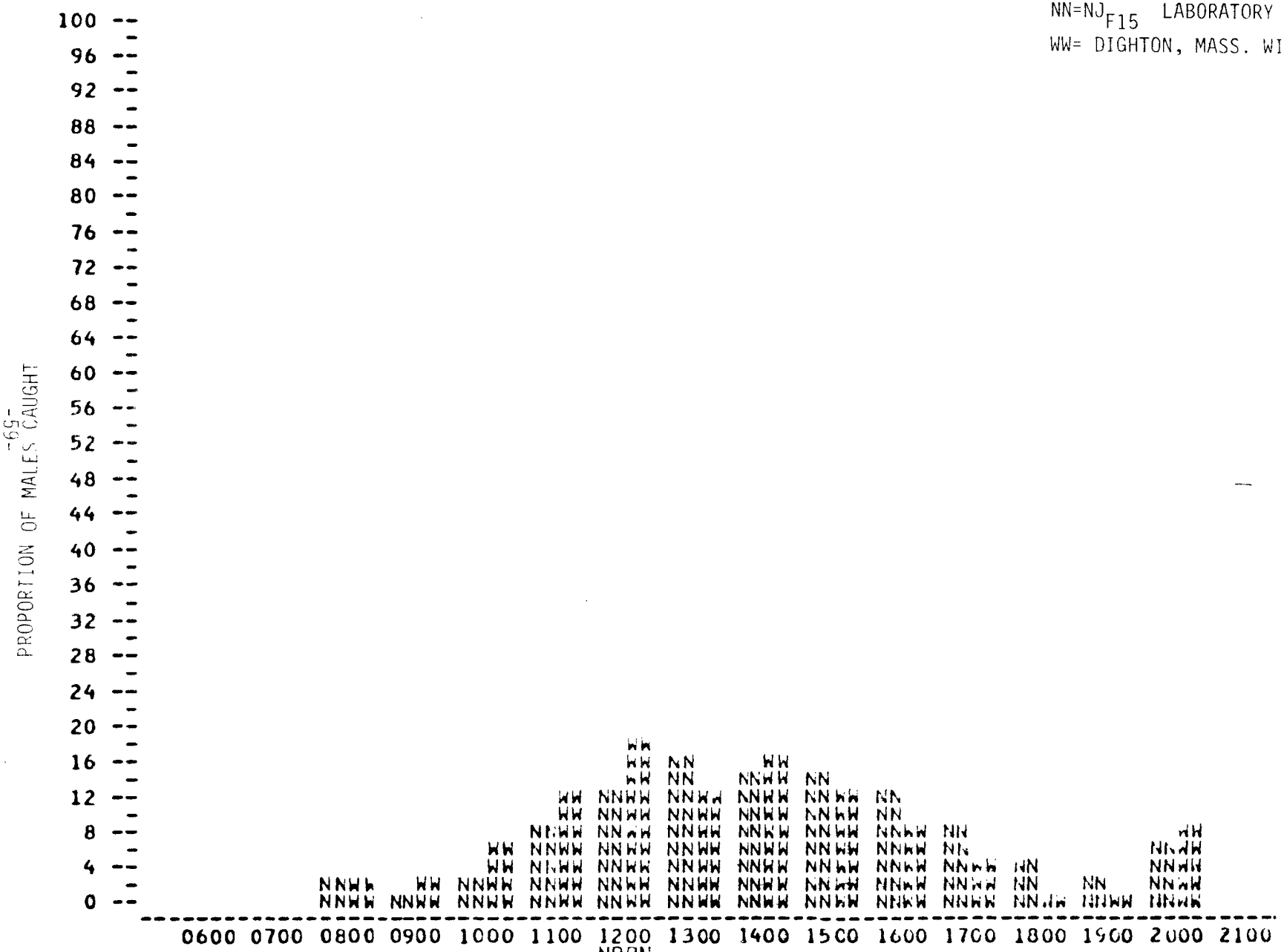


TABLE 2 PERIODICITY OF CAPTURE OF DIFFERENT STRAINS OF MALE GYPSY MOTHS 7/5 - 7/9

NN=NJ_{F15} LABORATORY REARED

WW= BUSHKILL, PA. WILD

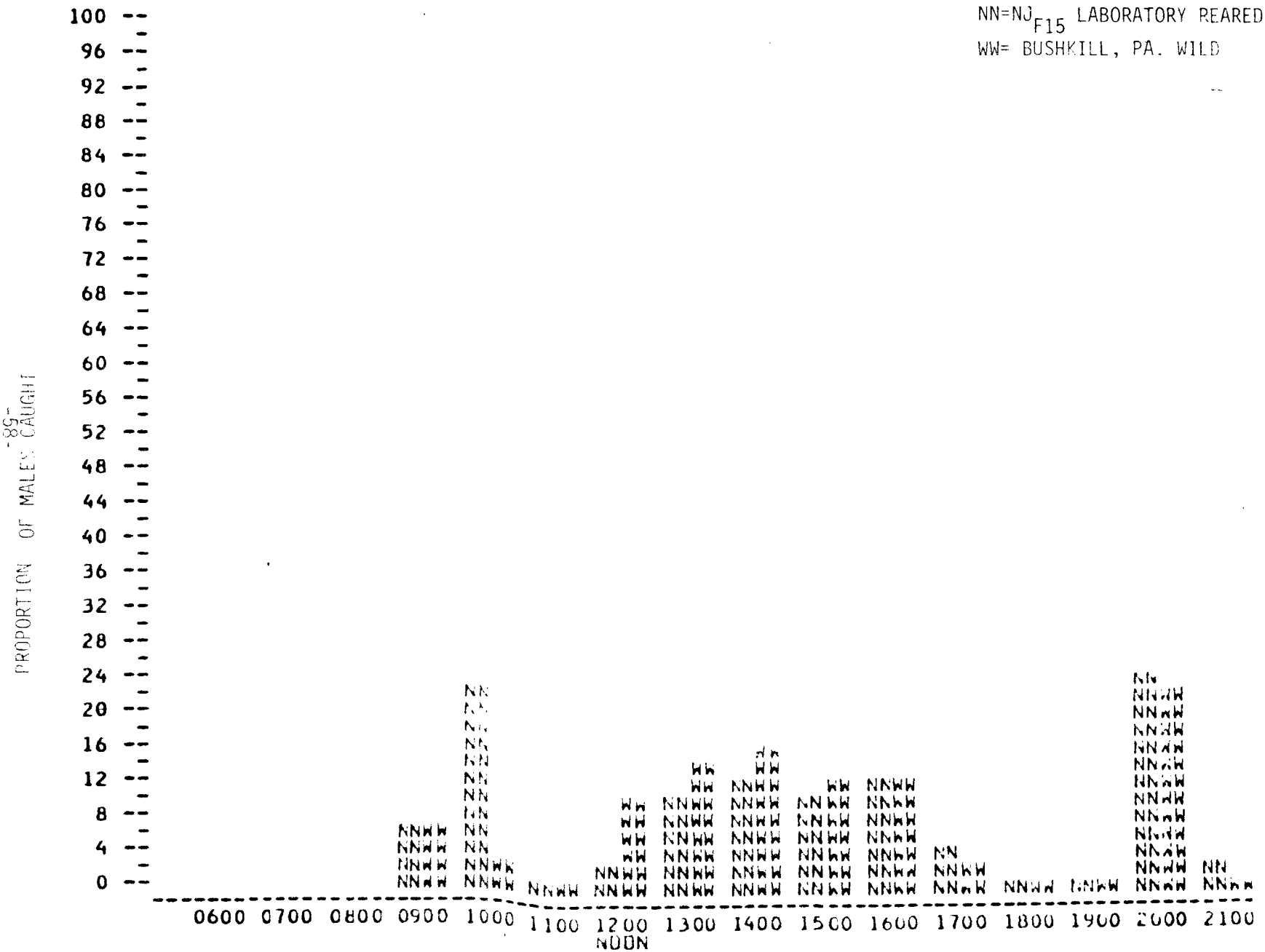
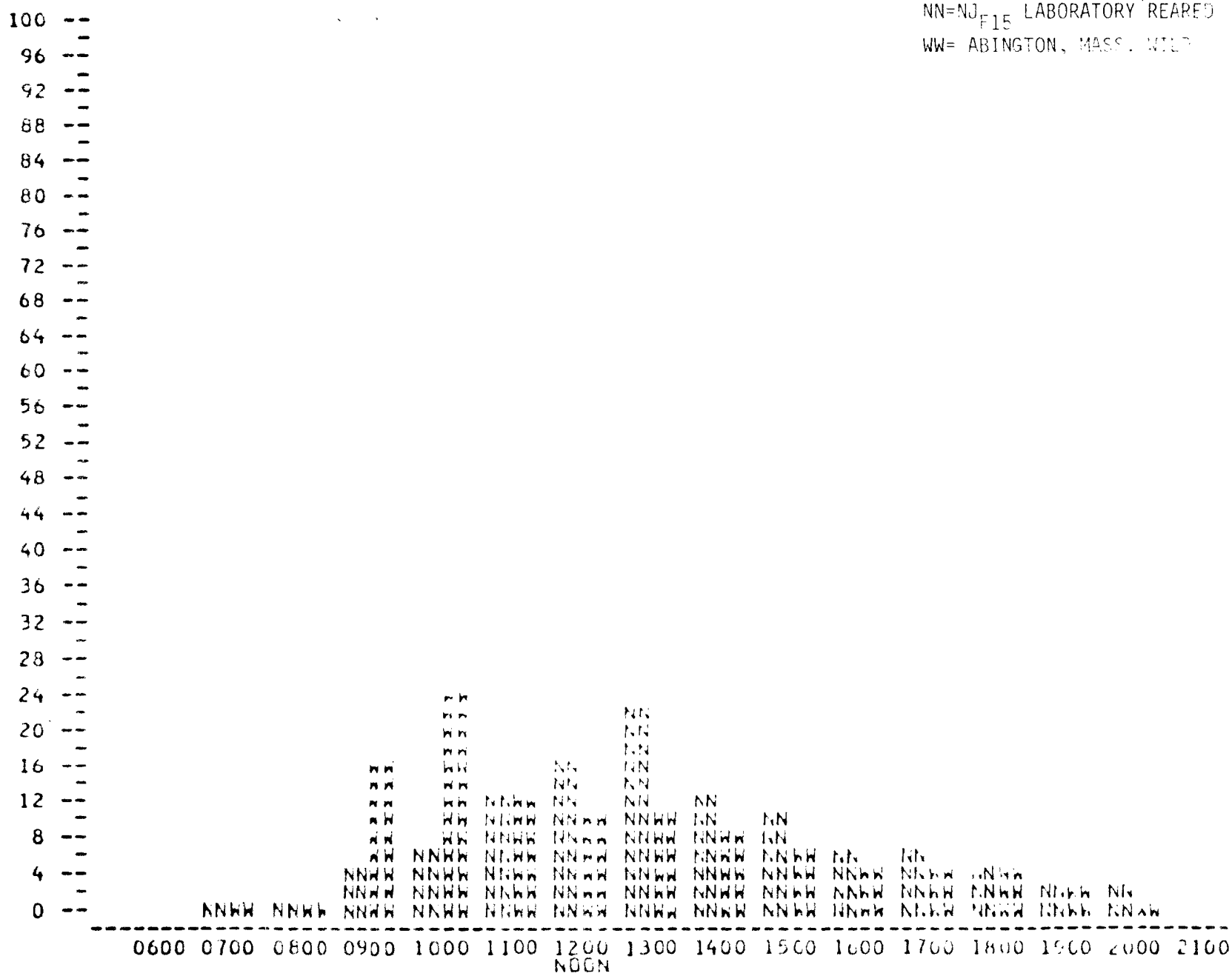


