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April 1, 1977 - September 30, 1977

Laboratory Report
Gypsy Moth Methods Development Laboratory
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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Project Number: GM 6.1.5
Project Title: Regulatory Treatments (Laboratory-Field)
Report Period: April 1, 1977 - October 30, 1977
Report Type: Interim
Project Leaders: Winfred H. McLane, J. A. Finney

House trailers and recreational vehicles are a major source of gypsy moth movement from regulated areas to non-infested localities. To minimize this movement, inspectors utilize many work hours inspecting trailer parks and campsites. This is done in a number of ways. Fall egg mass counts are made on the sites and in surrounding woodlands; a piece by piece inspection is conducted with any egg masses, pupae or larvae being removed; campers originating outside the regulated area are cautioned about the potential hazard and at times, insecticide treatments are used. The lack of effective treatment methods for recreational vehicles and mobile homes is a major deficiency in the APHIS gypsy moth regulatory program, and, therefore, experimental laboratory and field tests are conducted to develop improved techniques for dealing with these and other regulatory problems. Detergents and innocuous materials are appealing candidates for the treatment of these regulatory items. Such materials might also be used for the treatment of outdoor furniture.

Laboratory tests indicated that when applied directly to eggs, a number of commercial detergents were effective in preventing hatch (McLane, 1975-76).

Table 1. Laboratory tests with detergents and other materials

Material	Concentration	Total Hatch in 5 Samples Treated	Untreated
Light Water	10%	0	794
	5%	1032	1584
Liquid Floor Wax	10%	0	289
	5%	1222	1695
Top Job	100%	4	1858
	50%	0	995
Wax Remover	100%	36	763
	50%	5	804
Pine Scent	100%	0	1209
	50%	0	1192
Wisk	100%	991	1006
Ammonia	100%	479	777
Pink	100%	1104	1126

Table 1 cont'd.

Material	Concentration	Total Hatch in 5 Samples Treated	Untreated
Starch	100%	289	943
Joy	100%	1068	6[illegible]

All egg masses treated were sprayed to the point of runoff.

Although high concentrations were used in those laboratory tests, results were favorable enough to warrant additional testing in the field. Six materials were selected to be tested at various concentrations.

Table 2. Materials field tested.

Material	Percent Concentration
Light Water	5 - 10 - 25
Liquid Floor Wax	10 - 25 - 50
Top Job	10 - 25 - 50
Wax Remover	10 - 25 - 50
Pine Scent	10 - 25 - 50
Dimilin	0.06 - 0.1 - 1.0

Seven parallel lines were established on woodland in Dighton, MA. Criteria for selection of test location were: 200-400 large and healthy egg masses per acre and easily accessible, yet remote enough to avoid vandalism. Trees that had egg masses deposited on them were marked with colored survey tape, each line being identified by the color of the tape used. Lines were extended until 300 egg masses were located on each line within 2.1 m of the ground.

Table 3. Material used on each colored line.

No.	Material	Color
1	Top Job	Red
2	Floor Wax	Orange
3	Light Water	Blue
4	Check	White
5	Pine Scent	Yellow
6	Dimilin	White-blue
7	Wax Remover	White-orange

A hand-held 1 pint plastic container with an adjustable spray nozzle was used to treat the egg masses. Ten squirts (approximately 7.5 ml) were deposited on each egg mass from a range of 30-48 cm. Ten egg masses were treated at each dosage on a monthly schedule from August through April. At the end of each monthly treatment on the line, a tag was placed indicating the end and start of the treatments. Individual treated egg masses were marked with colored tacks (low dosage-white, median-red and high-blue). Of the 10 egg masses treated, 5 were left exposed to the elements for the rest of the season and the remainder were covered with 10x10 cm squares of tar paper. Tar papers were attached to the trees using staples. The lower edges of the tar paper flaps were left open to minimize fumigant effect.

During April all treated and control egg masses were taken to the laboratory for incubation and hatch. A special effort was made to keep the masses intact. Individual egg masses were placed in 100x15 mm petri dishes and held at 26.6°C and 50% RH. As hatch occurred, larvae were counted and removed from the petri dishes. As soon as an egg mass developed a hatch count of 100, recordings were discontinued and hatch from that mass was referred to as 100+.

In general, hatch was higher than expected based on previous laboratory data

Table 4. Average hatch of treated and untreated egg masses.

Treatment	8/3	8/31	9/30	10/29	11/26	12/30	2/1	2/28
Dimilin								
0.06 lb ai/g-c 1/	80	84	100+	100+	100+	90	87	94
0.06 lb ai/g-u 2/	79	100+	87	87	88	100+	77	76
0.1 lb ai/g-c	85	72	95	100+	96	100+	78	87
0.1 lb ai/g-u	74	65	100+	88	76	75	82	100+
1.0 lb ai/g-c	73	100+	88	87	81	93	64	82
1.0 lb ai/g-u	66	100+	82	85	100+	90	37	82
Light Water								
5%-c	100+	97	86	95	100+	87	83	85
5%-u	100+	46	95	99	100+	97	96	89
10%-c	87	92	100+	93	80	100+	85	77
10%-u	100+	73	84	77	94	84	72	71
25%-c	75	100+	75	91	93	62	81	90
25%-u	41	100+	100+	83	93	79	91	87
Pine Scent								
10%-c	74	100+	-	100+	94	39	86	73
10%-u	100+	75	-	67	100+	58	64	59

Table 4 cont'd.

Treatment	8/3	8/31	9/30	10/29	11/26	12/30	2/1	2/2[illegible]	[illegible]
Pine Scent cont'd									
25%-c	55	99	100+	100+	93	31	33	79	[illegible]
25%-u	3	71	100+	92	72	88	64	100+	[illegible]
50%-c	4	18	33	23	44	34	18	72	13
50%-u	45	30	68	13	61	50	54	69	100+
Top Job									
10%-c	33	91	74	63	78	51	88	100+	100+
10%-u	60	100+	100+	81	89	97	87	100+	94
25%-c	27	74	91	95	72	95	83	93	100+
25%-u	4	72	72	87	100+	90	86	93	100+
50%-c	41	77	87	43	76	94	86	94	95
50%-u	8	96	88	80	89	72	76	85	100+
Wax									
10%-c	100+	90	87	91	100+	89	88	90	68
10%-u	95	92	93	83	93	85	89	80	81
25%-c	83	99	100+	100+	89	62	78	88	97
25%-u	96	82	95	100+	92	90	91	93	94
50%-c	100+	77	96	100+	70	81	77	93	80
50%-u	89	100+	96	72	82	100+	70	91	100+
Wax Remover									
10%-c	83	84	100+	98	80	100+	85	63	84
10%-u	75	54	85	92	82	83	75	63	80
25%-c	75	78	86	78	56	89	54	100+	100+
25%-u	72	59	73	91	87	84	53	52	55
50%-c	55	75	87	84	75	100+	74	76	83
50%-u	60	87	68	84	70	82	60	93	97
Check-c	96	100+	88	100+	100+	78	100+	88	100+
Check-u	84	90	94	87	100+	100+	90	100+	100+

1/ Egg mass was covered with 10x10 cm tar paper.

2/ Egg mass was left uncovered after treatment.

Although there was slight reduction of hatch in most treatments, no treatment gave sufficient reduction to warrant its use as a regulatory treatment. Pine Scent at the 50% concentration reduced hatch in excess of all other treatments. Although Dimilin did little to reduce hatch extensive mortality occurred when larvae from treated egg masses were reared on artificial diet for 9-18 days. One hundred percent mortality of larvae from Dimilin treated eggs was observed. This aspect will be studied more extensively in the future.

It is difficult to explain why the materials tested were effective in reducing hatch in the laboratory and not in the field. Possibly, penetration of the test material into the individual egg masses was inadequate. Alternatively, a fumigation effect may have occurred during laboratory tests in which egg masses were incubated in petri dishes.

As a result of field tests, laboratory work was again started with the detergents and emulsifiers and a test was conducted using petri dishes and mesh net packets to search for fumigant effects of treatments.

Table 5. Egg masses treated with detergents and emulsifiers.

			Average Hatch of 5 Egg Masses	
Material	Concentration Dosage	Application Method	Incubated in petri dishes	Incubated in mesh net packets
Pine Scent	10%	10 ml-hand sprayer		65
	25%	"		8
	50%	"		9
Top Job	10%	10 ml-hand sprayer		100+
	25%	"		65
	50%	"		3
Orthene	.25 lb/g/a	10 ml-hand sprayer		100+
	.5 lb/g/a	"		100+
	1.0 lb/g/a	"		100+
Sevin 80S	.25 lb/g/a	1 ml-pipette		25
	.5 lb/g/a	"		18
	1.0 lb/g/a	"		5
Sponto AK 16-95	2%	1 ml pipette	0	0
	3%	"	0	0
	5%	"	0	0
Sponto 207	2%	1 ml-pipette	100+	100+
	3%	"	76	100+
	5%	"	38	100+
Triton X-100	3%	1 ml-pipette	100+	100+
	4%	"	.25	56
	5%	"	29	.25

The limited data did not indicate that fumigation action occurred in those laboratory tests using petri dishes (with the exception of Sponto 207). However, the screening still suggested that emulsifiers (and, to a lesser extent, Top Job and Pine Scent) prevent hatch. Therefore, a number of tests were conducted with emulsifiers.

Table 6. Hatch from egg masses treated with emulsifiers. (Dashes indicate no data taken).

Material	Concentration	Average Hatch of 5 Egg Masses Pipette Treatment of 1 ml	Pipette Treatment of 2 ml
Sponto 207	25%	-	0
	10%	-	0
	5%	38	0
	3%	100+	11
Sponto AK 16-95	25%	-	0
	10%	-	0
	5%	0	0
	3%	0	0
Sponto H-44C	10%	-	0
	5%	-	0
	4%	-	13
	2%	-	100+
Triton X-100	10%	-	0
	5%	0	0
	4%	-	5
	2%	-	5
Triton X-180	25%	-	0
	10%	-	0
	5%	28	2
	3%	-	8
Triton X-190	10%	-	0
	5%	-	0
	4%	-	6
	2%	-	3
Triton X-301	25%	-	0
	10%	-	0
	5%	-	36
	3%	-	65
Triton B 1956	25%	-	0
	10%	-	71
	5%	-	26
	3%	-	17

Table 7. Hatch from egg masses treated by 2 techniques with detergents

Material	Concentration	Average Hatch from 5 Egg Masses: Treatment with 2 ml by pipette	Average Hatch from 5 Egg Masses: Treatment with 10 ml hand sprayer
Light Water	10%	0	0
	25%	0	0
	50%	0	0
Top Job	10%	0	0
	25%	0	0
	50%	0	0
Pine Scent	10%	0	0
	25%	0	25
	50%	0	0
Light Water + Pine Scent	10%	0	0
	25%	0	0
	50%	0	0
Light Water + Top Job	10%	0	0
	25%	0	0
	50%	0	0
S-20546	10%	0	0
	25%	0	0
	50%	0	0
Check	-	100+	100+

In another series of tests, egg masses were treated with various insecticide dusts and liquid formulations.

Table 8. Hatch from egg masses treated with liquid formulations of insecticides.

Material	Application Rate Per Egg Mass (g/10 ml)	Average No. Hatch Per Egg Mass
Gardona 80S	1.49 g	>100
	.74 g	>100
	.37 g	>100
Orthene 75S	1.59 g	0
	.79 g	< 10
	.39 g	>100

Table 8 cont'd.

Material	Application Rate Per Egg Mass (g/10 ml)	Average No. Hatch Per Egg Mass
Sevin 80S	1.49 g	0
	.74 g	< 20
	.37 g	>100
Resmethrin (SBP 1382)	.597 g	0
	.147 g	0
	.035 g	< 10
FMC-45498 (Decis)	.125 g	0
	.023 g	0
	.008 g	> 50
FMC 33297	.479 g	0
	.119 g	< 30
	.023 g	> 60
Bioethanomethrin	.597 g	0
	.147 g	0
	.035 g	0
Pydrin (SD 43775)	.359 g	< 6
	.083 g	< 10
	.035 g	>100
DPX 5444	1.19 g	>100
	.267 g	>100
	.071 g	>100
Check	-	>100

Table 9. Hatch from egg masses treated with insecticide dusts.

Material	Percent of Active Ingredient	Average No. of Hatch per Egg Mass	Percent Mortality of Neonates Placed on Diet
Dimilin 25 WP	25	>100	100
	12.5	>100	100
Dimilin Oil Dispersable 25AI	25	>100	100
		>100	100
Dylox	80	0	-
	40	0	-

Table 9 cont'd.