TABLE 4 PERIODICITY OF EMERGENCE

7/18 - 7/23

NN=NJ_{F15} LABORATORY REARED

WW= ABINGTON, MASS. WILD

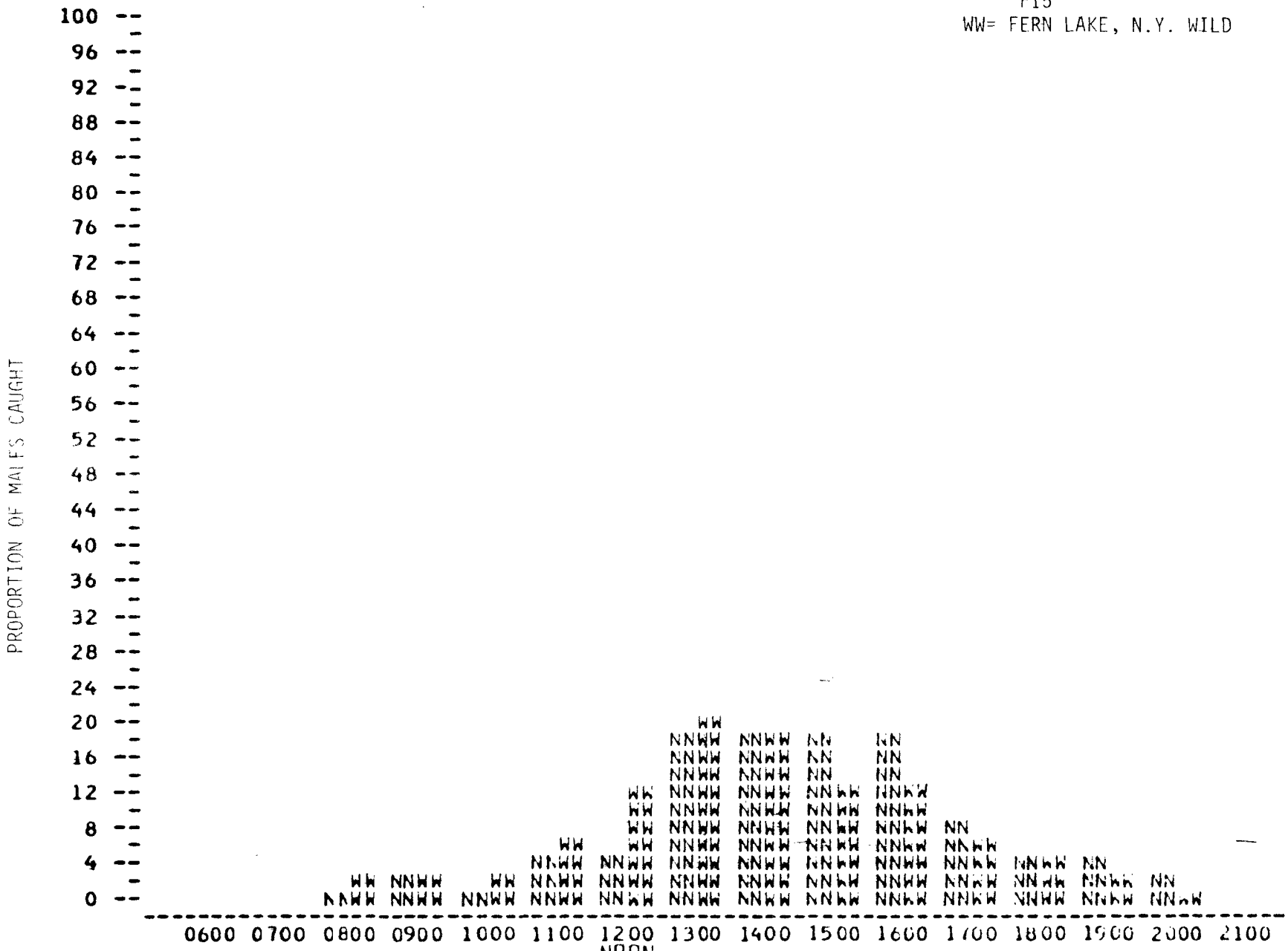


Property of Mass. Div. of Marine Fisheries

TABLE 5 PERIODICITY OF CAPTURE OF DIFFERENT STRAINS OF MALE GYPSY MOTHS 7/25 - 7/29

NN=NJ_{F15} LABORATORY REARED

WW= FERN LAKE, N.Y. WILD



Project Number: GM 7.2.7
Project Title: Evaluation of Commercial Controlled Release Dispensers of (+) Disparlure
Report Period: October 1, 1977 - March 31, 1978
Report Type: Final
Project Leaders: C. P. Schwalbe, V. C. Mastro, E. C. Paszek

Hercon dispensers used in the gypsy moth survey program are normally charged with 26 mg racemic disparlure. Since plans are underway to phase into the program the newly described (+) enantiomer, it was necessary to develop a dispenser design which makes more efficient use of the costly and rare material. Laboratory work leading to the development of candidate dispensers was performed by Dr. B. A. Bierl-Leonhardt, SEA-FR, BARC.

Six different dispenser designs were tested; dispenser size, lure type and lure concentration were varied. The dispensers were aged outdoors in delta traps for 0, 1, 2, 4 or 8 weeks. On March 11-16, 1978 the dispensers were placed into fresh delta traps and arranged in a randomized complete block design in an orange grove near Plantation, FL. Traps were stapled to stakes ca 1 m above ground; grid points were separated by 50 m. Traps baited with 100 µg (+)-disparlure on a cotton wick served as standards.

Laboratory-reared male moths, 1 day old were released in 36-49 locations within the plot. A total of 4,348 insects were liberated in 6 separate releases; 1,552 (35.5%) were recovered.

The attached Table gives specification details of dispensers tested and the average number of moths captured with each formulation. Traps with (+)-disparlure consistently captured more males than those baited with the racemic mixture. In nearly all cases, traps containing the Hercon laminated and mylar dispensers caught as many moths as the 100 µg cotton wick. All of the (+) enantiomer formulations remained effective for up to 8 weeks in the field. From these data, it was concluded that Hercon 5x25mm dispensers containing 1.6 mg (+)-disparlure per dispenser would be most effective in our 1978 survey program. The Mylar formulation was excluded because it was felt that Herculite may have technical problems in scaling up for large production of that design. The smaller (5x12.5 mm) Hercon strips, while requiring only 0.8 mg lure/dispenser, were excluded because their small size would make them very difficult to handle properly in the field.

Approximately 15,000 dispensers (5x25 mm, 1.6 mg lure/dispenser) were distributed to field locations for testing in 1978.

TABLE. Summary of trap data from preseason evaluation of pheromone dispensers. Plantation Fla. March 11-16, 1978

SPECIFICATIONS				WEEKS AGED				
Dispenser type	Size (mm)	mg lure/in^2	mg lure/ dispenser	0	1	2	4	8
Cotton wick	-	-	0.1 (+)	7.6	-	-	-	-
Mylar	25 x 25	1	1.0 (+)	7.9	8.4	6.9	6.5	9.1
Hercon	10 x 25	8.4	3.2 (+)	7.6	-	11.8	10.0	9.7
Hercon	5 x 25	8.4	1.6 (+)	9.9	6.0	8.5	14.2	8.0
Hercon	5 x 12.5	8.4	0.8 (+)	5.8	7.1	7.4	9.5	11.1
Hercon	25 x 25	26.0	26.0 (±)	1.7	1.5	1.5	1.3	0.7
Blanks	-	-	-	0	-	-	-	-

Note: 4,348 moths were released; 1,552 (35.5%) were recovered.

Project Number: GM 8.2.1
Project Title: Release Characteristics of Polyvinyl Chloride Disparlure Wicks
Report Period: October 1, 1977 - March 31, 1978
Report Type: Final
Project Leaders: R. Reeves, V. C. Mastro, J. Yarko

Introduction: Pheromone bait dispensers with controlled release characteristics are necessary for trapping programs. These dispensers should release a prescribed optimum amount of lure over a several week period to be efficient in attracting insects. Bait dispensers which could be prepared easily in the laboratory would be useful for testing purposes including: maximizing trap efficiency by alternating release rate; comparing trap-bait combinations; maximizing the use of bait placed in a dispenser; and designing dispensers for specific purposes (i.e. to approximate the release rate of female moths).

Presently in the gypsy moth program, cotton and Hercon wicks (a commercially prepared wick) are used for testing purposes. Cotton wicks are easily prepared but do not have uniform or long lasting release characteristics. Hercon wicks offer the advantage of desirable release characteristics but the disadvantage of little flexibility of adjusting bait strength or release rate in the laboratory.

It was the purpose of this study to investigate the release characteristics of laboratory prepared polyvinyl chloride bait dispenser. This method for dispensing lure has recently described and used successfully (Daterman 1974, Fitzgerald et al. 1973).

Methods and Materials: Wicks were prepared in the manner described by Daterman (1974). All wicks contained 5% disparlure by weight. The plastic formulation and lure were mixed (roto evaporated) for 3 hours before forming in molds and fusing. Molds consisted of 2 glass plates with 4-2mm spacer between. The finished wick were large flat sheets of disparlure impregnated polyvinyl chloride. From these sheets 3 different size wicks were cut (4 cm x 1.5 cm, 6 cm x 1.5 cm, 8 cm x 1.5 cm).

To determine the release characteristics of wicks, the system and procedures described by Schwalbe (Semiannual Report Oct. 1976 - March 1977) for pheromone emission monitoring were used. Wicks were placed in collection tubes and air passed over them for 24 hour periods at wick ages of 1, 2, 3, 10, 17, 24, 31 and 38 days (flow rate 50 ml/min, porapack Q used for collection substrate).

Results & Discussion: Emission data are presented on Fig. 1, 2 and 3. Although wicks had the surface area, initial weights differed because of variations in thickness. Emission rates for wicks of the same surface area were not combined because of differences in initial weight and, therefore, differences in lure content. Emission rates increased with surface area and initial weights (lure content). Rate of emission was nearly constant over time (table 1). The rate generally increased after 31 days. Wicks were held under room conditions which varied and this may account for some of the variation in release rate. This method provides a simple, quick laboratory technique for preparing wicks for test pruposes. The emission characteristics of a particular size (surface area) wick may be more standard if casting procedures were followed which produced wicks of the same weight (lure content).

Table 1. Mean emission rates over 38 days of Polyvinyl chloride disparlure wicks

Dimension of wick	Initial Total wick Wt.	$\bar{x}$ Release rate ug/24 hr	Standard Deviation
4 cm x 1.5 cm	1.1238 g	5.93	1.88
	1.2597 g	14.55	4.07
ca 2 mm	1.6925 g	17.19	1.39
	1.9869	21.08	3.60
6 cm x 1.5 cm	2.4075 g	22.95	5.15
ca 2 mm	3.4123	23.80	4.93
	2.5456	45.81	12.07
8 cm x 1.5 cm	2.9751	44.44	12.[illegible]
ca 2 mm	3.5334	45.04	9.16

Table 1. Emission Rates of Polyvinyl Chloride, Plastic Wicks (5% disparlure by wt.), Surface Area (4 cm x 1.5 cm x 2 mm)

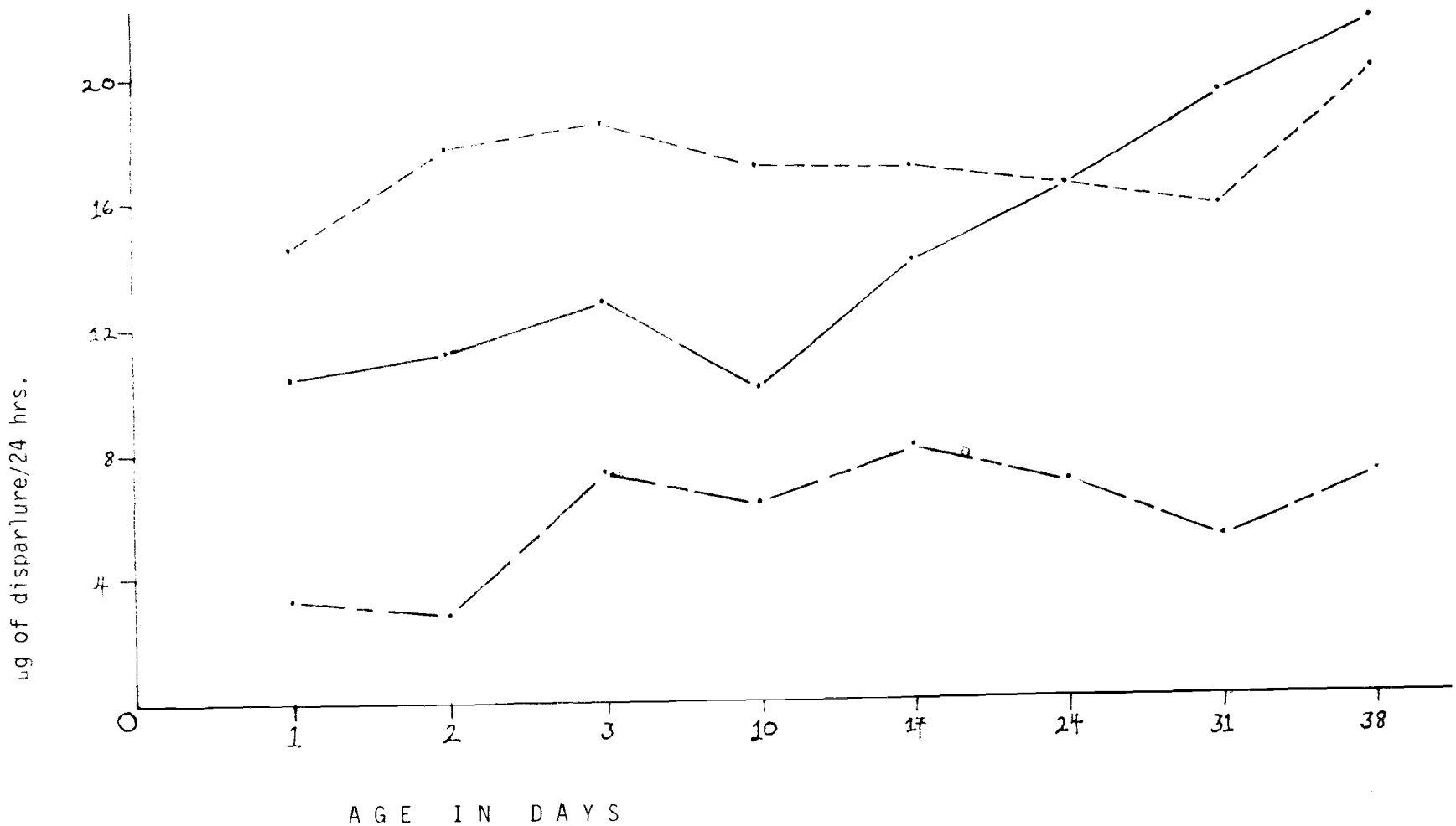


Table 2. Emission Rates of Polyvinyl Chloride, Plastic Wicks (5% disparlure by wt.), Surface Area (6 cm x 1.5 cm x 2 mm)

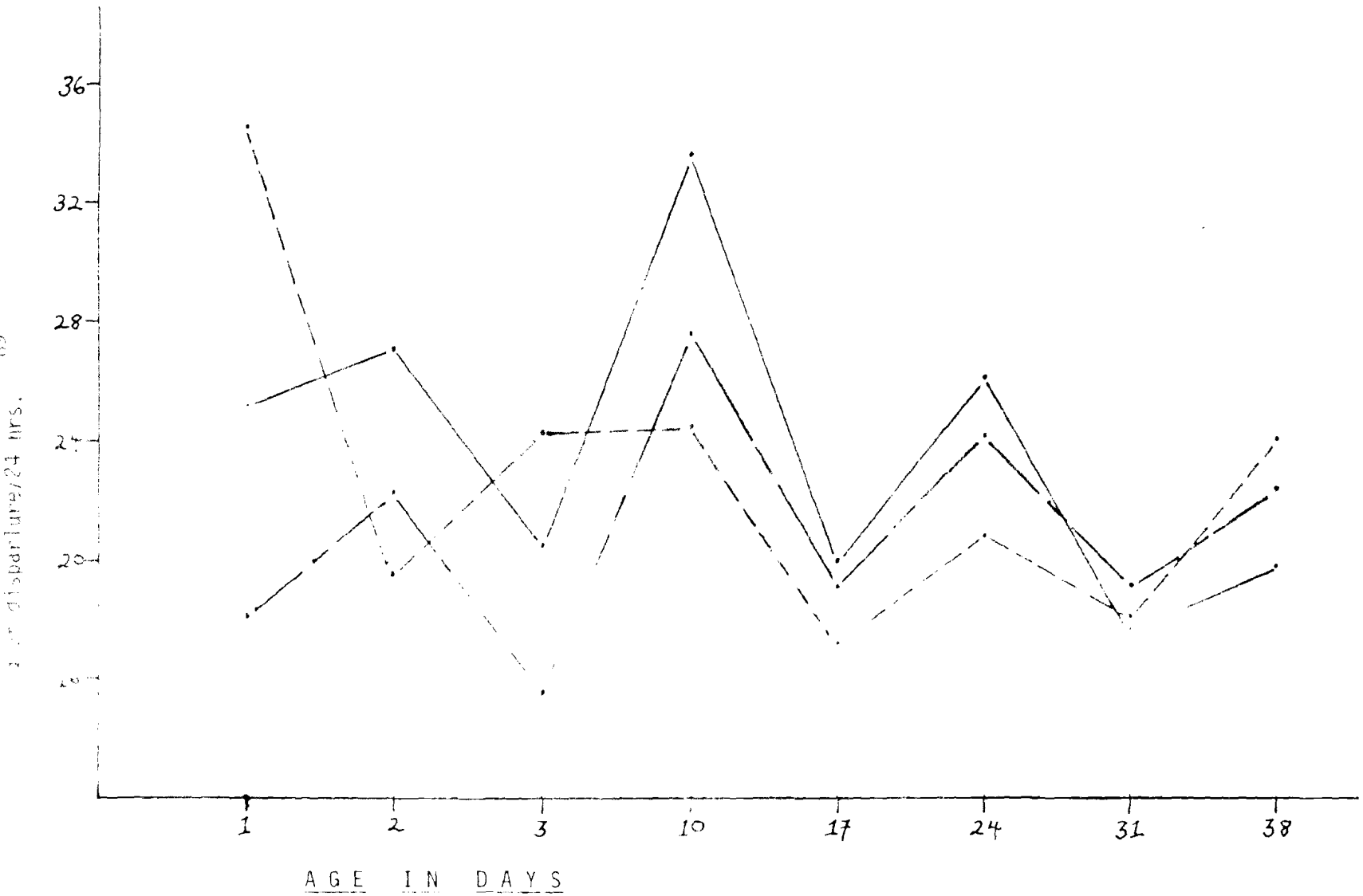
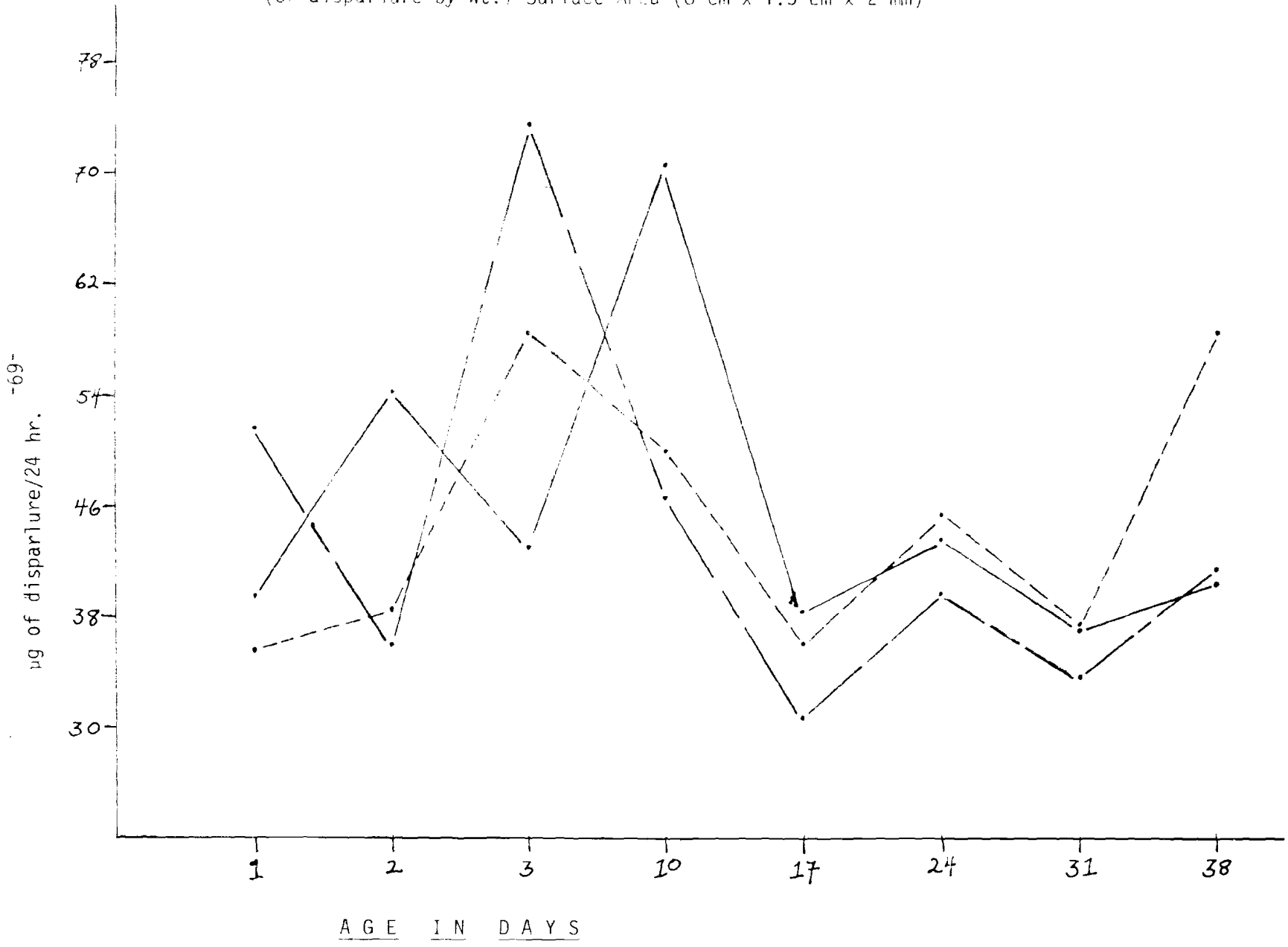


Table 3. Emission Rates of Polyvinyl Chloride Plastic Wicks
(% disparlure by wt.) Surface Area (8 cm x 1.5 cm x 2 mm)



Project Number: GM 8.2.2
Project Title: Radiological Sterilization of Male Gypsy Moths
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leader: C. P. Schwalbe

A series of tests is being conducted to determine the appropriate insect age: radiation dosage most suitable for sterilizing male pupae. All insects (NJF_{16}) were reared on artificial diet (B-4 or Modified Hornworm); hardened (dark) pupae were collected every other day. At the time of collection they were designated 0-1 day-old. Irradiation was performed in a Gamma cell 220 (Atomic Energy of Canada). After adult eclosion, male moths were mated with normal females (single-pair matings). After oviposition was complete, ca. 10 female moths were dissected to determine the presence of apyrene and eupyrene sperm in the bursa copulatrix and spermatheca. Resultant egg masses were embryonated and chilled via standard rearing procedures. After chilling for 90-180 days egg masses were dehaired and the number of eggs estimated volumetrically. A sample of 100 eggs was then taken from each egg mass and % embryonation and % hatch determined. Larvae that hatched were placed on standard diet and reared to the adult stage. These F_1 progeny (from irradiated fathers) were mated: Treated ♂ x Treated ♀; Treated ♂ x Normal ♀; Normal ♂ x Treated ♀ . Egg masses from these matings will be checked for hatch to determine the degree of inherited F_1 sterility.

At this time, most of the P_1 hatch tests have been completed and larvae are being reared. Therefore, this report will not include information on F_1 sterility tests.

Eclosion data from irradiated pupae are given in Table 1. Irradiation did not appear to affect the percent eclosion or length of pupal stage, except for those exposed to 20 krad at 4-5 days of age. This treatment also yielded large numbers of deformed adults with "crumpled" wings. Morphological abnormalities were not noted in other treatments.

Table 1. Adult Male Moth Eclosion Record

Treatment (dosage/age of pupae)	Irradiated			Control		
	n	% Emergence	$\bar{x}$ time to emergence (days)	n	% Emergence	$\bar{x}$ time to emergence (days)
2 krad/4-5 day	150	100	11.7	50	94	11.8
2 krad/6-7 day	49	96	12.2	-	-	-
2 krad/8-9 day	119	100	12.3	55	100	12.0
2 krad/10-11 day	136	97	11.4	71	92	11.6
8 krad/4-5 day	150	97	11.2	50	94	11.8
8 krad/6-7 day	146	99	13.6	60	98	13.6
8 krad/8-9 day	149	99	12.0	95	100	12.0
8 krad/10-11	130	93	12.0	40	98	12.2
10 krad/4-5 day	140	95	11.6	55	98	11.5
10 krad/6-7 day	148	[illegible]	12.6	60	98	13.6
10 krad/8-9 day	63	98	12.0	-	-	-
10 krad/10-11 "	120	[illegible]	12.2	40	98	12.2
15 krad/4-5 day	100	[illegible]	12.6	66	95	13.0
15 krad/6-7 day	149	97	11.9	56	100	10.8
15 krad/8-9 day	150	[illegible]	12.0	63	95	12.0
15 krad/10-11 "	150	79	12.2	45	100	12.4
20 krad/4-5 day	[illegible]	[illegible]	13.0	66	95	13.0
20 krad/6-7 day	[illegible]	[illegible]	11.9	56	100	10.8
20 krad/8-9 day	135	[illegible]	12.1	55	100	12.0
20 krad/10-11 "	107	[illegible]	11.6	45	100	12.4

Approximately 10 females from each treatment were dissected for determination of insemination. Results are in Table 2.