Material	Percent of Active Ingredient	Average No. of Hatch per Egg Mass	Percent Mortality of Neonates Placed on Diet
Rotenone	10	>100	100
Gardona 75S	75	< 25	100
	37.5	>100	100
Orthene 75S	75	< 20	100
	37.5	>100	100
Captan	15	<100	100
Malathion	7.5		
Methoxychlor	15		
Diazinon	40	< 3	100
Captan	5	>100	100
Methoxychlor	5		
Folpet	75	< 40	100
Check	-	>100	0

Larvae that hatched from dusted egg masses were placed on artificial diet shortly after hatch; mortality of larvae was 100% with all treatments. Work will continue with dust treatments.

Since treatment with emulsifiers looked promising in the laboratory, limited field tests were conducted in 1977. Individual laboratory reared egg masses were caged in screen packets and placed on the sides of oak and pine trees in back of the laboratory. Five egg masses were treated with each rate, allowed to stay in the field for 72 hours, and then collected and incubated in the laboratory at 26.6°C and 50% RH. Larvae were recorded as they emerged. Results are given in Tables 10 and 11.

Table 10. Results of field egg mass treatment no. 1 with emulsifiers using hand sprayer application method.

Material	Concentration	Average Hatch of 5 Egg Masses 5 squirts	10 squirts	15 squ
Triton X-100	10%	6	1	0
	5%	6	39	29
Triton X-180	10%	34	12	9
	5%	63	100+	89

Table 10 cont'd.

Material	Concentration	Average Hatch of 5 Egg Masses 5 squirts	10 squirts	15 squirts
Sponto 207	10%	1	0	4
	5%	76	76	90
Sponto AD 16-95	10%	41	0	0
	5%	21	22	0
Check (untreated)	-	100+	100+	100+
Check (H_2O treated)	-	100+	100+	100+

Table 11. Results of field egg mass treatment no. 2 with emulsifiers using hand sprayer application method.

Material	Concentration	Average Hatch of 5 Egg Masses 15 squirts	20 squirts	25 squirts	30 squirts
Triton X-100	10%	100+	80	76	86
Triton X-180	10%	100+	100+	96	100+
Triton X-190	10%	60	33	38	20
Sponto AK 16-95	10%	0	0	16	73
Sponto 207	10%	100+	100+	100+	77
Sponto H-44C	10%	5	0	0	0
Check (untreated)	-	100+	100+	100+	100+
Check (H_2O treated)	-	100+	100+	100+	100+

Results were favorable with Sponto AK 16-95 and Sponto H-44C, however, there was no correlation between rates of application.

Regulatory treatment work will continue with emulsifier and dust treatments and investigation into larval mortality after hatch from eggs treated with various insecticides.

Project Number: GM 6.1.6
Project Title: Field Testing of Juvenile Hormone Analogs for Ovicidal Activity
Report Period: April 1, 1977 - September 30, 1977
Report Type: Final
Project Leaders: Arthur P. Morris and C. P. Schwalbe

Juvenile hormone analogs have shown ovicidal action on some lepidopterous species. Dr. Jeff Granett (unpublished data) concluded that Altozar 4E and ZR 619-5E at 8g/qt sprayed on tree trunks, prevented gypsy moth hatch from egg masses deposited on recently treated surfaces. Also, his data suggested that ZR 619 discouraged the females from laying their full complement of eggs. Compounds with these properties can be useful for regulatory treatments to prevent the spread of gypsy moths to uninfested areas of the United States by recreational vehicles and regulated articles.

A series of tests was run during the summer of 1976 in Taunton, MA to determine if these compounds would prevent eggs laid on treated tree trunks from hatching, prevent females from laying their full complement of eggs or effect parasitization. It was also desired to find out how many eggs are generally not fertilized, i.e., not embryonated, to determine if such will adversely affect the computations of other categories of eggs.

Trunks of white and red oak trees were sprayed to the point of runoff with water emulsions of Altozar and ZR 619 at 3 g/l using a 1.5 gallon hand sprayer. Treatments were aged for 1, 3, 7, 14-15, 21-22 and 28 days before bioassay. At the end of each aging period 25 or more 1-3 day old mated females were imprisoned on each of 5 trees per treatment age for each compound. Two tree trunks per treatment age for each compound were used as controls.

Ventilated (punched), domed, plastic closures for 5 ounce Dixie Food Dishes were stapled to tree trunks to imprison the females.

Female moths and male moths for mating were generally from field collected pupae but laboratory reared adults of both sexes had to be used for 3 of the 12 tests. Laboratory males were used to supplement the wild ones on 3 occasions.

The females that deposited egg masses were recovered (generally after one day), placed in polyethylene bags and frozen until dissected and unlaid eggs per female counted. Egg masses were collected from the treated and control trunks during January-February, 1977 and stored outdoors in plastic petri dishes until 28 day hatch tests were run at 28°C and 56% RH. Each egg mass was then dissected to microscopically determine the number of eggs that failed to hatch, that were parasitized and that were unembryonated.

Results and Discussion:

1. Undeposited Eggs: Table 4, columns 5 and 6; Table 7, column 1. Neither compound, Altozar nor ZR 619, reduced the numbers of eggs laid by fe-

males on the treated tree trunks, i.e., neither discouraged egg deposition.

2. Hatched Eggs: Table 5, columns 5 and 6; Table 7, column 2. Altozar reduced the hatch of eggs 100% for one and 3 days, 98% for 7 days, 85% for 14 and 22 days and 59% for 28 days. Higher dosages will give better results. ZR 619 did not give 100% reduction of egg hatch for any treatment age but reduced hatch for all treatments except the 15 day one. The reductions in hatch were not consistent between time periods. Higher dosages will give better results but this compound is not effective enough for further testing.

3. Unhatched Eggs: Table 5, columns 9 and 10; Table 7, column 3. Altozar consistently prevented hatch of eggs for all ages of treatments, i.e., showed larger percentages of unhatched eggs on treated trunks than on the control trunks. Although not consistent between ages of treatments, this data verifies the validity of hatch data discussed in the preceding category (hatched eggs).

ZR 619 increased the percentages of unhatched eggs for all ages of treatments, except the 15 day one, over the percentages in the controls. This data verifies the results of the hatch data.

4. Parasitization of Eggs: Table 6, columns 5 and 6; Table 7, column 4. Altozar consistently showed lower percentages of parasitized eggs from treated trunks than from control trunks, except for the 14 day old treatments when they were equal. The progression of percentages from the shortest to the longest age of treatment was not consistent. Only the 7 and 22 day old treatments have significant differences between treated and control trunks. These points lead to a conclusion that Altozar had no effect upon parasitization rates.

ZR 619 consistently showed higher percentages of parasitization from treated trunks than from control trunks, except for the 3 day old treatment. The progression of percentages from the shortest to the longest ages of treatment was not consistent. There were no significant differences between the percentages on treated and control trunks. These points lead to the conclusion that ZR 619 had no effects upon parasitization rates.

5. Unembryonated Eggs: Table 6, columns 9 and 10; Table 7, column 5. The intention for counting unembryonated eggs was to determine if they were present in sufficient numbers to affect the validity of computations of percentages of eggs in the different categories, i.e., hatched, unhatched and parasitized. Less than one percent (1%) of the eggs were unembryonated (Tables 2 and 3, columns 5 and 6). Large numbers of collapsed eggs were present in several of the egg masses and it was impossible to tell if collapsed eggs were unembryonated (unfertilized) or embryonated. A one percent unembryonated figure may be low but they are not believed to have been present in high enough numbers to effect the validity of the computed percentages of hatched, unhatched or parasitized eggs.

Summary and Conclusions:

Altozar and ZR 619 reduced the hatch of eggs and increased the percentages of unhatched eggs but Altozar was superior. Altozar gave 100 reduction of hatch for 3 days and 98% reduction for 7 days. ZR 619 did not give 100% reduction of hatch for even one day. Altozar is being tested for prevention of hatch on trailers. ZR 619 has been dropped from the tests.

Neither Altozar nor ZR 619 had any effects upon deposition of eggs by females or upon parasitization rates of eggs deposited on treated surfaces.

The numbers of unembryonated eggs were not sufficiently high to effect the validity of the computations of hatched, unhatched or parasitized eggs.

Table 1. Compound, Female and Egg Mass Data, 1976 Field Tests.

		COLUMN					
		1	2	3	4	5	6
	Age of Treatment in Days	Number of Females Used		Recovered		# of Egg Masses Obtained	
Compound*		Treated	Control	Treated	Control	Treated	Control
Altozar 4E	1	36	14	32	14	31	13
	3	40	16	30	15	28	15
	7	36	15	32	13	32	10
	14	50	20	37	16	37	16
	22	35	14	25	11	25	11
	28	25	12	25	10	25	10
ZR 619-5E	1	53	21	26	13	26	12
	3	50	20	28	12	27	11
	7	42	18	39	14	41	14
	15	55	20	32	15	33	15
	21	30	15	25	12	24	12
	28	27	11	25	10	25	10

* All dosages were 8 g/l.

Table 2. Egg Counts from Females and Egg Masses Obtained from Altozar Tests, 1976

Compound	Treatment Age in Days	COLUMN 1 Unlaid Total 1/	2 Hatched	3 Unhatched	4 Numbers of Eggs Deposited Parasitized	5 Unembryonated	6 Total	7 Potential Total 2/
Altozar								
Treated	1	1238	0	3869	2211	47	6127	7365
Control		380	1196	239	880	11	2326	2706
Treated	3	1394	0	3834	1578	24	5436	6830
Control		619	1993	338	1030	6	3367	3986
Treated	7	1044	44	5738	1908	137	7827	8871
Control		660	1148	139	696	3	1986	2646
Treated	14	8218	905	10158	4493	59	15615	23833
Control		4471	3131	2347	2209	35	7722	12193
Treated	22	1059	364	3350	1845	45	5604	6663
Control		1350	820	383	895	19	2117	3467
Treated	28	805	1120	2024	1462	15	4621	5426
Control		256	1193	133	700	3	2029	2285
Totals						404	64777	
% Unembryonated							0.6	

1/ Numbers obtained by dissecting females exposed and then recovered.

2/ Numbers obtained by adding totals of unlaid and deposited eggs, i.e., columns 1 and 6 of this table.

Table 3. Egg Counts from Females and Egg Masses Obtained from ZR 619 Tests, 1976

Compound	Treatment Age in Days	COLUMN 1 Unlaid Total 1/	2 Hatched	3 Unhatched	4 Numbers of Eggs Deposited Parasitized	5 Unembryonated	6 Total	7 Potential Total 2/
ZR 619-5E								
Treated	1	7634	1681	9153	4089	113	15036	22670
Control		1797	2689	2441	1782	23	6935	8732
Treated	3	1519	1431	3404	2480	55	7370	8889
Control		592	1289	616	1028	26	2959	3551
Treated	7	1137	3442	2420	3565	50	9477	10614
Control		885	1184	607	992	27	2810	3695
Treated	15	4347	6895	2806	3382	6	13089	17436
Control		2254	2240	1217	1184	3	4644	6898
Treated	21	1398	2058	992	1233	1	4284	5682
Control		469	2031	265	776	0	3072	3541
Treated	28	888	3127	697	1297	2	5123	6011
Control		276	1705	185	571	0	2461	2737
Total						306	77260	
% Unembryonated							0.4	

1/ Numbers obtained by dissecting females exposed and then recovered.

2/ Numbers obtained by adding totals of unlaid and deposited eggs, i.e., columns 1 and 6 of this table.

Table 4. Numbers and Percentages of Unlaid Eggs

		COLUMN					
		1	2	3	4	5	6
		NUMBERS OF EGGS					
Compound	Treatment Age in Days	Potential Treated	Total 1/ Control	Unlaid Treated	Total 2/ Control	Percent Treated	Unlaid Control
Altozar 4E	1	7365	2706	1238	380	17	14
	3	6830	3986	1394	619	20	16
	7	8871	2646	1044	660	12	25
	14	23833	12193	8218	4471	34	37
	22	6663	3467	1059	1350	16	39
	28	5426	2285	805	256	15	11
ZR 619-5E	1	22670	8732	7634	1797	34	21
	3	8889	3551	1519	592	17	17
	7	10614	3695	1137	885	11	24
	15	17436	6898	4347	2254	25	33
	21	5682	3541	1398	469	25	13
	28	6011	2737	888	276	15	10

1/ Copied from Tables 2 and 3, column 7.