Table 2. Ability of Male Moths, Irradiated as Pupae, to Transfer Sperm to Normal Females. The Values Indicate What Percentage of Females Contained Both Apyrene and Eupyrene Sperm in both the Bursa Copulatrix and Spermatheca.

		Irradiation Dosage (krads)					
		2	8	10	15	20	0
Pupal age at Irradiation	4-5	9/10	9/10	9/9 a/	2/15	0/15	10/10
	6-7	10/10	-	-	8/10	9/10	10/10
	8-9	10/10	-	10/10 b/	-	8/10	-
	10-11	25/25	25/25	25/25	12/12	23/24	10/10

a/ One female did not contain sperm in the spermatheca.

b/ Sperm was not observed in one bursa.

Ability of male moths to transfer sperm is only impaired when they are irradiated with 15 or 20 krads at 4-5 days of age. Egg masses from those matings were very small and unembryonated. In all cases where sperm transfer occurred, both apyrene and eupyrene cells were observed.

After embryonation and chilling, egg masses were tested for embryonation and hatch. Data were separated into classes of greater than 100 eggs/mass (presumably mating occurred) and less than 100 eggs/mass from pairs where mating did not occur. Results of these tests are in Table 3.

Table 3. Size, embryonation and Hatch of Egg Masses Produced from Females Mated with Irradiated Males.

Age Class		Treated - 2 krads n	# eggs	% Embryonation	% Hatch	Control n	# eggs	% Embryonation	% Hatch
4-5	>100	56	846 (207)	95 (17)	47 (19)	17	869 (201)	99 (3)	58 (27)
	<100	0	-	-	-	1	46	0	0
6-7	>100	33	660 (191)	94 (11)	21 (16)	INCOMPLETE			
	<100	1	66	88	0				
8-9	>100	46	812 (174)	97 (7)	52 (17)	INCOMPLETE			
	<100	1	46	0	0				
10-11	>100	71	836 (239)	99 (5)	40 (23)	29	763 (180)	99 (1)	61 (21)
	<100	0	-	-	-	1	13	23	0
		Treated - 8 krads				Control			
4-5	>100	47	765 (227)	92 (20)	25 (18)	INCOMPLETE			
	<100	1	2	0	0				
6-7	>100	72	841 (222)	96 (13)	30 (15)	48	907 (223)	99 (1)	74 (25)
	<100	1	92	17	0	1	24	0	0
8-9	>100	89	803 (193)	97 (9)	21 (13)	69	710 (219)	93 (21)	58 (28)
	<100	1	99	0	0	2	57 (40)	44 (32)	21 (29)
10-11	>100	71	670 (171)	96 (7)	17 (11)	22	674 (230)	99 (3)	53 (28)
	<100	1	27	15	0	2	65 (29)	30 (42)	4 (5)

Age Class		Treated - 10 krads n	# eggs	% Embryonation	% Hatch	Control n	# eggs	% Embryonation	% Hatch
4-5	>100	39	731 (238)	91 (24)	28 (15)	18	773 (192)	99 (2)	83 (12)
	<100	4	14 (17)	20 (39)	0	1	3	0	0
6-7	>100	80	852 (221)	96 (16)	26 (17)	INCOMPLETE			
	<100	0	-	-	-				
8-9	>100	44	705 (183)	95 (14)	5 (6)				
	<100	0	-	-	-				
10-11	>100	65	700 (162)	97 (4)	18 (12)				
	<100	0	-	-	-				
		Treated 15 krads				Control			
4-5	>100	23	320 (188)	9 (26)	.17	INCOMPLETE			
	<100	21	36 (29)	5 (18)	0				
6-7	>100	57	831 (217)	92 (21)	8 (6)	24	861 (259)	95 (16)	61 (25)
	<100	2	44 (47)	0	0	1	55	89	4
8-9	>100	86	750 (174)	94 (13)	6 (5)	51	820 (215)	95 (19)	53 (29)
	<100	1	18	0	0	1	47	0	0
10-11	>100	50	903 (293)	90 (20)	4 (4)	13	802 (102)	98 (1)	81 (10)
	<100	1	13	0	0	0	-	-	-

Age Class		Treated - 20 krads n	# eggs	% Embryonation	% Hatch	Control n	# eggs	% Embryonation	% Hatch
4-5	>100	21	488 (227)	0	0	35	808 (222)	87 (30)	55 (24)
	<100	5	29 (30)	0	0	0	-	-	-
6-7	>100	65	859 (269)	87 (18)	1 (1)	INCOMPLETE			
	<100	3	37 (35)	0	0				
8-9	>100	50	751 (189)	92 (16)	1	23	847 (254)	94 (21)	58 (24)
	<100	1	10	0	0	0	-	-	-
10-11	>100	23	649 (197)	93 (8)	.22 (.42)	INCOMPLETE			
	<100	2	36 (1)	3	0				

The data above are somewhat incomplete, since all rough data are not fully collated. However, it is clear that at least 90% sterility can be induced by exposing pupae to 15 krads. Therefore, it is intended that insects evaluated in the field in 1978 will be treated with 15 krads at 6-7 or 8-9 days of age.

Larvae hatching from these egg masses are presently being reared. Resultant adults will be mated to determine incidence of F_1 sterility. This work will be more fully detailed in the next laboratory report.

Project Number: GM 8.2.3
Project Title: Gypsy Moth Mating Behavior
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leader: C. P. Schwalbe

In order to standardize certain mating porcedures used in connection with SMT investigations, a number of tests were conducted in which the effects of insect age, and duration of copulation on sperm transfer and egg fertilization were evaluated. These tests are briefly described below.

Adult insects of known age were individually paired at 10:00 a.m. and allowed to mate. The pairs were held in tubular plastic screen cages (8 cm dia. x 10 cm h); if mating did not take place by 4:00 p.m., the pair was excluded from data tabulation. Thus, all results are based upon insects which mated during that 6 hrs time interval.

Table 1. Effect of Insect Age on Precopulatory and Copulatory Time, Egg Mass Size and Embryonation.

♂ Age	♀ Age	n	% Mated	Precopulatory time (min)	Copulatory time (min)	Eggs/ female	% Embryonation
1	1	22	95	114 (96)	66 (23)	724 (227)	88 (28)
1	2	14	93	38 (66)	67 (35)	784 (148)	98 (2)
1	3	15	87	11 (11)	78 (20)	753 (221)	78 (22)
1	4	-					
1	5	26	38	41 (37)	75 (7)	694 (154)	69 (39)
2	1	8	100	5 (4)	48 (10)	742 (247)	96 (9)
2	2	15	100	28 (46)	58 (22)	771 (130)	97 (3)
2	3	15	93	13 (14)	71 (25)	657 (156)	81 (26)
3	2	12	92	33 (45)	87 (21)	862 (172)	97 (3)
3	3	15	100	30 (31)	72 (20)	775 (229)	79 (32)
3	4	22	86	39 (38)	68 (19)	750 (120)	83 (24)
3	5	12	58	89 (98)	59 (17)	537 (265)	74 (23)

After females oviposited, they were dissected. Generally, sperm cells were noted in those females that were observed mating. In one case (3 day ♂ x 5 day ♀) an inseminated female did not lay eggs.

In another experiment, 24-48 hr old moths were allowed to mate. After designated periods of time, matings were interrupted and females were dissected. Presence of apyrene and eupyrene sperm in the vulva, bursa copulatrix and spermatheca was noted.

Duration of Mating	n	Vulva Apyrene	Vulva Eupyrene	Bursa Apyrene	Bursa Eupyrene	Spermatheca Apyrene	Spermatheca Eupyrene
5 min.	10	0	0	0	0	0	0
10 min.	10	100	100	20	0	0	0
15 min.	10	100	90	70	40	0	0
30 min.	10	100	90	60	40	20	20
60 min.	10	100	80	70	60	70	60
120 min.	10	100	100	100	100	100	100
72 hr.	10	-	-	100	100	100	90

Transport of sperm to the bursa copulatrix begins within 10 minutes of copulation and is complete after 2 hours. Most copulations last for 60-80 minutes and it remains to be determined if the male is actively involved in translocation of sperm to the spermatheca. Insemination is complete after only 10 minutes of copulation. In one preliminary test, 4 females were mated for 10 minutes, after which the males were removed. Dissection 60 minutes later revealed sperm in the vulva, but none in the bursa or spermatheca.

Project Number: GM 6.3.5
Project Title: Development of Diet and Efficient Rearing Techniques
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leaders: Robert A. Bell, M. Shapiro, O. T. Forrester

A detailed report covering work conducted in this project will be included in the next laboratory report.

Project Number: GM 6.3.6
Project Title: Establishing Standards
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leader: John A. Tanner

Introduction:

The purpose of this project is to develop insect quality monitoring techniques and to routinely measure the quality of insects produced in the rearing facility. Data will be collected for each strain/generation and will be used to establish standards, develop life tables, and detect early changes from normal development so that corrective action can be taken.

Methods and Materials:

Table 1 is a compilation of data from previous attempts to measure the quality of gypsy moth strains reared at the Otis Center and is presented here as a reference. In future reports this table will be expanded and indepth description of the monitoring techniques will be presented.

Table 1. Performance of laboratory strains and a wild strain of gypsy moth under mass production.

	STRAINS[1,2]					
	NJF_{15}	NJF_{16}	$LUDF_{15}$	PAF_{7}	PAF_{1}[3]	PA(wild)
Eggs laid/female	775(125)	755(88)	732(90)	738(95)	574(74)	784(189)
% Hatch	56	33	37	29	15	13
Hatch Duration(Days)	4.2	4.1	4.1	4.0	4.6	3.4
Pupal weights						
Males (gms)	.61(.05)	.60(.02)	.59(.02)	.60(.02)	.51(.02)	.57(.06)
Females (gms)	2.10(.2)	1.98(.1)	1.98(.2)	1.99(.1)	1.77(.3)	1.77(.4)
Developmental Time (Days) (To adults)						
Male	45(3)	44(2)	44(1)	45(3)	48(2)	48(2)
Female	46(3)	46(3)	45(2)	46(3)	49(2)	49(2)
% Adult Deformative						
Male	6	3	6	4	6	11
Female	42	21	17	19	20	41
Sex Ratio Male:Female						
Total Adults	1.1	1.0	1.3	1.2	1.1	1.6
Normal Adults	1.9	1.2	1.6	1.4	1.2	2.5
% Mating Pairs	95	95	93	94	96	93
Male Longevity(Days)	6.1(.6)	6.3(.8)	6.2(.7)	6.1(.6)	5.7(.9)	5.9(.6)
% Adult Recovery						
Males	98	95	98	96	92	98
Females	94	93	90	97	96	92

1. - NJ = New Jersey
   Lud = Ludlow, Massachusetts
   PA = Pennsylvania
2. - $(\ )_1 = s\bar{d}$
3. - PAF^1 is one laboratory reared generation of the PA(wild)

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 Gypsy Moth
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leaders: Charles D. Owens, R. A. Bell, J. Tardif

Clean Air Filters

Last report showed that the colony rearing trailer had 5 times (533 to 91) more particles than the prototype trailer. An inspection of the HEPA filter on the colony trailer showed that they were not held tightly in place. The unit was slightly modified to improve the clean air filter. Some reduction in count was noticed but as this unit will be removed from the system shortly, it was decided not to rebuild the filter holding unit.

A Coulter Particle Counter has been rented for monitoring the various work areas in mass rearing. The units will also be used to determine the real efficiency of the various grades of filters against scales and hairs of the gypsy moth. The filter to be tested has efficiency ratings of 60, 80, 95 and 99.97%.

Clean Air System for Handling Larvae and Pupae

A table was built in the infest room (see 6.3.9) for 10 workers. Air is drawn away from the workers through 2 inch slots at the back of the counter by a fan mounted within the plevum under the table. The air is forced out through 60% HEPA filter back into the room. Four stations have a hose from central vacuum units for drawing away the skins and silk that are in containers when harvesting pupae. The work area is so made that containers on trays can be moved down the center length of the table so workers on either side can work from one tray. A 30' hose can be connected to one of the central vacuum outlets for cleaning the whole room.

Air Handling in New Adult Room

Presently the temperature control in the adult room is part of the collector system. In the new adult room the temperature control system is separate from the collector system. The collector will be placed out doors so the emptying of the containers will not contaminate the building. The air will be returned to the room through a HEPA filter. The temperature control system will discharge air all through ceiling grills into the room and return through grills near the back of the counter. This makes all air movement to pass by the workers before passing over the moths and eggs. All area including halls will be under positive pressure and clean air, so entering or leaving the area will not draw dirty air into the area.

Project Number: GM 6.3.9
Project Title: Evaluation and Development of Automated Procedures for Rearing Gypsy Moths
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leaders: Charles D. Owens, J. Tardif, R. A. Bell

A new kitchen, containers storage, diet storage, weighing and infesting rooms were built. The kitchen is 19 x 36'. The floor has an epoxy cove all around the room and an epoxy threshold at the doors to prevent water from running under the walls or out the doors. Epoxy floor paint was used on the floor. Therefore, very hot water or steam can be used to wash the floor. A floor drain was located under the steam kettle. An industrial thermostatic steam and hot water mixing valve with 25' of high temperature hose was installed to do the cleaning of equipment and floors.

The boiler that was used in the original kitchen was installed to furnish higher temperature steam (147°C). The time to bring the water to boil has been reduced considerably. A new mixing unit (Breddo Food Liquefier) has been placed on order and when it is received the mixing operation will be automated. It is planned to automatically fill the kettle with water, heat to boiling, time mixing of agar, possible cool down to 80°C, and a fixed time to mix in diet. This will help to eliminate some of the variables in the diet quality due to temperature or the length of time of mixing. The only other automation planned at this time is to convey the containers past the filler automatically.

The storage room for containers is 12 x 13' with a door to kitchen and one to the general storage area. This area is under clean air and holds a supply of containers for several days. Therefore, the kitchen workers do not go out of the clean area while making and dispensing of diets. The diet storage area is 12 x 26' with a weighing area at one end. It has double door access from the general storage area for bringing in supplies. Also there is a door to the kitchen. The room is held at 10°C to prevent loss of quality in the ingredients and a deep freezer is in the room to hold vitamin mix and the weighed out batches of ingredients. An exhaust hood is located over the weighing scales to reduce the spread of dust in the room. A central vacuum outlet is provided for cleaning up the whole room.

The infest and pupae harvesting room is 20 x 36' with a 20 x 6' table in the center. The work table will accommodate 10 workers for infesting containers. Tray carts can be passed through from side to side or down the center of the table. A low velocity air system like the one built in the portable table, reported last time, was built into the work table.

Also each station can have a vacuum hose connected to a central system to draw cast-off skins and webbing away from workers when transferring insects or harvesting pupa.

All handling of containers after they are filled will be on trays (20 x 23"). Twenty-eight trays are placed on carts for transporting and also to be held during rearing.

Egg Storage Room

A conveyor system was installed in the 8 x 16' egg chamber. The conveyor is 84' long and takes 180 days to make a complete cycle. A time clock moves the chain each day 5.6". Plastic wheels are used on the trollies because of the light load of eggs per trolley. But they do not rotate as freely as they should due to the cool temperature in the chamber. The conveyor makes 2 - 90° turns and 5 - 180° turns in the chamber, therefore, the pull on the conveyor chain is much more than was originally calculated. Replacing the wheels with ball bearing type will reduce some of the load. The chain sprocket is driven by a gear motor with 2.8 rpm output which drives a gear reducer (120 to 1) thats connected to the sprocket. As of late the temperature has held very close and appears that more uniform results or response of eggs to chilling may occur.

Mass Rearing Facility:

A new room for handling mating and egg harvest is being built. Egg cleaning facility will also be included in this room as well as the entrance to the egg chilling room. The large cycloid collectors will be placed out doors so when the containers are emptied, scales will not get all over the building. All holdings of pupae, eggs and adults will be over the work counter so all the air flow in the room will pass by the workers before it flows by the holding area. A room to perform quality control measurements and a room for making experimental diets is also being built.

Two of the mobile homes, one used for rearing and one for kitchen, will be removed.

A prefab unit 14 x 36' and a constructed in place 14 x 57' will be used for rearing of insects. The new rooms will have perforated ceiling for air to enter the room. The return will be a one inch slot near the floor on each side of the room, where the air will flow up in a false wall cavity. When complete all areas will be under clean air and connected by clean air hallways. All insects will be moved about the holding room on carts.

Project Number: GM 6.3.15
Project Title: Development of Efficient Methods for Gypsy Moth Virus Production and a Pilot Plant Facility
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leaders: Martin Shapiro, R. A. Bell, J. A. Tanner

A detailed report covering work conducted in this project will be included in the next laboratory report.

Project Number: GM 7.3.3
Project Title: Weight Changes in Gypsy Moth Egg Masses During Pre-Diapause Development
Report Period: November, 1977 - March 30, 1978
Report Type: Final
Project Leader: Othel T. Forrester, R. A. Bell

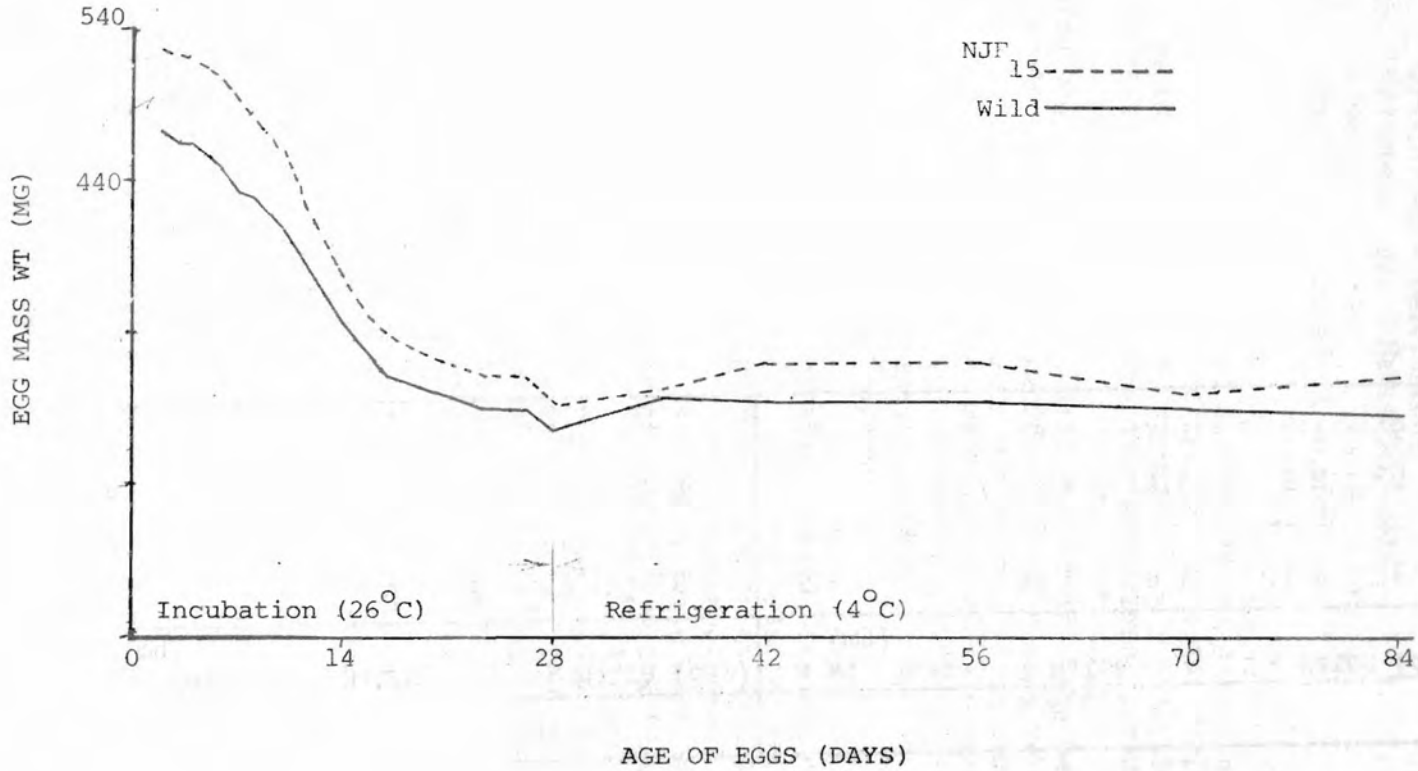
Weight losses in gypsy moth egg masses during the pre-diapause development period can be used to estimate the time required to enter diapause. This study was designed to quantify weight changes in newly oviposited egg masses to determine the optimum pre-diapause development period.

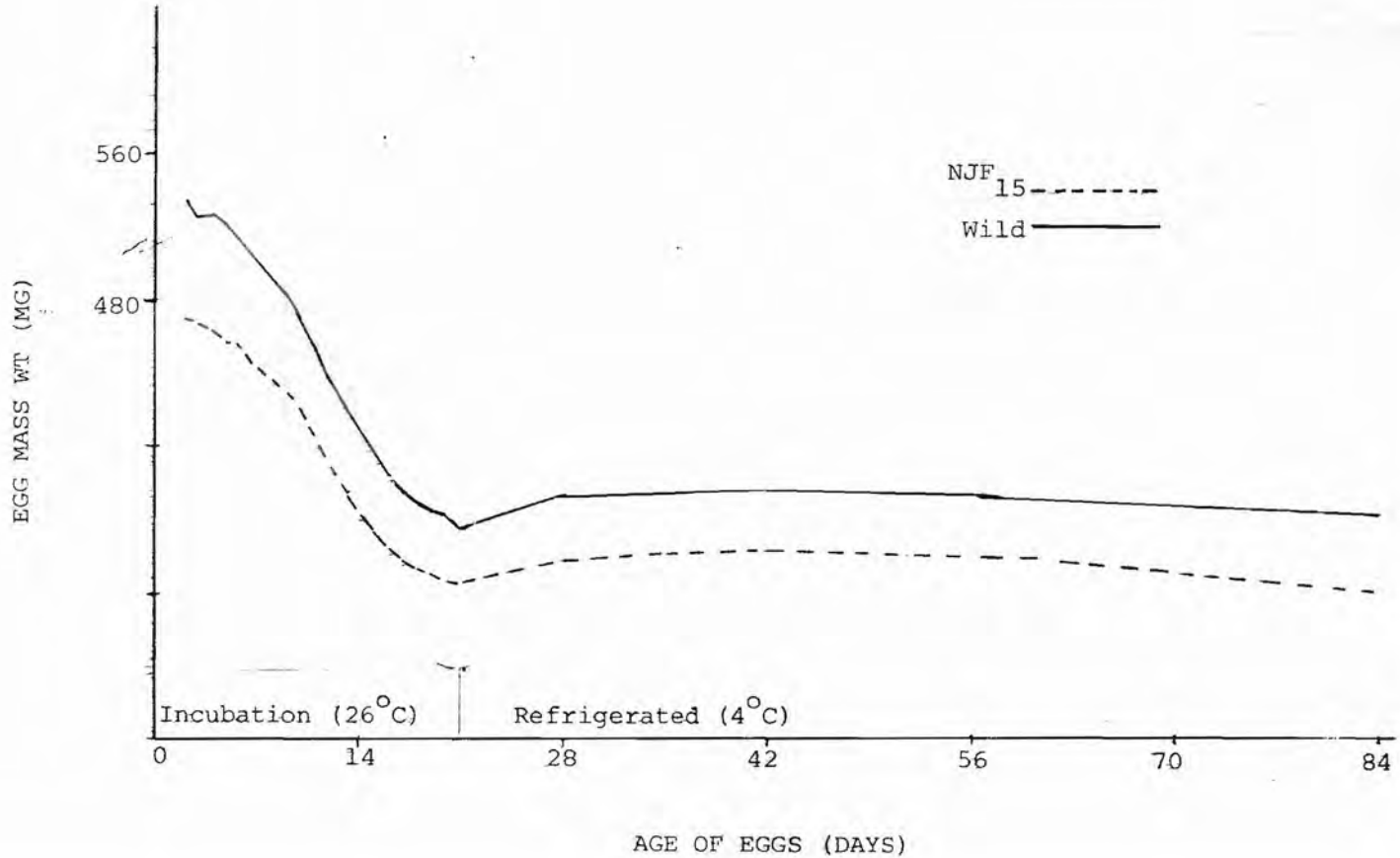
Laboratory reared New Jersey F_{15} and feral gypsy moths were mated and allowed to oviposite on brown craft paper. The egg masses were collected 48 hours after mating. The excess paper was trimmed about 5mm from each egg mass to maintain the shape of each mass and facilitate handling. Ten NJF_{16} and 10 feral egg masses were used for each treatment. The egg masses were weighed daily during the pre-diapause development period and at 7 and 14 day intervals for 84 days during diapause.