2/ Copied from Tables 2 and 3, column 1.

Table 5. Numbers and Percentages of Hatched and Unhatched Eggs.

		COLUMN									
		1	2	3	4	5	6	7	8	9	10
		NUMBERS OF EGGS									
Compound	Treatment Age in Days	Deposited Treated	Total 1/ Control	Hatched Treated	Total 2/ Control	Percent Treated	Hatched Control	Unhatched Treated	Total 3/ Control	Percent Treated	Unhatched Control
Altozar 4E	1	6127	2326	0	1196	0	51	3869	239	63	10
	3	5436	3367	0	1993	0	59	3834	338	71	10
	7	7827	1986	44	1148	1	58	5738	139	73	7
	14	15615	7722	905	3131	6	41	10158	2347	65	30
	22	5604	2117	364	820	6	39	3350	383	60	18
	28	4621	2029	1120	1193	24	59	2024	133	44	7
ZR 619-5E	1	15036	6935	1681	2689	11	39	9153	2441	61	35
	3	7370	2959	1431	1289	19	44	3404	616	46	21
	7	9477	2810	3442	1184	36	42	2420	607	26	22
	15	13089	4644	6895	2240	53	48	2806	1217	21	26
	21	4284	3072	2058	2031	48	66	992	265	23	9
	28	5123	2461	3127	1705	61	69	697	185	14	8

1/ Copied from Tables 2 and 3, column 6.

2/ Copied from Tables 2 and 3, column 2.

3/ Copied from Tables 2 and 3, column 3.

Table 6. Numbers and Percentages of Parasitized and Unembryonated Eggs.

		COLUMN									
		1	2	3	4	5	6	7	8	9	10
		NUMBERS OF EGGS									
Compound	Treatment Age in Days	Deposited Treated	Total 1/ Control	Parasitized Treated	Total 2/ Control	Percent Treated	Parasitized Control	Unembryonated Treated	Total 3/ Control	Percent Treated	Unemb. Control
Altozar 4E	1	6127	2326	2211	880	36	38	47	11	1	0
	3	5436	3367	1578	1030	29	31	24	6	0	0
	7	7827	1986	1908	696	24	35	137	3	2	0
	14	15615	7722	4493	2209	29	29	59	35	0	0
	22	5604	2117	1845	895	33	42	45	19	1	1
	28	4621	2029	1462	700	32	34	15	3	0	0
ZR 619-5E	1	15036	6935	4089	1782	27	26	113	23	1	0
	3	7370	2959	2480	1028	34	35	55	26	1	1
	7	9477	2810	3565	992	38	35	50	27	1	1
	15	13089	4644	3382	1184	26	25	6	3	0	0
	21	4284	3072	1233	776	29	25	1	0	0	0
	28	5123	2461	1297	571	25	23	2	0	0	0

1/ Copied from Tables 2 and 3, column 6.

2/ Copied from Tables 2 and 3, column 4.

3/ Copied from Tables 2 and 3, column 5.

Table 7. Percentages of Eggs in Each Category

Compound	Age of Treatment in Days	COLUMN 1 Unlaid	2 Hatched	3 Unhatched	4 Parasitized	5 Unembryonated
				Percentage		
Altozar 4E						
Treated		17	0	63	36	1
Control	1	14	51	10	38	0
% Decrease			100		5	
Treated		20	0	71	29	0
Control	3	16	59	10	31	0
% Decrease			100		6	
Treated		12	1	73	24	2
Control	7	25	58	7	35	0
% Decrease		52	98		31	
Treated		34	6	65	29	0
Control	14	37	41	30	29	0
% Decrease		8	85		0	
Treated		16	6	60	33	1
Control	22	39	39	18	42	1
% Decrease		59	85		21	0
Treated		15	24	44	32	0
Control	28	11	59	7	34	0
% Decrease			59		6	
ZR 619-5E						
Treated		34	11	61	27	1
Control	1	21	39	35	26	0
% Decrease			72			
Treated		17	19	46	34	1
Control	3	17	44	21	35	1
% Decrease		0	57		3	0
Treated		11	36	26	38	1
Control	7	24	42	22	35	1
% Decrease		54	14			0
Treated		25	53	21	26	0
Control	15	33	48	26	25	0
% Decrease		24		19		

Table 7 cont'd.

Compound	Age of Treatment in Days	COLUMN 1 Unlaid	2 Hatched	3 Unhatched	4 Parasitized	5 Unembryonated
				Percentage		
ZR 619-5E						
Treated		25	48	23	29	0
Control	21	13	66	9	25	0
% Decrease			27			
Treated		15	61	14	25	0
Control	28	10	69	8	23	0
% Decrease			12			

Project Number: GM 6.1.7
Project Title: Field Testing of Altozar 5E and Dimilin 25 W Against Egg Masses Laid on Treated Surfaces of Recreational Vehicles
Report Period: April 1, 1977 - September 30, 1977
Report Type: Preliminary
Project Leaders: Arthur P. Morris, C. P. Schwalbe

A previous test series with Altozar 4E at 8 g/l applied to oak tree trunks and aged for 1, 3, 5, 7, 14, 22 and 28 days prior to egg mass deposition upon them prevented hatch for 3 days and reduced hatch 59% after 28 days. Also, field tests with Dimilin W 25 indicate that larvae hatching from egg masses laid upon treated surfaces will not develop beyond the 2nd instar.

Compounds with these properties are needed for regulatory treatments to prevent the spread of gypsy moths to uninfested areas of the United States by recreational vehicles and other regulated items. Tests have been initiated to determine if Altozar and Dimilin, when applied to such vehicles, will affect hatch or larval development from eggs deposited upon surfaces treated with these compounds.

A house trailer, 12 x 50 feet, supported by steel I beams and covered on the bottom with heavy craft paper painted with a thin layer of asphalt tar and a travel trailer, 8 x 14 feet, supported by steel C beams with an exposed bottom of galvanized sheet steel were used in these tests. Types of construction material on which treatments were applied were tar coated craft paper, galvanized sheet steel and steel beams.

A heavy plastic curtain, extending from the bottom of the trailers to the ground, was taped approximately ½ the length of each trailer to effectively isolate each half. The structural components were marked off into sections so that treatments would age for the desired number of days, i.e., 1, 3, 5, 7, 14 and 28 days. A control surface for each section was kept untreated with a waterproof plastic.

One-half of each trailer bottom was sprayed with 16 g/l of Altozar, plus 30 ml of Chevron spray sticker, plus water to 1000 ml of total spray solution. The other half of each was sprayed with 115 g/l of Dimilin, plus 30 ml of Chevron spray sticker, plus 1000 ml of water. All spray was applied to the point of runoff with a 1.5 gallon sprayer.

Thirty or more mated 1-3 day old females were imprisoned on each section after the treatments had aged for the appropriate time periods; 12 or more similar females were imprisoned on control surfaces. Vented 5 ounce plastic Dixie Food Dish Closures (4" diameter) were used to imprison the females on desired surfaces. Females were from field collected pupae, except for the 28 day old treatments. Laboratory reared males were mated with the females for all treatments, except those for one and three days when they were used to supplement wild males.

The egg masses laid on the treated surfaces are, at present, aging in place. The numbers of egg masses obtained for each treatment age per compound are shown in the following table. The numbers of egg masses laid on the galvanized steel bottom were so low that this component was abandoned after the one and three day aged treatments.

Egg masses will be removed from trailers during January-February, 1978 and hatching tests will be conducted. First instar larvae will be placed in petri dishes with artificial diet and observed through the 2nd instar. Percentage of hatch and survival will be tabulated for comparison and for analysis.

Table 1. Females Used and Egg Masses Obtained on Trailers - 1977

			Construction Material											
			Tar Paper				Steel Beams				Galvenized Steel			
			Treated		Control		Treated		Control		Treated		Control	
Compound*	Treatment Age in Days	Date Females Placed	♀♀ Used	EM's Obtained	♀♀ Used	EM's Obtained	♀♀ Used	EM's Obtained	♀♀ Used	EM's Obtained	♀♀ Used	EM's Obtained	♀♀ Used	EM's Obtained
Altozar 5E	1	July 6	30	27	12	10	30	19	12	8	33	6	11	4
	3	July 8	30	28	12	12	31	18	13	7	30	3	12	2
	7	July 12	30	28	12	10	30	26	12	10	-	-	-	-
	14	July 19	30	28	12	8	30	18	12	6	-	-	-	-
	28	Aug 2	36	14	12	5	35	14	14	4	-	-	-	-
Dimilin W25	1	July 6	30	28	12	7	30	22	12	7	35	2	16	0
	3	July 8	30	29	12	11	30	14	12	11	35	3	15	0
	7	July 12	30	26	12	12	30	27	16	16	-	-	-	-
	14	July 19	35	22	12	10	30	20	12	8	-	-	-	-
	29	Aug 3	37	17	12	6	41	10	12	0	-	-	-	-

* treatments made July 5, 1977

Project Number: GM 7.1.0
Project Title: Contact Toxicity of Registered Insecticides Against Gypsy Moth Larvae
Report Period: April 1, 1977 - September 30, 1977
Report Type: Interim
Project Leaders: Winfred H. McLane, J. A. Finney

It is important that the stage of larval development is known during an insecticide application program. If larvae are too large, treatment may not be efficacious and additional insecticide may be needed to kill the later instars. Alternatively, adequate control of small larvae may require less insecticide. Tests are being conducted to determine the LD_{50} of 2nd, 3rd, 4th and 5th instar laboratory reared gypsy moth larvae when treated topically with the major registered insecticides used in campground treatments. To date, Sevin 80S, Dimilin 25 WP and Orthene 75S have been tested. A starting dosage was taken from previously published work by McLane, 1974. All larvae used for the experiments were newly moulted, hand picked insects from the NJF_{16} strain.

Chemicals were mixed in acetone prior to testing and stored in sealed vials under refrigeration when not in use. Dosage-mortality data were obtained by application of one microliter of acetone containing the insecticide to the dorsal metathoracic region. This was done using a 50 microliter syringe and repeating dispenser calibrated to deliver 1/50th of its capacity. Dosage-mortality curves were obtained by treating groups of larvae with 7 dilutions of each insecticide. Tests consisted of treating 20 larvae with each of the 7 dosages on consecutive days until a minimum of 100 larvae were exposed to each dilution. Treated 2nd and 3rd instar larvae were held in groups of 20 in plastic dishes (94 mm x 48 mm) containing 60 ml of artificial diet. Treated 4th and 5th instar larvae were held 10 per dish. Chamber conditions were 26°C and 60% RH. Mortality readings with Sevin and Orthene tests were made at the end of 48 hours. Readings were made up to 12 days with Dimilin. Criterion for death was no reaction when larvae were prodded gently with a dissecting needle.

Although a statistical analysis has not been completed at this time, basic raw data are available.

In a number of the Dimilin treatments where there was larval survival, pupation was abnormal and survival to adulthood was rare. This aspect of the work will be studied in greater depth in the future.

There are still a number of topical tests to be conducted before the study is complete.

Table 1. Percent mortality 48 hours after treatment with Sevin 80S.

Treatment	Larval Instar II	III	IV	V
20 μg/μl				98
10 μg/μl				82
5 μg/μl	100	98	98	72
2.5 μg/μl	82	88	79	38
1.25 μg/μl	73	75	57	20
0.60 μg/μl	60	61	31	9
0.312 μg/μl	52	41	15	4
0.156 μg/μl	45	28	3	0
0.078 μg/μl	51	8	0	0
0.0039μg/μl	23	3	0	0
Acetone Check	0	0	0	0
Untreated Check	0	0	0	0

Table 2. Percent mortality 48 hours after treatment with Orthene 75S.

Treatment	Larval Instar II	III	IV	V
5 μg/μl	98	99	98	47
2.5 μg/μl	99	98	78	33
1.25 μg/μl	97	88	44	6
0.62 μg/μl	80	58	13	1
0.312 μg/μl	52	25	2	0
0.156 μg/μl	19	0	0	0
0.078 μg/μl	0	0	0	0
Acetone Check	0	0	0	0
Untreated Check	0	0	0	0

Table 3. Percent mortality 10 days after treatment with Dimilin 25 W.