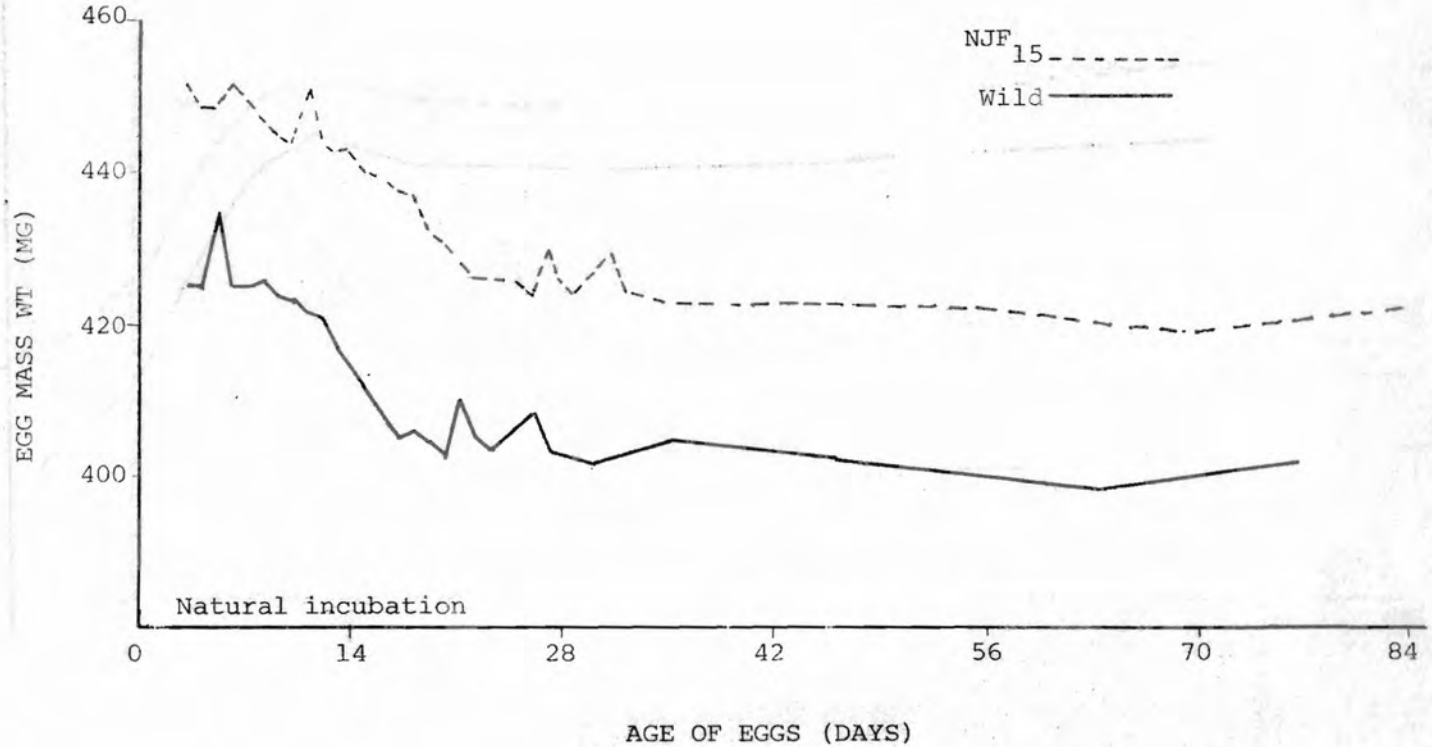
Six months after the designated embryonation period, the egg masses were dehaired; 50 egg samples were drawn from each egg mass and incubated at 25°C. The best hatch in the feral egg masses and in the New Jersey F_{15} egg masses was obtained with 21 days pre-diapause development and natural conditions. However, when egg masses of either strain were held outdoors under natural conditions, the duration of hatch increased from ca. 6 to ca. 12 days. It is also noteworthy that some hatch (24%) occurred in the feral strain after only 14 days of pre-diapause and 6 months of chilling. The NJF_{16} eggs did not hatch when treated similarly. The optimum pre-diapause development period under laboratory conditions (25°C) appears to be 21 days. The mean weight loss, hatch time and % hatch is given in Table 1. The mean weight losses are given in Figures 1 - 4.)

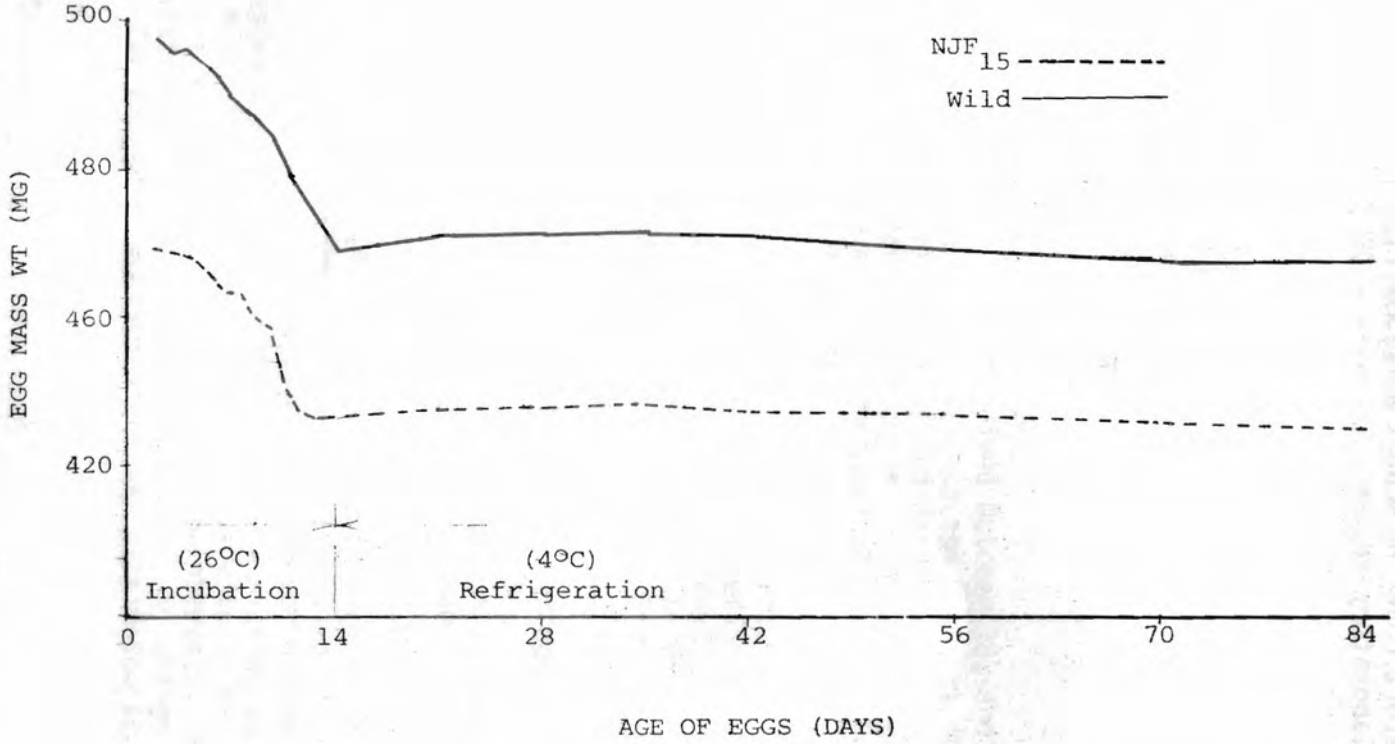
Table 1.

Pre-diapause Development Period	Feral			NJF16		
	$\bar{x}$ wt. loss (mg)	% Hatch	$\bar{x}$ Hatch (day)	$\bar{x}$ wt. loss (mg)	% Hatch	x Hatch (days)
Natural	5.2	93.3 ± 8.4	13.5 ± .6	5.6	94.6 ± 9.4	11.6 ± .6
14 days 26°C	5.7	23.6 ± 18.5	5.2 ± .3	4.8	0	--
21 days 26°C	7.6	94.4 ± 5.0	6.5 ± .3	8.0	87.8 ± 14.1	5.8 ± .5
28 days 26°C	9.2	38.8 ± 25.7	6.3 ± .1	8.7	79.6 ± 17.0	6.7 ± .6


540
440
EGG MASS WT (MG)
NJF 15
Wild
Incubation (26°C)
Refrigeration (4°C)
0
14
28
42
56
70
84
AGE OF EGGS (DAYS)


560
480
EGG MASS WT (MG)
NJF15
Wild
Incubation (26°C)
Refrigerated (4°C)
0
14
28
42
56
70
84
AGE OF EGGS (DAYS)


460
440
420
400
EGG MASS WT (MG)
NJF15
Wild
Natural incubation
0
14
28
42
56
70
84
AGE OF EGGS (DAYS)


500
480
460
420
EGG MASS WT (MG)
NJF15
Wild
(26°C)
Incubation
(4°C)
Refrigeration
0
14
28
42
56
70
84
AGE OF EGGS (DAYS)

Project Number: GM 7.3.4
Project Title: Insect Production and Distribution
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leaders: Leonard F. Kennedy, J. J. Baker, O. T. Forrester

A detailed report covering work conducted in this project will be included in the next laboratory report.

Project Number: GM 7.3.5
Project Title: Evaluation and Colonization of Gypsy Moth from the San Jose, California Infestion
Report Period: March 31, 1977 - March 31, 1978
Report Type: Final
Project Leaders: Othel T. Forrester, R. A. Bell, J. J. Baker

An infestation of Gypsy Moth was detected near San Jose, California, in early October of 1976. Egg masses were requested for colonization, to determine developmental rate, diapause requirements. parasitism incidence of virus, and general quality of the population.

Methods and Materials:

The first shipment of 3 egg masses was received October 18, and stored at 4°C. After one month chilling one-third of each egg mass was incubated at 26°C, 60% RH hatch began December 15, and ended January 23 A second shipment of 13 egg masses was received December 1. Hatch had occurred during shipment in 3 of the egg masses. One-half of each egg mass was incubated and the remaining one-half was stored.

The first portions of the egg masses used were not surface sterilized so virus could be detected. Two rearing methods were used. Neonate from the portions of the first 3 egg masses were reared individually on modified hornworm diet in 1½ oz. Thunderbird styrene plastic cups with paper lids. Larvae were transferred after 14 days to 45ml diet in 3 oz. ME-3 Sweetheart plastic cups with paper lids.

Neonates from the portions of the second shipment of 16 egg masses were aggregately reared on 45ml of diet at 10 insects per 3 oz. ME-3 plastic cups with paper lids and transferred to fresh diet in ME-3 cups with 45ml of diet. The number of insects per cup was reduced from 10 to 5 per cup at transfer.

The remaining portions of egg masses were removed from chilling in early May. These egg mass portions were surface sterilized with 10% Formalin solution for one hour and rinsed with cold tap water for one hour. The resulting neonates were aggregately reared on a 75ml high wheat germ diet at 8 insects per 6 oz. M-6R Sweetheart plastic cups with paper lids. Diet changes and transfer was planned for 14 and 21 day intervals. However, a bacterial contamination of the diet made it necessary to change diet at 3 day intervals. The bacterial contamination was eliminated at 26 days development.

Results and Discussion:

The time required to complete hatch in the first portion of the egg masses was considerable. The mean time to complete hatch was 30.6 days. This extended period of hatch is not unusual in egg masses chilled for a short period of time (two months or less).

The time required to complete hatch in the portions of egg masses which received chilling treatment was 4.6 days $\pm$ 1.5 days. The hatch ranged from 0-99%. The establishment of the California insect on artificial diet was exceptional, 95% of the insects established. This is 5% higher than is generally expected in our standard laboratory strain. (ninety-seven percent of second portion infested survived to pupation.

The incidence of disease was low, 0.7% of the insects lost showed symptoms of virus. There was no evidence of parasitism.

The first portion of the 16 egg masses yielded 225 egg masses. The second portion yielded 383 egg masses for a total of 608 egg masses. The mean egg mass weight was 571mg or about 750 eggs per mass.

The sex ratio of the first portion of the egg masses was 57.6% male and 42.4% female. The sex ratio of the second portion was 51.5% and 48.5% female. The developmental time from neonate to pupae is 4 of 5 days longer than our standard strain.

The California strain has been established as a laboratory colony and is now in F_3 generation.

Project Number: GM 8.3.2
Project Title: Rearing Techniques and Production Costs
Report Period: October 1, 1977 - March 31, 1978
Report Type: Interim
Project Leaders: Othel T. Forrester, L. Kennedy, J. Baker

The following rearing techniques are those in effect March 31, 1978. However, they are subject to change as new procedures are developed. The primary purpose of this report is to establish standard operating procedures and to aid in calculating the changes in costs of insect production that will result from various changes in techniques. Costs of each operation are expressed per 1000 insects.

Diet Preparation:

Clean, sanitary technique is essential in the efficient production of gypsy moths. All diet mixing, blending and dispensing equipment must be clean and sanitized prior to preparing diet. General procedures to follow are listed below:

1. Measure 9 liters of water into the mixing kettle
2. Start blender and add agar slowly
3. Bring water-agar solution to a boil for 5 minutes
4. Cool the water-agar solution to 80°C
5. Add the diet ingredients and blend for 10 minutes
6. Dispense 70 ml infest (Hornworm) diet into 67 ME-6R plastic cups. Dispense 90 ml of transfer (hi wheat germ) diet into 67 Dixie plastic cups. Cool diet to room temperature (about 45 minutes)
7. Clean dispenser, mixer, blender and store for next use
8. Clean kitchen work areas

Labor 70 minutes - $5.59

Neonate infestation:

Infestation of neonate larva should be done under a hood or other device with sufficient air flow to move insect setae away from the worker. A respirator should be worn while handling insects for additional protection. The following procedures must be used:

1. Wipe down all work surfaces with an 80 ppm solution of chlorine dioxide. (.5 g Oxine/gal water)
2. Using a sanitized 10/120 artist brush, place 15 neonate larvae on the diet supplied in a Sweetheart plastic XE-6 or ME-6R cup with AS 306 lid. Do not infest larvae that are not active. Dip the brush in a 40 ppm solution of chlorine dioxide (.25 g of "Oxine"/gal water) each time a different incubation dish is used. It is imperative that the strains be kept separate. Work with only 1 strain at a time.

3. Put trays of infested diet in R-4 for holding. Mark the tray with strain, date of infestation and diet batch number. Containers should be upside down and inverted after 2 days.

4. Clean work surface and wipe down with an 80 ppm solution of chlorine dioxide.

5. Return all unused lids and equipment to the proper storage place.

6. Discard all unused diet and trash.

7. The floor of the infestation room must be swept and mopped at the end of each day with chlorine dioxide, pine oil or lysol.

Labor cost for infesting larvae	$2.65
Container & lid cost	$2.15
Diet cost	$2.50
Cost	$7.30

Larval Transfer:

Larval transfer must be done under a hood or other device with sufficient air flow to move insect body hair and other irritants away from the worker. A respirator must be worn while transferring larvae. Hearing protection should be used if noise levels interfere with normal conversation. All unsafe or hazardous working conditions should be reported to the supervisor. The following transfer procedures must be used.

1. Clean work surface and wipe down with an 80 ppm solution of chlorine dioxide.

2. Check containers for diseased or otherwise abnormal insects, and fungal or bacterial contamination. If diseased or contaminated insects are observed, close the container, clean and sanitize hands, tools and the work area before continuing. Enter the contamination or disease in the contamination control log book.

3. Place diet cup (90 ml in L0311 Plastic Dixie cup) in a 16 oz. 2186SE untreated paper Dixie cup.

4. Transfer 21 day old larvae from the original container (ME-6R or XE-6 Sweetheart plastic cup) into the 16 oz. container with sanitized forceps, and close with a 3068 transparant plastic Dixie lid.

5. Mark trays with infestation date, transfer date, diet batch numbers and strain.

6. Return transferred larvae to R-4 and hold for an additional 14 days.

7. Clean the work area. Wipe all work surfaces with an 80 ppm solution of stabilized chlorine dioxide and return all materials to their proper storage place.

8. Discard all unused diet and empty trash cans.

9. Sweep and mop the floors daily. Dust each Wednesday and Friday.

Cost of transfer:

1.	Labor	$3.50
2.	Dixie 2186 cups	$1.99
3.	Dixie 3086 lids	$1.69
4.	Dixie L0311 diet cups	$0.66
5.	Diet	$3.00
	Cost	$10.84

Pupal Harvest:

Pupae must be harvested under a hood or other device with sufficient air flow to move larval body hair, frass, cast skins and other irritants away from the worker. Hearing protection must be used if noise levels are high enough to interfere with normal conversation. A respirator must be worn and gloves should be worn. Gloves must be worn if skin sensitivity is noticed. All working conditions believed to be unsafe or hazardous should be reported to the supervisor. Pupae will be harvested 35 days after infestation. Harvesting procedures are as follows:

1. Clean and wipe the work surface with a 80 ppm solution of stabilized chlorine dioxide.

2. Before opening the containers, check for diseased or abnormal insects, fungus or bacterial contamination. If contamination or dead insects are noticed do not open the container! Record the contamination or disease in the contamination and disease log book and report it to the supervisor. If a container is found to be contaminated after opening, close it, clean hands, tools and work surface before continuing harvest.

3. Use forceps to harvest pupae and spins. Harvest all pupae and spinups (pre-pupae) discard all remaining larvae.

4. Sex will be made by size and antennal ridges. The location of the anal pore will be used to determine those not readily determined by size; spinups will be sexed by size alone.

5. Female pupae will be stored at 25/16 oz. 2186SE paper Dixie cups with a 3086 plastic lid. Male pupae will be stored at 50/16 oz. 2186SE paper Dixie cups with a 3086 plastic lid. Male and female pupae will be stored in the same manner as male and female pupae.
   Pupae and pre-pupae will be stored in R-3 for eclosion and mating.

6. Clean the work area and wipe down work surfaces with an 80 ppm solution of stabilized chlorine dioxide, sanitize tools and empty trash. Floors should be swept daily and mopped on Wednesday and Friday.

Labor Pupal harvest	$4.50
Container & lid	$1.62
Cost	$6.12

7. Store all equipment in its proper place.

Mating, Egg Harvest and Embryonation:

Mating and egg harvest must be done under a hood or other device with sufficient air flow to move insect body hair and wing scales away from the worker. Hearing protection must be used if noise levels are high enough to interfere with normal conversation. A respirator (3M brand 08710) must be worn while handling adult moths and harvesting egg masses. It is imperative that the strains be kept separate, only one strain should be handled at a time. If there is any doubt about the origin of an insect do not use it!

1. Clean work surfaces and wipe down with an 80 ppm solution of stabilized chlorine dioxide.
2. Line a 1 gallon seal right ice cream container with wrapping paper.
3. Place 25 - 1 day old female moths and 25 chilled (1 min. in the refrigerator) - 1 day old male moths into the containers and lid. Mark the container with the strain, date mated and the number of mated pairs.
4. Harvest the egg masses 3 days after mating. And store at 100 egg masses/16 oz. 2186SE paper Dixie cup with a 3086 plastic lid. Scrape egg masses from the wrapping paper with small spatula and discard the paper. The 1 gal. container should be reused. (Be certain that the labels are removed from the containers.)
5. Unused adults should be discarded after the required number of moths have been mated.
6. Clean work surface, sanitize tools and store in their proper place. Empty trash and sweep the floors, mop on Wednesday and Friday.
7. Egg masses must be held in Room 3 for 28 days before they are chilled.

Labor cost/250 egg masses	$2.50
Container & Lid	$0.12
Cost	$2.65

Weighing Diet Ingredients:

Diet will be pre-weighed in 10 liter or 20 liter batch sizes. The ingredients must be weighed in the exact sequence as listed in the recipe. Each batch will be check weighed and the weight and batch number recorded on the bag. Pre-weighed diets must be stored in the freezer. The weighing and diet storage area must be kept clean, orderly and sanitized. Ingredients must be in clearly labeled containers with date received and quality control number.

All containers and balances must be cleaned and sanitized after each use. Diet weighing cost 10 or 20 liters is $0.50.

Preparing egg masses for hatch:

Remove egg masses from the chilling chamber. Put 20-30 egg masses per pouch and emerse in 10% formalin solution for 1 hour. Remove egg masses and rinse in running cold tap water for 1 hour. Dry the egg masses and put 5 egg masses in each petri dish. Mark each dish with the strain and generation, date mated and date incubated. Incubate the egg masses in the larval holding chamber.

Labor		$0.50
Petri dish		$0.04
	Cost	$0.54

Quality control costs must also be a part of the total rearing cost. However, these costs are not available at this time. Some of the items not included in these costs are utilities, supervision costs, fringe benefits or administrative costs.

Estimated production cost/1000 is $33.54.

HIGH WHEAT GERM DIET

	Ingredient	Amount/Liter	Ingredient Cost/Liter
1.	Wheat Germ	120 gm	$.0432
2.	Casein	25 gm	.0425
3.	Salt Mix	8 gm	.0240
4.	Sorbic Acid	2 gm	.0218
5.	Methyl Paraben	1 gm	.0069
6.	Vitamin Premix	10 gm	.0624
7.	Agar	15 gm	.1524
8.	Water	800 ml	
		Total	$.3595
			$.36/L

Note: Add $.0194 per gm of Aureomycin used.

ABSTRACT

Duplicate experiments were conducted in Massachusetts and Maryland to evaluate 7 aerially applied controlled-release formulations of disparlure for gypsy moth mating disruption. The incidence of mating of female moths was significantly less in treated plots than in the untreated plots. Depending upon the formulation sprayed, mating in treated plots over the season was reduced 83% to 21% from that in control plots in MA; the mating reduction in MD treatment plots varied from 97% to 19%. Several formulations reduced mating throughout the entire flight season. In plots treated with the most effective formulation (1976 2% NCR microcapsules), the mating reduction dropped to 73% in MA during peak flight, when most male moths were present. With this same formulation, the mating reduction in MD, where more sparse populations existed, was at least 88% throughout the test.

INTRODUCTION:

The development of efficacious and environmentally acceptable techniques for the control of insect pests has received considerable research effort. An appealing potential component of a pest management strategy is the disruption of mating behavior of the target insect through permeation of the atmosphere with its pheromone(s). Indeed, this novel approach to insect control has been studied in at least 25 species of serious insect pests. In many of these research programs, controlled release of the pheromone (or other behavior-modifying chemicals) into the environment has been achieved through the use of stationary pheromone dispensers, such as planchets, polyethylene caps, nylon mesh, teflon cavities, plastic strands and hollow fibers. Unless the mating disruption approach is intended to be used only in rather restricted areas, it is necessary to develop sprayable formulations of pheromone which can be applied over large infested areas with conventional aerial spray equipment. An aerially applied microcapsular formulation of disparlure (cis-7,8-epoxy-2-methyloctadecane), the synthetic sex attractant of the gypsy moth, Lymantria dispar (L.), was field tested in 1973 (Schwalbe et al., 1974). In those tests, the incidence of mating of female moths in simulated populations in the test plots treated with 15 g disparlure/ha was significantly lower than that in untreated control plots. Disruption was observed throughout the 6-week test period. Subsequently, similar formulations (gelatin-based microcapsules containing 2 2% active ingredient in xylene) have been tested in several other pheromone research programs studying the Oriental fruit moth (Gentry et al., 1974, Gentry et al., 1975), redbanded leafroller (Cardé et al., 1975, Taschenberg et al., 1974), codling moth (Moffitt, 1973), spruce budworm

(Sanders, 1976) and the grape berry moth (Taschenberg et al., 1974). These studies have demonstrated the need for further refinement of controlled-release technology.

In order to have utility in a gypsy moth management program, a formulation must be uniformly distributed in a forest environment and withstand exposure to wide fluctuations in temperature, rainfall and humidity. Furthermore, it must remain effective throughout the 6 to 8 week flight season of the adult moth. A program for laboratory screening and evaluation of potentially useful slow release formulations has been conducted. Formulations thus developed are identified as candidates for field evaluation. This report describes the results of field tests conducted on seven controlled-release formulations of disparlure.

MATERIALS AND METHODS

Field tests to determine the relative efficacy of 7 formulations of disparlure for disruption of adult gypsy moth mating were conducted in duplicate experiments in Massachusetts and Maryland. This design was chosen to test effectiveness in different population densities and histories and, potentially, varying climatological conditions. The test area in MA was in Fall River State Forest. Forest composition there was mixed hardwood with predominantly white oak; gypsy moth populations have been relatively low and stable for 22 years. The MD study site was located in Cecil County. This region has been infested since 1971 and is located within the expanding "fringe" of the generally infested zone of the U. S. Intensive searches for egg masses were conducted in this area; too few egg masses were discovered to accurately estimate density, but it may be described as very sparse, much less than 1 egg mass/ha. Because populations were so low, systematic egg mass surveys were not conducted.

Thirty-six plots, 16 ha each, were established in both the MA and MD test areas in completely randomized block design. Individual plots were separated by at least 400 m. Eight plots in MA were randomly selected for intensive survey of egg masses. Ten subplots, each 0.4 ha and uniformly distributed, were established within each plot and these subplots, representing 25% of the total area, were intensively searched for egg masses. Approximately 8 man hours were spent scouting each subplot. To further estimate population density and to monitor larval development, pupation and adult emergence in the 8 plots which were surveyed for egg masses, 15 cm wide burlap bands (larval traps) were placed around host trees of 10 cm dbh, or greater. One hundred bands were distributed in each plot and they were examined every 3 days until adult eclosion was complete.