Treatment	Larval Instar II	III	IV	V
0.078 μg/μl	99	83	12	15
0.039 μg/μl	98	39	6	7
0.019 μg/μl	95	21	2	7
0.009 μg/μl	81	3	1	1
0.004 μg/μl	25	0	0	0
0.002 μg/μl	7	0	0	0
Acetone Check	0	0	0	0
Untreated Check	0	0	0	0

Project Number: GM 7.1.1
Project Title: Laboratory Screening of Candidate Pesticides Against the Gypsy Moth
Report Period: April 1, 1977 - September 30, 1977
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 potentially useful against the gypsy moth, evaluating this data and selecting suitable materials for field testing. All of the tests herein reported were conducted by standard techniques with oak seedlings and 2nd instar larvae.

Two new growth regulators (Bay Sir 6874 and 8514) were compared with Dimilin 25 WP.

Material	Company	Dosage 1/	Weather	% Mortality	Days After Treatment
Bay Sir 6874	Chemagro	0.25		97	9
Dimilin 25 W	Thompson-Hayward	0.25		92	9
Bay Sir 6874		0.125		97	9
Dimilin 25 W		0.125		97	9
Bay Sir 6874		0.06		100	9
Dimilin 25 W		0.06		100	9
Bay Sir 6874		0.03		100	9
Dimilin 25 W		0.03		100	9
Bay Sir 6874		0.015		100	9
Dimilin 25 W		0.015		100	9
Bay Sir 6874		0.0078		86	9
Dimilin 25 W		0.0078		88	9
Bay Sir 6874		0.0039		89	9
Dimilin 25 W		0.0039		91	9
Check		No Treatment		0	9

When Bay Sir 6874 was mixed with crop oil supplied by Chemagro, treated plants showed signs of phytotoxicity. This was mostly observed when applications of a gallon per acre were applied.

Material	Company	Dosage	Weather	% Mortality	Days A-- Treatm-
Bay Sir 8514-25W	Chemagro	0.25		100	9
Dimilin 25 W	Thompson-Hayward	0.25		99	9
Bay Sir 8514-25W		0.125		98	9
Dimilin 25 W		0.125		98	9
Bay Sir 8514-25W		0.06		97	9
Dimilin 25 W		0.06		99	9
Bay Sir 8514-25W		0.03		100	13
Dimilin 25 W		0.03		100	13
Bay Sir 8514-25W		0.015		100	13
Dimilin 25 W		0.015		100	13
Bay Sir 8514-25W		0.0078		100	13
Dimilin 25 W		0.0078		100	13
Bay Sir 8514-25W		0.0039		100	12
Dimilin 25 W		0.0039		98	12
Bay Sir 8514-25W		0.0019		98	12
Dimilin 25 W		0.0019		100	12
Bay Sir 8514-25W		0.0009		95	12
Dimilin 25 W		0.0009		100	12
Bay Sir 8514-25W		0.0004		52	11
Dimilin 25 W		0.0004		55	11
Bay Sir 8514-25W		0.0002		32	11
Dimilin 25 W		0.0002		64	11
Bay Sir 8514-25W		0.0001		22	11
Dimilin 25 W		0.0001		18	11
Check		No Treatment		0	13
Bay Sir 8514-EC	Chemagro	0.25		96	12
Dimilin 25 W	Thompson-Hayward	0.25		98	12
Bay Sir 8514-EC		0.12		100	12
Dimilin 25 W		0.12		98	12
Bay Sir 8514-EC		0.06		98	12
Dimilin 25 W		0.06		97	12
Check		No Treatment		0	12

Material	Company	Dosage	Weather	% Mortality	Days After Treatment
Bay Sir 8514-TECH	Chemagro	0.25		100	14
Dimilin 25 W	Thompson-Hayward	0.25		95	14
Bay Sir 8514-TECH		0.12		100	14
Dimilin 25 W		0.12		94	14
Bay Sir 8514-TECH		0.06		100	14
Dimilin 25 W		0.06		99	14
Bay Sir 8514-TECH		0.03		100	13
Dimilin 25 W		0.03		94	13
Bay Sir 8514-TECH		0.015		100	13
Dimilin 25 W		0.015		99	13
Bay Sir 8514-TECH		0.0078		100	13
Dimilin 25 W		0.0078		99	13
Check		No Treatment		0	14

Material	Company	Dosage 2/	Weather	% Mortality	Days After Treatment
Bay Sir 8514-TECH	Chemagro	0.06		93	9
Bay Sir 8514-TECH		0.03		89	9
Bay Sir 8514-TECH		0.015		91	9
Bay Sir 8514-TECH		0.0078		85	9
Bay Sir 8514-TECH		0.0039		92	9
Bay Sir 8514-TECH		0.0019		93	9
Check		No Treatment		0	9

Material	Company	Dosage 1/	Weather	% Mortality	Days After Treatment
Bay Sir 8514-25W	Chemagro	0.06	1"	100	13
Dimilin 25 W	Thompson-Hayward	0.06	1"	94	13
Bay Sir 8514-25W		0.03	1"	93	13
Dimilin 25 W		0.03	1"	99	13
Bay Sir 8514-25W		0.015	1"	100	13
Dimilin 25 W		0.015	1"	97	13
Check		No Treatment	1"	0	13

Material	Company	Dosage 1/	Weather	% Mortality	Days After Treatment
Bay Sir 8514-25W	Chemagro	0.06	3"	98	10
Dimilin 25 W	Thompson-Hayward	0.06	3"	96	10
Bay Sir 8514-25W		0.03	3"	100	10
Dimilin 25 W		0.03	3"	100	10
Bay Sir 8514-25W		0.015	3"	95	10
Dimilin 25 W		0.015	3"	94	10
Check		No Treatment	3"	0	10
Dimilin 25 W	Thompson-Hayward	0.06		95	15
Dimilin-OD		0.06		98	15
Dimilin 25 W		0.03		99	15
Dimilin-OD		0.03		98	15
Dimilin 25 W		0.015		100	15
Dimilin-OD		0.015		96	15
Dimilin 25 W		0.0078		100	15
Dimilin-OD		0.0078		91	15
Dimilin 25 W		0.0039		100	15
Dimilin-OD		0.0039		88	15
Check		No Treatment		0	15
FMC-47628-TECH	FMC	0.025		97	8
FMC-47628-TECH		0.125		88	8
FMC-47628-TECH		0.062		71	8
FMC-47628-TECH		0.031		10	8
FMC-47628-TECH		0.015		1	8
Check		No Treatment		0	8
FMC-50215-TECH	FMC	1.0		100	7
FMC-50215-TECH		0.5		100	7
FMC-50215-TECH		0.25		100	7
FMC-50215-TECH		0.125		100	7
FMC-50215-TECH		0.062		92	7
FMC-50215-TECH		0.031		48	6
FMC-50215-TECH		0.015		10	6
FMC-50215-TECH		0.007		0	6
Check		No Treatment		0	7

Material	Company	Dosage	Weather	% Mortality	Days After Treatment
FMC-50215-TECH	FMC	0.5	1"	100	6
FMC-50215-TECH		0.25	1"	99	6
FMC-50215-TECH		0.12	1"	94	6
FMC-50215-TECH		0.06	1"	63	6
FMC-50215-TECH		0.03	1"	44	6
Check		No Treatment	1"	0	6

1/ Applied at 1 gallon/acre

2/ Applied at 1 quart/acre

The number of tests conducted was somewhat limited due to field work and a lack of submitted candidate insecticides. However, the new growth regulators from Chemagro looked as good as Dimilin in laboratory testing and that company will be looking to develop Bay Sir 8514. However, at this time not enough work has been conducted with the material to warrant field testing in 1978 using aerial application.

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: April 1, 1977 - September 30, 1977
Report Type: Interim
Project Leaders: Robert G. Reeves, P. Yelton

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

Methods and Materials

Diet: The diet used in these tests was Yamamoto's Tobacco Hornworm diet which has been modified for gypsy moth. Two to 3.5 l batches of diet were blended in a Waring blender and poured into 2- or 3-ounce Solo cups. These cups were put into a 16 ounce (paper can) cup and 15 larvae were infested into each 16 ounce container.

Insecticide solution: Stock solutions of insecticides were made by weighing 25 grams of each insecticide into a 1 l Erlenmeyer flask and diluting to 600 ml with water. All dilutions of the pesticide for diet incorporation were taken from the stock solution; the solutions were not used after they had been stored for 1 week.

Contamination of diets: Insecticide stock solutions were diluted with water and acetone at concentrations to yield the desired ppm or ppb of the pesticide in the diet. The concentration level of insecticide was based on total weight of the diet and appropriate dilutions were incorporated into the diet with a burette while the diet was blending.

Results

Table # 1 presents results from tests completed to the point that adult moths have emerged, mated and egg masses are in storage. Erratic results were obtained from tests with all 4 insecticides because the diluted insecticide settled to the bottom of the burette prior to being incorporated into the diet. Originally, the dilutions were made with water, but we found that the addition of acetone to each dilution enhanced uniform insecticide distribution.

Observations

1. Sevin 80S - All concentrations of Sevin in diets resulted in large numbers of larvae becoming infected with virus, therefore, several tests had to be repeated. At dosages yielding mortality rates greater than 70%, larvae molting from fourth to fifth instar could not shed their head capsules or the exuvia from the posterior section of their bodies.

2. Dylox 80S - Larvae exposed to Dylox in their diet have significantly softer than normal setae. Concentrations of Dylox that cause more than 75% mortality result in incomplete, deformed pupae and inhibited emergence of adult moths.

3. Orthene 75S - Orthene concentrations in the diet causing more than 75% mortality result in rampant cannibalism. Surviving larvae devour the dead as quickly as they die. Survivors undergo incomplete molts from 4th to 5th and 5th to 6th instar by retaining their head capsules. The larvae are very fragile, much smaller than normal and retain more body setae than the controls. The resulting pupae are smaller than controls, and male and female pupae are virtually identical in size.

4. The Dimilin tests have been repeated because of the aforementioned problems of the insecticde settling in the burette. Survivors of the 80% mortality treatment prey upon insects that reach the pupal stage. The setae on the larvae are hard and very sharp and larval mortality resembles that caused by virus.

Table 1.

Sevin 80S

Instar	ppm	% Mortality
I	1.5	9
	4.0	39
	5.0	61
II	8	20
	10	36
	12	60
III	3	24
	7	20
	10	10
	12	80
	14	94
Orthene 80S		
I	0.5	10
	1.0	14
	2.0	16
	2.5	32
	3.0	58
II	4.0	59
	5.0	60
III	1.5	11
	5.0	69
	6.0	91
Dylox 80S		
I	1.5	8
	3	55
	4.5	12.5
	5	30
II	7	60
	8	96
	9	76
	10	92
III	7	81

Table 1 cont'd.

Dimilin 25 WP

Instar	ppb	% Mortality
I	0.01	71
	0.05	23
	0.1	9
	0.15	17
	0.25	7
	0.25	22
II	0.4	14
III	0.25	12

Project Number: GM 7.1.3
Project Title: The Effects of pH and Temperature on the Chemical Stability and Toxicity of Dimilin, Dylox, Carbaryl and Orthene
Report Date: April 1, 1977 - September 30, 1977
Report Type: Preliminary
Project Leaders: Robert G. Reeves, J. Yarko

The objectives of this project are to determine the stability of Dimilin 25 W, Dylox 80S, Orthene 75S and Sevin 80S at various temperatures and pH values and to determine the pesticidal value of each insecticide at 25 and 50% hydrolization against gypsy moth larvae.

Methods: Each insecticide will be mixed in water with the pH varying from 5 10 and exposed to temperatures from 15°C to 35°C. The half life of each mixture will be determined using gas chromatographic analytical procedures. The pesticidal value will be established for each chemical using oak seedlings and 2nd instar gypsy moth larvae.

Preliminary Work: The extractions and analytical procedures for Sevin 80S have been completed and work is in progress with this insecticide.

Project Number: GM 7.1.7
Project Title: 1977 Field Studies of Dimilin W-25
Report Period: April 1, 1977 - September 30, 1977
Report Type: Interim
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.

Conclusions:

At the time of this writing, prism point fall egg mass counts are still being collected. All data will be tabulated in final form in the next laboratory report. However, preliminary data are presented in attached Table 1.

Visual defoliation estimations were made at pre-spray and at peak defoliation in increments of 20%. In all cases both pre-spray and post spray estimates fell within the 0-20% defoliation range.

Each treatment plot evaluated for eradication had 200 burlap bands distributed in the center 10 acres (total of 600 bands per treatment). Table 2 gives the totals for each treatment. It should be noted that the 26 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 2 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.

Leaf and duff samples were collected both pre- and post-spray for chemical analysis.

In brief summary, it appears that all applications of Dimilin may give a 100% reduction in egg masses.

Table 1. 1977 Prism Point Egg Mass Counts, Pike County, PA.