Seven candidate controlled-release formulations of disparlure were selected for field evaluation. The laboratory testing procedures used and data supporting these selections are reported elsewhere (Plimmer et al., 1977). Descriptive details of the formulations are presented in Table 1. The NCR formulations consisted of aqueous suspensions of plastic-coated, gelatin-walled capsules containing a 3:1 xylene : amyl acetate solution of disparlure (2%, 10%, or a mixture of these 2). The Stauffer material was an aqueous suspension of cross-linked polyurea microcapsules containing a xylene solution of disparlure; 75% of the capsules were 2% and 25% were 10% in lure. The MGK material was a slurry of particles of a paraffin wax-inorganic salt matrix containing 2% disparlure. The Conrel fibers were hollow (8 mil ID) plastic fibers, 2.3 cm in length, filled with 30% disparlure in hexane. Disparlure (racemic) incorporated into the formulations was synthesized by ChemSampCo, Columbus, Ohio. It contained 3.4% of the trans isomer and 1.8% of the corresponding hydrocarbons.

Aerial application of the formulations was accomplished with a USDA, APHIS Agtruck aircraft. Guidance was provided by marking the corners of each plot with white cloth bags placed over saplings. The saplings were cut and wired to the highest accessible portion of corner trees in such a way that the bag protruded through the canopy. Helium-filled white or yellow display balloons were also raised on certain corners to facilitate plot identification and aircraft guidance. Radio communications were maintained among the ground crew, airport mixing operations, and the spray plane. The MA plots were treated on 6-7 July and those in MD were sprayed with NCR, MGK, and Stauffer formulations on 24-25 and 30 June.

The Conrel fibers were applied on 15 July in MD, too late in the season for realistic evaluation. Conventional aerial spray equipment was used for all liquid formulations; special equipment was designed and constructed for distribution of the Conrel fibers. Serious foaming of the MGK material occurred during its agitation in the aircraft tank, however, this was remedied by addition of Antifoam A and Varsol. The remaining formulations were applied with minimal operational difficulties. The degree of mating disruption was monitored by determining the incidence of mating of female moths placed in treated and control plots. Female pupae were collected from moderately defoliated infestations in NJ, PA, NY, VT and ME. Pupae were returned to the laboratory and held at 22-23°C, 16 hour photoperiod until adult emergence. As the supply of feral females was depleted on 29 July, laboratory-reared insects were substituted thereafter. Female moths 1-2 days old were placed under slab shelters attached to the boles of oak trees. Twelve female moths were placed in each plot; they were collected and replaced with new moths on the third day they were in the field. After collection, female moths were held individually in 1.5 oz. plastic creamers at 15°C (MA) or in cardboard cylinders (MD). The bursae copulatrices and spermathecae of collected females were excised and examined under phase-contrast microscopy. Presence or absence of sperm was used as the indication of mating history. Any eggs that were associated with female moths were also collected and independently checked for embryonation at least 4 weeks after collection. In MA, incidence of parasitization of eggs from the study plots was determined by counting the number of parasites (largely Oencyrtus kuwanii (Howard)) that emerged from the egg masses while in MD parasitization was noted, but percentage parasitism was not assessed.

RESULTS AND DISCUSSION

Intensive systematic visual survey in MA revealed that pretreatment population density was ca. 2 egg masses/ha. Although only 8 out of 36 plots were surveyed in this manner, observations made in the course of establishing plots indicated that population density throughout the study area was uniform. At least one egg mass was observed in each plot. As mentioned earlier, systematic survey was not conducted in the MD test site. However, several locations were intensively searched for egg masses and very few were discovered. It is estimated that population density was less than 1 egg mass/ha.

Eight hundred burlap bands (larval traps) were placed around host trees in 8 MA test plots (100 bands/plot). Thirty-one male and 57 female pupae were found under these bands. Adult emergence from those pupae occurred from 19 July to 8 August and 21 males (67%) eclosed. Peak male eclosion was from 24-28 July; 11 males (52%) emerged during that 4 day period. Forty-five (78%) female moths emerged from 24 July to 11 August. Peak female emergence was from 30 July to 3 August; 25 females (55%) emerged during that 4 day period.

In MD 800 burlap bands were placed around host trees in eight test plots (100 bands/plot). However, only one plot had sufficient numbers of larvae to monitor for pupation and adult emergence. Two additional sites in Cecil County (outside of the plot boundaries) had populations high enough to warrant monitoring, and these were included in observations on seasonal development.

The maximum number of larvae found on any one day at the most active MD location was 46 (17 June). At this site the first pupa was found on 11 June with a peak of 34 pupae found on 27 June. Female moths and egg masses were located at this site on 7 July. Dates for first pupation at the other two locations were 17 and 23 June.

The earlier emergence in MD, while expected, led to complications since feral females for evaluation had to be collected further north. Consequently, male flight in MD was well underway before evaluation could begin and was over in MD (by 30 July) well before it was in MA. Useful data could be obtained only for the first 8 sampling dates, thus, the results from MD are necessarily based upon fewer returned moths than those from MA.

Field collected female moths were used to monitor mating activity in the MA test plots until 29 July after which they were no longer available. Laboratory-reared insects were used for the remainder of the MA tests. Recovery of these females in the MA tests was 61.6% and 56.5% for field collected and laboratory-reared insects, respectively.

The incidence of mating of female moths in the test plots is presented in Table 2. The data are totals of all fertilization determinations (based on dissection and egg embryonation) performed during the test period. In MD, some females that were inseminated in disparlure-treated plots laid unembryonated egg masses. The phenomenon was not observed in the control plots and was only occasionally noted in the MA experiments.

In Figures 1 and 2, the incidence of mating in the test plots in MA is plotted against time after treatment. Daily variations in mating values have been minimized by plotting 3-point running averages. In this way, trends observed throughout the course of the test can be more clearly identified. Two figures were prepared since MGK, Stauffer, 1976 2% NCR and Control B plots were sampled on days alternate the Conrel, 1976 10% NCR, 1976 4.3% NCR, 1975 2% NCR and Control A plots. These curves illustrate that most mating in treated plots occurred during the period when mating in the control areas was the greatest. This period of peak mating was shortly after the time when most adult male moth eclosion took place (24-28 July). Diminishing effectiveness of treatment as adult male emergence increased also indicates that the degree of disruption is population-density dependent.

Late in the season (28-44 days after application) when the male moth population dropped to a lower level, the disruption in mating was again 90% or better in MA with the 1976 2% formulation. This is in contrast to results of tests with less effective formulations, especially MGK, Stauffer and Conrel. Those formulations although effective in the early part of the season, generally failed to appreciably reduce mating success during the second half of the test (i.e., after peak male flight); the reduction mating with these formulations was about 50% in mid August and 0% in the last 2 weeks of the test. Either these materials do not weather well or they release most of the formulated disparlure early in the season.

The corresponding curves prepared for the MD data show similar trends, although only the last half of the adult flight season was monitored because of the early male eclosion and the unavailability of feral female moths early in the season. Figure 3 shows the percent mating observed in the Maryland control plots and those treated with the two most effective formulations, 1976 2% NCR and 1976 4% NCR. At the time of peak mating in the control plots, both formulations caused 88% mating reduction.

The rates of parasitization of eggs collected in the test plots were 39.3 (n=43) and 43.3 (n=85) parasites/egg mass in the 1976 2% NCR and control plots, respectively. Since the purpose of this evaluation was to determine if parasitization was affected by disparlure treatment, only eggs from plots treated with the most effective formulation (1976 2% NCR) and the corresponding control plots were tested. Treatment of test plots with this formulation of disparlure had no effect on the rate of parasitization of eggs by O. kuwanii. Cameron and Rhoads (1973) concluded that the pupal parasite, Brachymeria intermedia (Nees) (Hymenoptera: Chalcididae) would not be affected in the field by disparlure treatment.

SUMMARY

Disparlure has been incorporated into controlled-release formulations that can be applied over large infested areas with conventional spray equipment. These tests show that the 1976 2% NCR microcapsular formulation was more effective in disrupting successful mating than other treatments tested. The degree of disruption attained is affected by the amount of pheromone released into the test area. The differences in effectiveness among the 7 formulations may be ascribed to differential disparlure release characteristics or to differences in environmental stability. Therefore, it is possible that improved formulations which deliver disparlure more efficiently may be more efficacious. Caro et al. (1977) calculated that only 2.3 and 12.3% of the disparlure applied in the form of 1976 2% and 10% NCR formulations, respectively, was found in air samples over a 66 day test period based on currently accepted micrometeorological techniques. Our experiments also give evidence that mating disruption through air permeation is population density dependent. Before this technique can be integrated into a gypsy moth management program, the population density limits within which biologically meaningful mating disruption can be expected must be defined.

ACKNOWLEDGEMENTS

We thank Mr. Jack Henderson, USDA, APHIS for the application of the formulations to the test plots and for the construction of the equipment used to apply the Conrel fibers. We are also grateful to Mr. C. S. Hood, MA, DNR, for his permission to use State Forest lands in MA for these tests, and to Mr. J. G. R. Tardif, USDA, APHIS, for his assistance in the mixing and loading of the test formulations and to the summer employees who were instrumental in the conduct of the experiments.

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Footnotes

[1] Lepidoptera: Lymantriidae

[2] Received for publication _____. Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA. This study was funded in part by the USDA Expanded Gypsy Moth Research and Development Program.

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Table 1. Microdispersable controlled-release formulations of disparlure field tested. All formulations were applied to achieve a dosage of 20 g disparlure/ha.

Formulation	Particle Type	Particle Size (u)	Sticker	Rate(l/ha)
1975 2% NCR	Microcapsules	50-400	1.1% Rhoplex B-15	5.2
1976 2% NCR	Microcapsules	50-250	1.1% RA-1645	5.2
1976 4% NCR 1/	Microcapsules	50-250	1.1% RA-1645	2.6
1976 10% NCR	Microcapsules	50-250	1.1% RA-1645	1.1
Stauffer	Microcapsules	10-40	Unknown	2.1
MGK	Matrix	100-200	1.1% RA-1645	3.5
Conrel	Hollow fibers	2/	Polybutene and Hydroxyethyl cellulose + RA-1645	10^5 fibers/ha

1/ Mixture of 1976 2% and 1976 10% NCR formulations.

2/ 8 mil ID x 2.3 cm long.

Table 2. Incidence of mating of female gypsy moths in test plots in MA and MD. Totals from 4 replicated treatment plots and 8 control replicates.

MASSACHUSETTS TESTS

	Field Collected Females 12-29 July		Laboratory Reared Females 30 July-27 August		TOTAL 12 July-27 August	
	No. Determined	% Fertile	No. Determined	% Fertile	% Fertile	% Reduction
1976 2% NCR	116	5.2	274	11.3	9.5	83.2
1976 4.3% NCR	89	14.6	200	17.5	16.6	70.6
1975 2% NCR	91	11.0	221	20.8	17.9	68.3
1976 10% NCR	85	17.6	214	22.0	20.7	63.4
Conrel	103	20.4	216	31.0	27.6	51.2
Stauffer	110	20.9	270	34.1	30.3	46.4
MGK	132	33.3	282	50.0	44.7	20.9
Control	215	63.3	490	53.5	56.5	0

MARYLAND TESTS

	Field Collected Females 9-30 July 1/		
	No. Determined	% Fertile	% Reduction
1976 2% NCR	73	1.4	97.1
1976 10% NCR	148	2.7	94.5
1976 4.3% NCR	99	4.0	91.8
1975 2% NCR	91	8.8	81.9
Stauffer	94	20.2	58.4
MGK	109	39.4	18.9
Control	181	48.6	0

1/ Only the latter portion of the flight season in MD could be monitored because of unavailability of female moths prior to 9 July.

FIGURES

Figure 1. Mating of female gypsy moths in plots treated with 20 g/ha disparlure incorporated into MGK, Stauffer and 1976 2% NCR formulations. Treatments were applied on 6-7 July. Field-collected moths were used to monitor mating activity from 6-29 July. Laboratory-reared insects were used thereafter. Fall River, MA. 1976.

Figure 2. Mating of female gypsy moths in plots treated with 20 g/ha disparlure incorporated into Conrel, 1976 10% NCR, 1976 4.3% NCR and 1975 2% NCR formulations. Treatments were applied on 6-7 July. Field-collected moths were used to monitor mating activity from 6-29 July. Laboratory-reared insects were used thereafter. Fall River, MA. 1976.

Figure 3. Mating of female gypsy moths in plots treated with 20 g/ha disparlure incorporated into 1976 2% NCR and 1976 4.3% NCR formulations. Treatments were applied on 24-25 June, 1976; monitoring of mating activity in plots began two weeks later. Cecil Co, MD. 1976.

Schwalbe et al. Controlled Release Formulations for Gypsy Moth

Disparlure

Gypsy Moth

Controlled Release

Lymantriidae

Mating Disruption