Dimilin Dosage	# of Applications	Plot #	# Spring Egg Masses/A	# Fall Egg Masses/A
0.06 lb/gal/A	1	1	870	0
		7	421	0
		30	843	- 1/
0.06 lb/gal/A	2	10	1824	0
		13	2610	0
		25	929	-
0.03 lb/gal/A	2	11	2257	0
		20	1053	0
		27	2340	-
0.03 lb/gal/A	3	16	891	0
		17	297	0
		22	719	0
0.06 lb/.5 gal/A	2	8	1201	0
		24	1203	0
		26	2487	-
0.03 lb/.5 gal/A	2	3	823	0
		5	1135	0
		18	564	0
0.03 lb/.5 gal/A	3	9	2402	0
		15	1286	0
		21	1089	0
0.06 lb/.5 gal/A	1	6	957	0
		14	1287	0
		29	944	-

Table 1 cont'd.

Dimilin Dosage	# of Applications	Plot #	# Spring Egg Masses/A	# Fall Egg Masses/A
0.03 lb/.5 gal/A	1	2	719	0
		4	1852	0
		12	3217	-
		19	1193	-
		23	893	0
		28	1187	-
Checks		1	245	213
		2	476	345
		4	1254	2109
		5	4686	2147
		6	3200	1194
		7	751	275
		10	1519	-
		3	2839	-
		8	1969	-
		9	3000	-

1/ Blanks indicate data not yet collected.

Table 2. 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

Project Number: GM 7.1.9
Project Title: 1977 Field Evaluation of 5 Insecticides Applied by Ground Equipment.
Report Period: April 1, 1977 - September 30, 1977
Report Type: Final
Project Leaders: Larry L. Herbaugh, W. H. McLane, J. A. Finney

Each year Animal and Plant Health Inspection Service personnel treat campground trailer parks and other hazardous sites for the control of gypsy moth larvae. Many of the treatments (largely Carbaryl) are applied with ground equipment (mist blowers). During the past year, two new insecticides, Dimilin and Orthene have been registered for gypsy moth control. It is necessary that minimum effective dosages be established to maximize control with minimal environmental contamination. Registered pesticides and new materials that show promise in controlling gypsy moth larvae in laboratory tests must be adapted to effective field use.

The test herein described had the following objectives:

1. Determine the minimal effective dosage of Sevin 80S, Orthene 75S and Dimilin 25 WP for regulatory control of gypsy moth larvae when applied with ground equipment.

2. Determine the insecticidal value for gypsy moth control of FMC 45498 (Decis) and Pydrin applied with ground equipment.

Methods: One hundred and ten plots were established along remote roads in a predominantly oak forest containing a healthy population of gypsy moth eggs in Pike County, PA. The spray plots were 200 feet wide and 200 feet long with a small dirt road dividing them. Within each one acre plot, two 1/40 acre subplots or sampling units were established, one on each side of the road. Ribbons were placed near the road at both ends of the 200 foot plots with tags identifying each plot. The two subplots were tagged for identification and all data were collected from them.

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

Conclusions: Each treatment was evaluated from the standpoint of larval and egg mass reduction and foliage protection. Defoliation estimations were made by visual examination at the termination of larval feeding. Those results are summarized in Tables 1 through 6.

The proposed objective of the tests with Sevin 80S and Orthene 75S was to determine the minimum effective dosage that results in 95% larval reduction. This degree of control was achieved in both 1976 with 3 replicates and again in 1977 with 2 replicates at 2.00 lbs. active ingredient per acre mixed with 5 gallons of water for Sevin 80S. Orthene 75S, under the same circumstances, gave 95% or better larval mortality at 1.50 lbs. active ingredient per acre mixed with 5 gallons water.

Dimilin was used under an experimental use permit that would establish a dosage response to give 95 control of gypsy moth larvae. As one can see in Table 3, this was not accomplished at any of the applied rates with 4 replicates of each. However, it should be noted that due to the high level of infestation and the slower mode of action with growth inhibitors, migration into one acre plots was inevitable.

FMC 45498 and Pydrin were tested to establish their pesticidal value. Based on the standard of 95 or better larval control, this was accomplished with FMC 45498 at 0.025 lbs. active ingredient per acre mixed with 5 gallons of water. Pydrin attained the same goal at 0.025 lbs. as well as at the minimum dosage of 0.003 lbs. ai/acre. It should be noted that the lower dosage did not give the foliage protection nor the egg mass reduction that 0.025 lbs. ai/acre did.

To summarize the tables very briefly, all materials tested with the exception of Dimilin showed a 95% gypsy moth larval control when applied to one acre forested plots with a mist blower.

Table 1. Sevin 80S, 1977 (2 replicates):

Dosage lbs ai/A	Avg.# Spring Egg Masses/A 2/	Avg. # Fall Egg Masses/A 2/	% Change	Average # Pre-Spray Larvae 3/	Average # Post-Spray Larvae 4/	% Change	% Defoliation
0.12 1/	750	40	95	41	52	127	80 - 100
0.25	1100	140	87	30	28	7	80 - 100
0.50	2380	620	74	22	11	50	50 - 70
1.00	1750	90	95	15	5	67	20 - 40
1.50	2300	30	99	14	4	71	0 - 20
2.00	1900	0	100	27	1	96	10 - 30
Sevin 80S, 1976 (3 replicates):							
0.12	-	-	-	-	-	-	-
0.25	1000	713	29	26	5	81	80 - 100
0.50	1046	326	69	22	2	91	20 - 40
1.00	1653	80	95	23	2	91	40 - 60
1.50	1663	126	92	22	1	95	40 - 60
2.00	1373	40	97	26	1	96	0 - 20

Table 2. Orthene 75S, 1977 (2 replicates):

0.12	885	40	95	44	11	75	55 - 75
0.25	2640	10	99	37	3	92	20 - 40
0.50	1820	80	96	37	4	89	10 - 30
1.00	1820	30	98	64	1	98	40 - 60
1.50	3140	30	99	20	1	95	0 - 20
2.00	2700	130	95	24	1	96	0 - 20

Table 2 cont'd. Orthene 75S, 1976 (3 replicates):

Dosage lbs ai/A	Avg.# Spring Egg Masses/A	Avg. # Fall Egg Masses/A	% Change	Average # Pre-Spray Larvae	Average # Post-Spray Larvae	% Change	% Defoliation
0.12	-	-	-	-	-	-	-
0.25	606	453	25	29	6	79	0 - 20
0.50	640	526	18	25	3	88	0 - 20
1.00	480	386	20	26	2	92	0 - 20
1.50	533	113	79	29	0	100	0 - 20
2.00	446	33	93	22	0	100	0 - 20
Table 3. Dimilin 25 WP, 1977 (4 replicates):							
0.001	3308	0	100	88	9	90	80 - 100
0.002	1900	0	100	109	37	66	80 - 100
0.005	3218	0	100	73	121	166	80 - 100
0.01	2745	0	100	82	72	12	80 - 100
0.03	2348	0	100	82	66	20	75 - 95
0.06	2015	0	100	48	61	127	70 - 90
Table 4. FMC 45498, 1977 (4 replicates):							
0.003	3185	175	95	60	20	77	50 - 70
0.006	3781	35	99	37	5	86	25 - 45
0.012	3360	20	99	22	7	68	0 - 20
0.025	1570	0	100	36	1	97	10 - 30
0.05	1995	35	98	30	1	97	15 - 35
0.10	1717	95	94	12	0	100	0 - 20

Table 5. Pydrin, 1977 (4 replicates):

Dosage lbs ai/A	Avg.# Spring Egg Masses/A	Avg. # Fall Egg Masses/A	% Change	Average # Pre-Spray Larvae	Average # Post-Spray Larvae	% Change	% Defoliation
0.003	1030	145	86	20	4	98	40 - 60
0.006	1345	45	97	61	21	66	50 - 70
0.01	1115	110	99	32	6	81	30 - 50
0.025	2768	20	99	79	1	99	15 - 35
0.05	2100	5	99	69	1	99	30 - 50
0.10	2650	5	99	86	1	99	35 - 55

Table 6. Average of 10 Untreated Plots (1977)

Checks	2007	236	88	60	63	105	76 - 96

1/ All dosages in lbs. active ingredient per 5 gallons H_2O per acre.

2/ Egg masses per acre based on 1/40 acre actual counts.

3/ Based on number of larvae within 3 feet of a chest high 15 foot string.

4/ Same as 3 but taken 5 days after spray.

5/ All pre-spray defoliation was 0-20%.

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

In the last report (October, 1976 - March, 1977) we reported on the development and calibration of a Porapak Q system for collecting airborne pheromone. Most of those experiments were conducted with Hercon dispensers or other types of disparlure evaporating devices. Collection efficiency was ca. 70% (70% of released pheromone was collected in the Porapak trap). Subsequently, large numbers of females, both singly and in aggregate were tested in the apparatus under constant temperature (26°C) and 16L:8D photoperiod. At least 300 female moths have been tested and pheromone has never been detected. The level of sensitivity is estimated to be 200 ng released/collection period; many females were tested for emission for 3-4 days.

We have recently abandoned this approach temporarily and have initiated experiments to quantify the pheromone contained within the pheromone gland. The technique was quite simple, procedurally. Pooled samples of 5-10 excised abdominal tips were soaked in 5 ml CH_3Cl for 10 minutes, filtered through glass wool and rewashed with three 2 ml aliquots of CH_3Cl. The extract was concentrated to 0.5 ml for GLC analysis. No cleanup procedures were required. Excessive vortexing of the tissue in the solvent resulted in increased contamination and, therefore, occasional gentle swirling was the only mixing used. Similar procedures applied to appendages and heads of female moths did not reveal peaks similar to disparlure.

In the first experiment, tips from female moths (NJ_{15}) of known age were analyzed for disparlure. Results are in Table 1.

Table 1. Disparlure content in abdominal tips of female moths aged 6-90 hours.

Age (hrs)	# Females	ng Collected	$\bar{x}$ (ng)
6	20	196	9.8
16	20	152	7.6
24	20	0	0
48	15	196	13.0
58	10	148	14.8
68	20	333	16.7
80	10	304	30.7
90	10	236	23.6

The data indicate that pheromone content increases as insect age increases and drops after 80 hours. However, the time of the day in which extractions were conducted is not known and, as is shown later in this report, there is a strong diurnal periodicity of pheromone content in abdominal tips.

Another experiment tested the relative efficiencies of acetone, hexane and methylene chloride as solvents for extracting disparlure. Minor contaminant peaks (none interfering with disparlure) were observed with 1 hour soaking in CH_3Cl. Large interfering peaks occurred in the acetone and hexane injections. However, the solvent peak (CH_3Cl) on the resultant chromatograms was often large, reducing the sensitivity of the analytical procedure and another test was run comparing CS_2 and CH_3Cl at two different soaking times.

Table 2. Comparison of disparlure extraction efficience of carbon disulfide and methylene chloride.

Solvent	Extraction Time	# Tips (PA)	ng Collected	Contaminant Peaks
CS_2	5 minutes	10	288	2
CS_2	30 minutes	10	263	3
CS_2	-	-	-	2
CH_3Cl	5 minutes	10	561	0
CH_3Cl	30 minutes	10	500	0
CH_3Cl	-	-	-	0

Analysis of the CS_2 samples revealed much smaller solvent peaks, but the reduced recovery (compared to CH_3Cl) dictated the selection of CH_3Cl as the working solvent. Also, 5 minutes was chosen as the standard extraction time for future tests. Future testing may lead to drying the CH_3Cl extract and reconstituting with CS_2.

In an experiment designed to determine the periodicity of pheromone content in tips, over 120 female moths were collected between 0900 and 1500 and held at 26°C with 16L:8D photoperiod. At regular intervals thereafter, duplicate samples of 8-10 pooled tips were clipped and analyzed for disparlure. The results of the experiment are shown in Figure 1. There is clearly a strong periodicity of pheromone content during the first 60 hours post-emergence. Peak content consistently was at 2000 hours, and declined thereafter until 0400-0800. Approximately twice as much disparlure was recovered from females 58 hours old than from those 32 hours old possibly indicating intensified calling behavior. A further experiment is in progress to determine if pheromone content increases until 2200 hours when scotophase sets in. From these data, it appears that photoperiod is the triggering mechanism for pheromone production.

Obviously, the pattern of tip pheromone content cannot be translated into emission profiles. In fact, peak attractancy in the field occurs at ca. 1200 hours at a time when disparlure concentrations in the gland are rather low. These relationships will be the subject of future experiments. Also, we will be determining the effects of altered photoperiod, mating and age on the pheromone profiles.

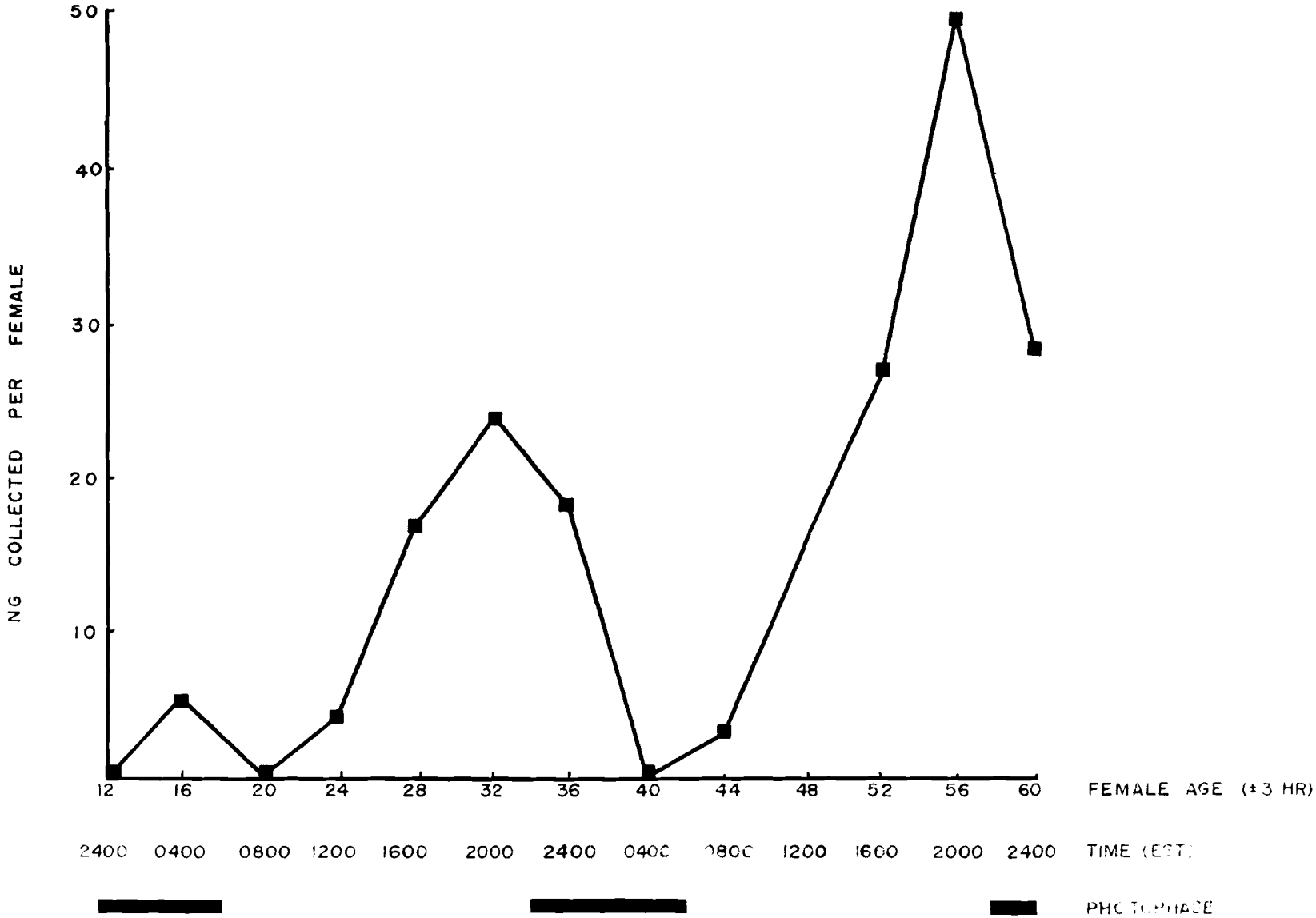


Figure 1. [illegible] female [illegible] abdominal [illegible] and time of day.

Project Number: GM 6.2.8
Project Title: Field Evaluation of Controlled Release Formulations of Disparlure for Mating Disruption
Report Period: April 1, 1977 - September 30, 1977
Report Type: Interim
Project Leaders: Charles P. Schwalbe, E. C. Paszek

A manuscript is currently in draft form and upon finalization will be submitted to the Annals of the Entomological Society of America and included as a Final Report in the Laboratory Report.

Project Number: GM 7.1.1
Project Title: Efficiency of (+) Disparlure-Baited Traps in [illegible] Estimating Male Moth Density and Delimitation of the [illegible] Infestation
Report Period: April 1, 1977 - September 30, 1977
Report Type: Final
Project Leaders: Charles P. Schwalbe, E. C. Paszek

Introduction:

Gypsy moth adult detection surveys are normally conducted in areas outside the regulated zone by placement of pheromone-baited survey traps in high risk (recreational) sites or on grid patterns with trap densities ranging from 1 trap/3 square miles to 1 trap/45 square miles. When a male moth is captured in such a detection trap, additional traps are deployed in an array surrounding the "find" for the purpose of confirming the presence of an infestation and delimiting its range. Currently, trap density in these grids is 9 traps/square mile. Since it is likely that a significantly more attractive lure (comprised of the (+) enantiomer of disparlure) will be used in future survey programs, it is necessary to determine what grid pattern of traps will give a reliable sample of the population within the array.

Factors which are expected to influence the sampling efficiency of traps in arrays are (1) pheromone release rate (PRR) and (2) trap spacing or intertrap distance (ITD). Array efficiency can be measured by determining the percentage of male moths that are captured in the traps after release. Theoretically, as PRR increases and ITD decreases, a point will be reached where recovery of released moths is significantly reduced due to confusion or some form of trap interaction. Conversely, as PRR decreases and ITD increases, recovery is again expected to decline because less of the area under survey will be within the "active space" of the trap.

Objectives:

1. Determine the PRR and ITD of traps in arrays that recovers the highest percentage of released male moths, thus giving the highest probability of detection and best sample for estimating male moth density. Relationships among male moth capture, PRR and ITD will be determined.

2. Determine if the pattern of moth capture in test plots reflects the location at which moths were released.

3. Compare the relative attractancy of (+) and (±) disparlure-baited traps in arrays.

Test plots were established in a mixed hardwood woodland in the Freetown Fall River State Forest, Watappa Reservation section of the Fall River Watershed and adjacent private land belonging to Acushnet Sawmils, Cranberry Corporation of America, Clarence Clark, and David McLaughlin. The native moth population was very low in these areas.

Twelve plots were set up in 3 arrays, one treatment in each array. The smallest 4 plots were 49 ha, gridded in an 8x8 pattern with 64 traps space 87 m apart. The middle size plots were 110 ha, gridded in a 6x6 pattern w 36 traps spaced 175 m apart. The largest were 196 ha, gridded in a 4x4 pa tern with 16 traps spaced 350 m apart. The minimum distances between plots was 0.8 km. Traps were baited with 10, 100 or 1000 μg (+) or 2000 μg (±) d parlure. All traps within a plot contained similar baits and no keeper wa used.

Standard Delta traps were freshly made up in the laboratory and baited wit dental roll cotton wicks clipped with a hairpin that was pushed through th wall of the trap. Traps were made up in separate groups to prevent cross-contamination and sealed in disposable plastic bags. They were initially put out 7/6/77 and replaced with new wicks on 7/12/77 - 7/13/77 and again 7/19/77 - 7/20/77. They were removed 7/27 - 7/29 at which time they were r turned to the laboratory for analysis of residual disparlure.

Male pupae were field collected from areas of building populations in easte Massachusetts, Pennsylvania and New York. They were held outdoors in well-ventilated screenhouses in aggregates of 50 in 16 ounce squat cups with cle plastic covers. Emerged moths were collected daily at 6-7 AM and transferr to a cool chamber (5-8°C) where they were marked on the wings with Rhodamin B dye. They were immediately placed in sytrofoam coolers packed with coola and transported to the release sites. Moths were released every other day the 12 plots and traps were checked each day following release. A record w made of the number marked and unmarked moths captured. Day of release was coded by marking the right, left or both wings. If more than 10 moths were captured in a trap, the cotton wick was transferred to a new trap which replaced the old one.

A total of 8 releases were made over a period of 16 days (7/6 - 7/21) in ea of the 12 plots. The average number of moths per release was 140 and appro imately 13,000 moths total were released in the test.

Results:

The recovery in traps of released insects is shown in Table 1. Clearly, as the distance between traps increases, percent recovery decreases. Also, th highest recapture rates were generally obtained in plots with traps baited with 100 μg (+). This diminution of effectiveness with pheromone concentra tions greater than 100 μg was more clearly demonstrated in tests reported elsewhere. In nearly all cases, traps baited with the (+) enantiomer of d parlure caught more moths than those baited with the racemic mixture. If t released moths in these tests are behaviorally similar to those which eclos and disperse naturally in sparse populations in the field, then the values Table 1 represent the probability of detection of male moths in the corresp ing arrays. Meteorlogical conditions, sex ratios, mating histories, predat and other variables are likely to affect percent capture. Furthermore, al moths were released from a central point of maximum distance from traps. they had been randomly released within the grids, the percent recovery wou likely be greater.

Table 1. Percent Recovery of Male Moths Released into Traps [illegible] Fall River, 1977.

		ATTRACTANT (μg)			
		10 (+)	100 (+)	1000 (+)	2000 (+)
ITD	88	21.5	35.4	27.0	9.4
	175	8.6	7.9	16.7	1.1
	350	2.8	5.1	0.6	0.9

The pattern of capture of moths in a representative plot (PRR = 100 μg +; ITD = 175 m) is shown in Figure 1. In all cases, most males were captured in traps closest to the point of release and the pattern accurately reflects the point of origin. The distribution of all captured insects in 12 test plots is given in Table 2. The highest recovery was in the grid with ITD = 87 m and PRR = 100 μg (+). There, 22.7% of the released moths were captured in the 4 traps 64 m from the central release point. As mentioned above, recovery decreases when traps are widely spaced. However, even in plots with ITD = 350 m, most moths are caught in the 4 center traps closest to the release point.

Data from these experiments also relate to the dispersal behavior of the gypsy moth. In Table 3, the distribution of captured moths in each of the 4 quadrants of the test plots is presented. Generally, more moths are recovered in the SW quadrant than in any of the other 3 plot areas. The "prevailing" wind during these tests was from the SW, however, large numbers of insects were captured "down wind", indicating that there may be a significant amount of non-oriented dispersal following release. Meteorlogical conditions in a forest environment are extremely variable and anemotactic flight would tend to be random. It should also be noted that, although the numbers are rather small, distribution in "racemic" plots is quite random and does not tend toward larger recovery in the SW quadrant as do most (+) plots.

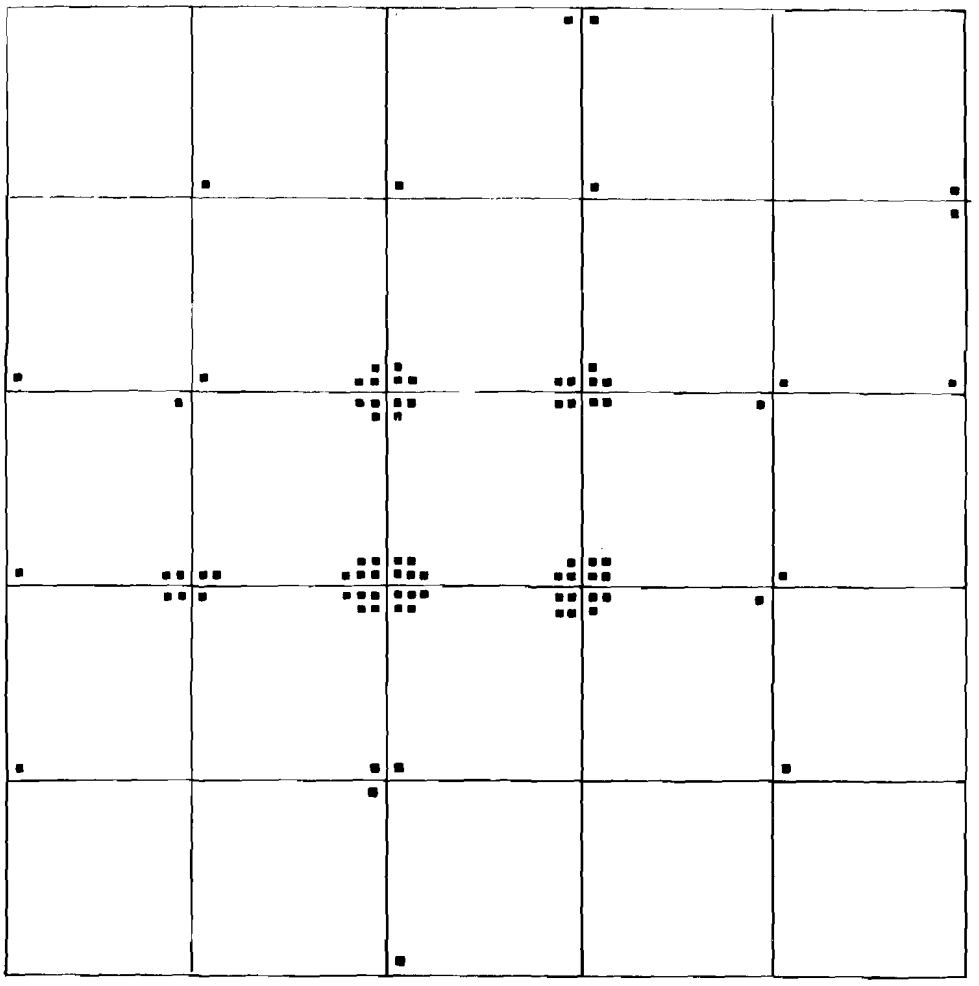


Figure 1.
Pattern of capture of male gypsy moths released centrally arrays of traps baited with 100 μg (+) disparlure. ITD = 175m. Fall River, MA, 1977.

Table 2. Distribution of Male Gypsy Moths Released into the Center of 3 Grids of Traps, 8x8, 6x6 and 4x4, Baited With the Following Lures:

	Distance From Release Point	2000 µg ±		1000 µg +		100 µg +		10 µg +	
		No. Males Captured	% of Released Males Captured	No. Males Captured	% of Released Males Captured	No. Males Captured	% of Released Males Captured	No. Males Captured	% of Released Males Captured
ITD = 87m (8x8)	64	48 (4) 1/	4.00	153 (4)	12.75	239 (4)	22.74	114 (4)	10.84
	135	27 (8)	2.25	59 (8)	4.91	62 (8)	5.89	48 (8)	4.56
	187	7 (4)	0.58	19 (4)	1.58	10 (4)	0.95	16 (4)	1.33
	223	10 (8)	0.83	31 (8)	2.58	26 (8)	2.47	17 (8)	1.61
	256	9 (8)	0.75	24 (8)	2.00	19 (8)	1.80	10 (8)	0.95
	307	6 (12)	0.50	19 (12)	1.58	8 (12)	0.76	9 (12)	0.85
	336	5 (8)	0.41	10 (8)	0.83	1 (8)	0.38	6 (8)	0.57
	377	3 (8)	0.25	8 (8)	0.66	2 (8)	0.19	5 (8)	0.47
	436	0 (4)	0	1 (4)	0.08	3 (4)	0.28	1 (4)	0.09
ITD = 175m (6x6)	116	8 (4)	0.66	107 (4)	8.91	55 (4)	5.23	74 (4)	7.04
	277	2 (8)	0.16	55 (8)	4.58	18 (8)	1.71	7 (8)	0.66
	372	0 (4)	0	8 (4)	0.66	2 (4)	0.19	1 (4)	0.09
	448	2 (8)	0.16	16 (8)	1.33	6 (8)	0.57	2 (8)	0.19
	507	1 (8)	0.08	14 (8)	1.16	3 (8)	0.19	6 (8)	0.57
	617	1 (4)	0.08	3 (4)	0.25	0 (4)	0	1 (4)	0.04
ITD = 350m (4x4)	247	7 (4)	0.58	4 (4)	0.33	37 (4)	3.52	16 (4)	1.52
	550	4 (8)	0.33	4 (8)	0.33	13 (8)	1.23	10 (8)	0.95
	733	0 (4)	0	0 (4)	0	4 (4)	0.38	3 (4)	0.28

1/ Numbers in parentheses are the number of traps at the indicated distances from the release point.

Table 3. Distribution of Moths Caught in the 4 Quadrants of Test Plots. Fall River, 1977.

	Treatment	Total Moths Captured by Quadrant SW	SE	NW	NE	Plot Total	% Released Males Captured
ITD = 87 m	2000 μg ±	16	41	40	18	115	9.5
	1000 μg +	73	63	95	93	324	27.0
	100 μg +	158	132	39	44	373	34.5
	10 μg +	86	44	53	43	226	20.9
ITD = 175 m	2000 μg ±	2	4	5	3	14	1.16
	1000 μg +	74	30	51	48	203	16.9
	100 μg +	33	17	17	17	84	7.9
	10 μg +	30	11	42	8	91	8.6
ITD = 350 m	2000 μg ±	4	3	0	4	11	0.91
	1000 μg +	0	5	3	0	8	0.66
	100 μg +	23	15	10	6	54	5.13
	10 μg +	16	4	5	4	29	2.75

The capture of native male moths in traps in the test plots is shown in Table 4 and are used to illustrate that higher proportions of males are consistently caught in (+) baited traps than in those containing (±) disparlure. No other trends are immediately obvious in the data.

Table 4. Unmarked, Native Moths Captured in Traps in Arrays. Fall River, 1977.

ITD (m)	LURE (μg) 10 (+)	100 (+)	1000 (+)	2000 (±)
87	910	1731	2158	489
175	748	962	1461	671
350	517	1305	435	253

Project Number: GM 7.2.3
Project Title: Capture of Male Gypsy Moths by Traps in Operational Grid
Report Period: April 1, 1977 - September 30, 1977
Report Type: Final
Project Leaders: Charles P. Schwalbe, L. T. Mundy

Delta traps with Hercon wicks containing racemic disparlure are currently used for detection purposes (often in a non-random fashion) near areas of "high risk". When evidence of an infestation is discovered, it is necessary to re-survey (generally the following year) to confirm the presence of an infestation (indicated by the capture of additional male moths). Egg masses may or may not be found. Traps are normally placed ½ mile apart on grid patterns in these surveys.

With the anticipated implementation of the (+) enantiomer of disparlure as trap bait, it is necessary to determine what the probability of detection of traps is at distances operationally useful. It is assumed that closer grid patterns increase the probability of detection. Four traps per square mile (½ mile intertrap distance or ITD) is the "tightest" grid that can currently be logistically and economically tolerated. Therefore, this study was designed to determine what percent of male gypsy moths within a grid of traps (ITD = ½ mile) are captured.

OBJECTIVES:

1. Determine the relative efficiency of Delta traps distributed at operational arrays and baited with (+) and (±) disparlure.

2. Determine the probability of detection or percent capture of male gypsy moths by traps in operational grids.

A 5 mile x 5 mile forested and uninfested area in central Michigan was selected as the study site. Traps were placed ½ mile apart on a 10 x 10 grid pattern. For purposes of comparing the relative efficiency of the (+) enantiomer and racemic mixtures of disparlure, each trap site contained two traps. Each trap was baited with 100 μg of (+) disparlure or 200 μg (±) disparlure and separated by ca. 15-20 m at each site.

One-2 day old male moths (laboratory-reared NJ_{15}) were released into the centers of the 4 center sections (640 A). Moths were marked to designate point of origin and all traps were checked every other day. Approximately [illegible] males were liberated at each release and there were 16 separate releases (totalling 2369 moths) made during June 18-27. The results of this test are given in Table 1.

Table 1 Capture of male gypsy moths in traps with ITD = ½ mile and baited with 100 μg (+) or 200 μg (±) disparlure. Mt. Pleasant, MI, 1977.

	No. Captured	% Captured	Range
100 μg (+)	22	.92	(0 - 4.0%)
200 μg (±)	16	.67	(0 - 3.1%)

In these tests, there were only minor differences in catch between traps baited with (+) and (±) disparlure (.92% vs. .67% recovery, respectively). Additional testing will be necessary to determine if this diminution of attractancy of (+) at large distances from the release point is real or an artifact caused by the proximity (15-20 m) of the (+) and (±)-baited traps at each site.

It is illustrative to examine the distribution of captured insects within the study area as shown on Figure 1. For purposes of clarity, data from the 4 release sections have been pooled to a common release point indicated by X on the figure. Dashes indicate trap sites, open and closed circles represent moths captured in (+) and (±) baited traps, respectively. Most insects (74%) were captured in the square mile of release and only 26% of those captured migrated out of the section where they were released. This indicates that dispersal in such areas is not great. No insects were captured in traps "2 sections" away from the release point (not shown on Figure 1).

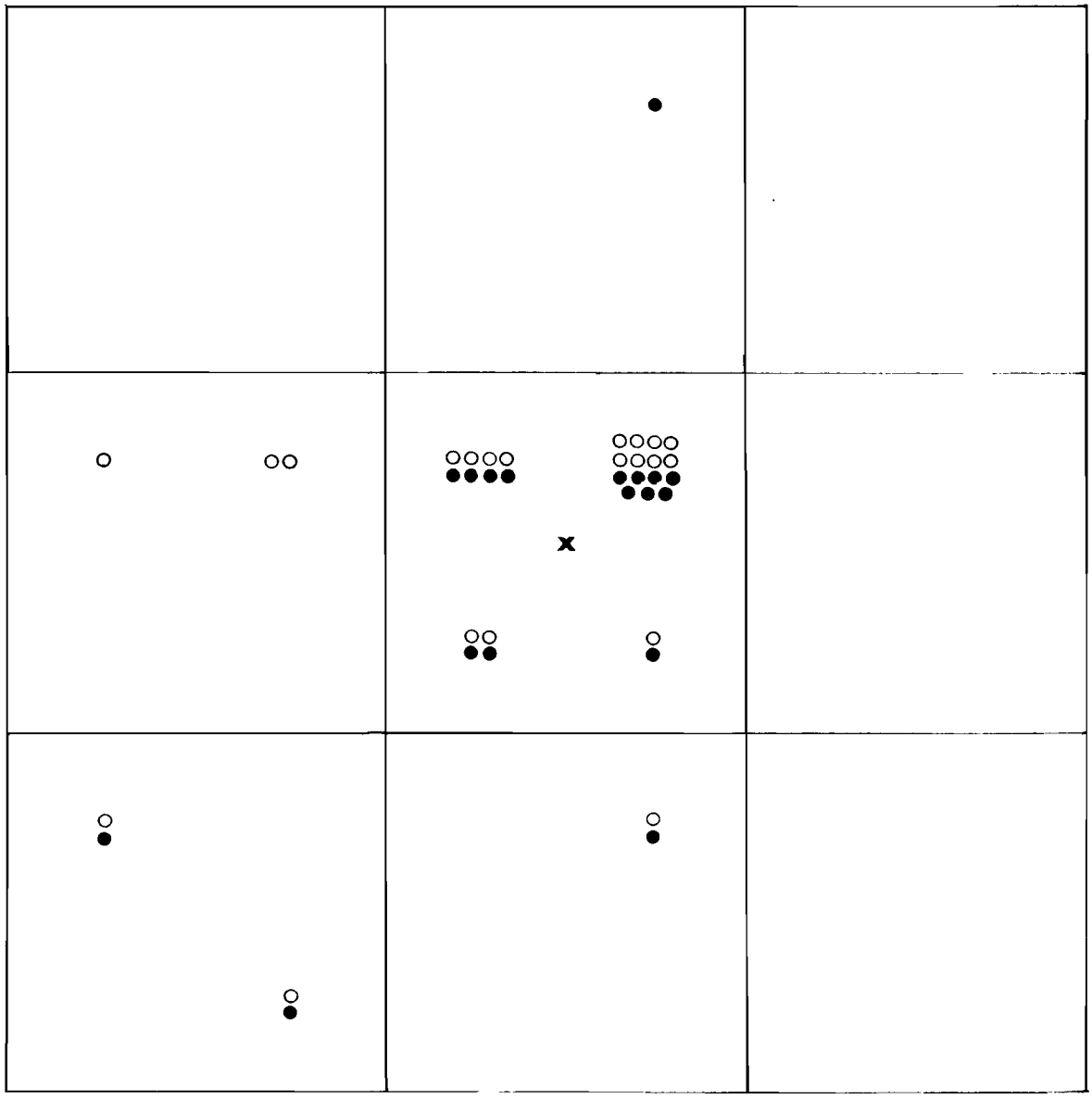
In another smaller test, 36 trap sites were established on a grid pattern over 1 square mile. Each trap site contained 2 traps (100 μg (+) and 200 μg (±)) separated by 20 m. Twelve hundred male moths were released in the center of the grid during 6-22 to 6-27. Captures are presented in Table 2.

Table 2. Capture of male moths centrally released in a grid of traps with ITD = 1000' (1200 male moths released).

	No. Captured	% Captured
100 μg (+)	152	12.7
200 μg (±)	45	3.8

In this experiment, ca. 3.4 times more moths were captured in (+)-baited traps than in those baited with (±) disparlure. Other tests in MA indicate that as ITD decreases, the differences in capture between (+) and (±)-baited traps increase.

MT PLEASANT, 1977



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: May 31, 1977 - October 1, 1977
Report Type: Interim
Project Leader: Victor C. Mastro

Introduction: Comparisons of the competitiveness of male gypsy moth populations or evaluations of mating disruption tests can, at the present time, only reliably be monitored with feral virgin female moths. Even this procedure may prove to be inadequate with possible differences in female quality from various wild populations. Additionally, the expense and logistics of these operations are burdensome.

The purpose of the work reported below was to develop a monitoring technique which could be used in the above types of tests. The specific objectives were to:

1. determine if females (laboratory reared and wild) placed in traps were comparable to feral females placed on the boles of trees
2. determine if synthetic pheromone baited traps were comparable to feral females placed on the boles of trees and
3. compare the eclosion, dispersal and sexual response of feral and laboratory strains of male gypsy moths.

Preliminary Testing: Preliminary studies before the natural flight season were designed to determine:

1. what synthetic pheromone concentration would be appropriate for baiting traps and
2. to evaluate different marking techniques for male gypsy moths.

Plots for these purposes were established using a 5x5 grid (50 meter spacing between points). The center point of the grid was used as a male release point and the remaining stations were randomly assigned the following treatments:

5 stations	1 μg (+) disparlure baited traps
5 stations	10 μg (+) disparlure baited traps
5 stations	100 μg (+) disparlure baited traps
4 stations	1000 μg (+) disparlure baited traps
5 stations	2000 μg racemic disparlure baited traps

Male moths (one day old, virgin) were released from the center of a plot for two consecutive days. The test was repeated once on two separate plots. All male moths used in this test were from a strain which has been maintained for fifteen generations in the laboratory. The original stock to establish the colony was collected in New Jersey. Hereafter this strain shall be referred to as NJ_{15}.

Results of the test described above are presented on Table 1.

Table 1. Number and Percentage of Male Gypsy Moth Captured Using Different Bait Concentrations of (+) Disparlure and Racemic Disparlure

Treatment (μg of cis-7,8-epoxy-2-methyloctadecane/trap)	Total No. Males Recovered Rep 1	Rep 2	$\bar{x}$ No. Males Recovered/Trap Rep 1	Rep 2
1 μg (+)	64	71	12.8	14.2
10 μg (+)	72	80	14.4	16.0
100 μg (+)	103	116	20.6	23.2
1000 μg (+)	52	54	13.0	13.5
2000 μg racemic	64	58	12.8	11.6

The data indicate a dose-response relationship exists when using (+) disparlure in a trap. Trap catch increased consistently for traps baited with one to one hundred micrograms of (+) disparlure. However, a decrease in catch was noted when traps were baited with one thousand micrograms. The performance of racemic disparlure in a trap was nearly as effective as the lowest and highest dosages of the (+) disparlure. The results of this test indicated a trap baited with 100 μg of (+) disparlure was best suited for further testing against feral females.

A suitable male marking technique was necessary for the planned "in season" field test. To test two possible techniques a series of trials were combined with the previously described test. The purposes were to find a marking technique which did not affect male performance and which could be used on site in the field with freshly eclosed adults. Groups of male moths (NJ_{15}) were marked in two ways: syringe application on the wings with alcohol solutions of rhodamine B or methylene blue or day-glo fluoroscent dust applied with a brush. Table 2 summarizes the results of these tests.

Table 2.

Plot	No. Males Released: Alcohol Carried Dyes	Day-Glo Dust	Unmarked	No. & Percentage of Male Recovered: Alcohol Carried Dyes		Day-Glo Dust		Unmarked	
1	200	200	200	31	16%	50	25%	77	39%
2	247	250	250	89	36%	115	46%	139	56%

Marking with day-glo dust was chosen for use in the summer's testing. Further testing with males marked in this manner indicated that it did not impair a male m[illegible] response to pheromone. In addition, the ease of application to newly eclose[illegible] insects made it more suitable for field use.

Methods and Materials: In-season testing objectives, as stated earlier, were to compare monitoring tools which could be used for evaluating male competitiveness and to compare the eclosion, dispersal and response to pheromone of different strains of male gypsy moths. Plots for these tests were established using a 6x6 square grid (Latin square) with a 50 meter spacing between points. The thirty-six points defined by this grid were randomly assigned one of the following treatments:

1. A feral female placed on the bole of a tree (ca. 1.5 meters high). Females were allowed to seek their own resting and calling sites.
2. A trap baited with a feral female.
3. A trap baited with a PA_1 (laboratory reared) female.
4. A trap baited with a NJ_{15} (laboratory reared) female.
5. A trap baited with 100 μg (+) disparlure.
6. A trap with no bait.

Traps were constructed of hardware cloth (1.27x1.27 cm grid) and measured 25.4 cm x 30.5 cm. In the center of the trap a 10.2 cm x 10.2 cm section of screening was removed to facilitate bait placement. In use traps were coated with Tack Trap and stapled on a tree with the long side of the trap parallel to the tree bole. The baits were placed in small circular cages (7 cm diameter x 2 cm) and hung in the center of the trap. Bait cages were constructed of hardware cloth (0.3 cm x 0.3 cm grid, bottom of cage; 0.6x0.6 cm grid sides of cage) and a plastic petri dish cover (7.2 cm diameter x 0.6 cm) which served as a top. In use the bait cage was hung so that the clear plastic cover faced away from the tree bole.

Each trap-bait combination was represented six times within a plot. All traps were placed 1.5 meters high on tree boles. Female baits were one day old, virgin moths and were replaced daily. Cotton wicks containing 100 μg (+) disparlure were placed out on the first day of the test and were not replaced with fresh wicks during the course of the test.

The geographic center of the plot was used as a release station for male moths and was ca. 35 meters from the nearest trap. Eclosion boxes (0.6x0.6x0.3 m deep) with open tops were placed at the center points and provisioned with adequate numbers of male pupae to result in ca. 300 male moths of each strain eclosing per day for a three day period. However, because pupation dates of feral and laboratory insects were not known precisely, males were allowed to eclose for an additional day in two replicates. Males were marked with day-glo fluorescent dust after eclosion and before their wings had fully hardened. Marking males during this period of development produced only minor disturbance to the insects. Marking colors were changed daily to segregate between day of eclosion and strain (i.e., feral, NJ_{15}, PA_1) of moth. Emergence times and dispersal times were recorded from 7 AM to 9:30 PM.

Observers were stationed by feral females placed on the boles of trees from 9 AM to 4 PM. Behavioral observations were recorded on any male flying near or appearing to orient to the trap. Any male attempting to mate with a female moth was captured. After 4 PM and until the next morning, these females were removed from the tree and placed in traps. All other trap-bait combinations were moni-

tored every hour from 8 AM to 8 PM and males were collected for later laboratory determination of marking code. In addition, when personnel were available, behavioral observations were made on all trap-bait combinations.

Observations were initiated the first day of eclosion and were continued until one day after eclosion had terminated. Traps were monitored from the first day of eclosion and continued until at least 3 days after eclosion had stopped.

Temperature, relative humidity, wind direction, wind speed and solar radiation data were collected continuously on site to determine if and how these factors influence emergence, dispersal or trap catch.

Results and Discussion: All laboratory determinations of male marking codes has now been completed. A majority of the data is in the process of being coded for computer analysis. However, some preliminary results have been tabulated which may be indicative of trends. Table 3 presents the number of males eclosed and captured for the summer's "in season" test.

Table 3. Numbers of Male Gypsy Moths (*Lymantria dispar*) Eclosed, Released and Recaptured for Two Laboratory Strains and Four Feral Strains.

	Eclosion			Captures		
Date	Strain			Strain		
	Feral	Laboratory		Feral	Laboratory	
	Bushkill, PA	NJ_{15}		Bushkill, PA	NJ_{15}	
7/5/77	40	71				
7/6/77	321	375		TOTAL 272	142	
7/7/77	754	492		% of eclosion captured		
7/8/77	427	286		18%	12%	
TOTAL	1542	1224				
	Dighton, MA	NJ_{15}		Dighton, MA	NJ_{15}	
7/11/77	152	131				
7/12/77	118	143		TOTAL 408	510	
7/13/77	162	176		% of eclosion captured		
7/14/77	221	325		62%	66%	
TOTAL	653	775				
	Abington, MA	NJ_{15}	PA_1	Abington, MA	NJ_{15}	PA_1
7/18/77	484	698	26			
7/19/77	352	571	46	TOTAL 475	1066	85
7/20/77	399	581	36	% of eclosion captured		
TOTAL	1235	1850	108	38%	58%	79%
	Fern Lake, NY	NJ_{15}	PA_1	Fern Lake, NY	NJ_{15}	PA_1
7/25/77	238	158	13			
7/26/77	127	110	10	TOTAL 144	195	-
7/27/77	71	182	16	% of eclosion captured		
7/28/77	80	282	-	28%	27%	-
TOTAL	516	732	38			

Percentages of males eclosing from each strain which were recaptured appear to be very similar within each replicate. In the first replicate the recoveries for both strains were very low, 18% and 12%. This low percentage recovery may be explained by three factors: (1) the difference between the number of moths eclosing and the number dispersing; (2) mild temperatures or (3) by intense vertebrate predation (both avian and mammalian) noted in this test. Of the three, the latter, vertebrate predation, appears largely responsible. Noticeable avian predators keyed in on the release site and consistently took large numbers of moths. However, further analysis may indicate temperature is an additive factor.

The second and fourth replicates both had very nearly the same percentages of feral and laboratory (NJ_{15}) males recovered. The third replicate however, had a much lower percentage of feral males recovered. Further analysis may indicate if this is a real difference and, if so, the reason for the poor recovery.

Generally, the NJ_{15} laboratory strain males appear to be able to find pheromone sources as well as feral males. More complete analysis of the data is being undertaken to determine if the types of pheromone sources (i.e., natural or artificial, distance from the release point) different strains of males seek out are similar. Also, the timing of capture of different strains is being studied. Preliminary results indicate that the NJ_{15} strain periodicity of activity (response to traps) may be shifted slightly. In several replicates, peak capture of feral males occurred one to two hours before peak capture of NJ_{15} males. It should be noted that peak activity of both strains did not always occur during the same hour period and further analysis of climatic data will be needed to determine if one or more factors is or are the modifying mechanism(s). Moreover, the activity peaks have not as yet been segregated by age of moths within the same strain. This segregation may point out more differences or explain part of the shift that is now evident.

Periodicity of eclosion of the NJ_{15} males appears similar to that of feral strains tested (Tables 4, 5, 6 and 7). However, on some days the patterns appear to be out of phase. Analysis of climatic conditions may discern the reasons for this variation. Dispersal of males from eclosion boxes also appears to follow a similar pattern for laboratory and feral insects (Tables 8, 9, 10 and 11). Preliminary results again indicate this activity may be temperature mediated. Generally, periodicity of dispersal of NJ_{15} males from eclosion chambers was similar to feral insects. However, some lag in the dispersal of the laboratory strain is noted on several days. Analysis and further testing will be needed to determine if these are critical differences.

A cursory comparison of trap bait combinations has been completed. A full analysis including the variables of trap position, wind direction, wind speed, temperature and the age, strain and timing of insect capture will have to be done before definite conclusions can be drawn. Given that these variables are not included, indications are that the NJ_{15} and PA_1 laboratory reared females performed as well as feral females when placed in traps. Periodicity of capture for all trap bait combinations appears to be similar including traps baited with (+) disparlure.

Conclusions. [illegible] be done before all the information from [illegible] into further testing. However, two major [illegible] laboratory reared (NJ_{15}) males are a[illegible] the feral populations tested, [illegible] dispersal or laboratory females [illegible] ity of some of the activi[illegible] However, analysis will have [illegible] no, if so, how this affects [illegible] throughout this paper, analysis [illegible] below and final conclusions [illegible] These results should also give us a better [illegible] dispersal and mate finding and [illegible]

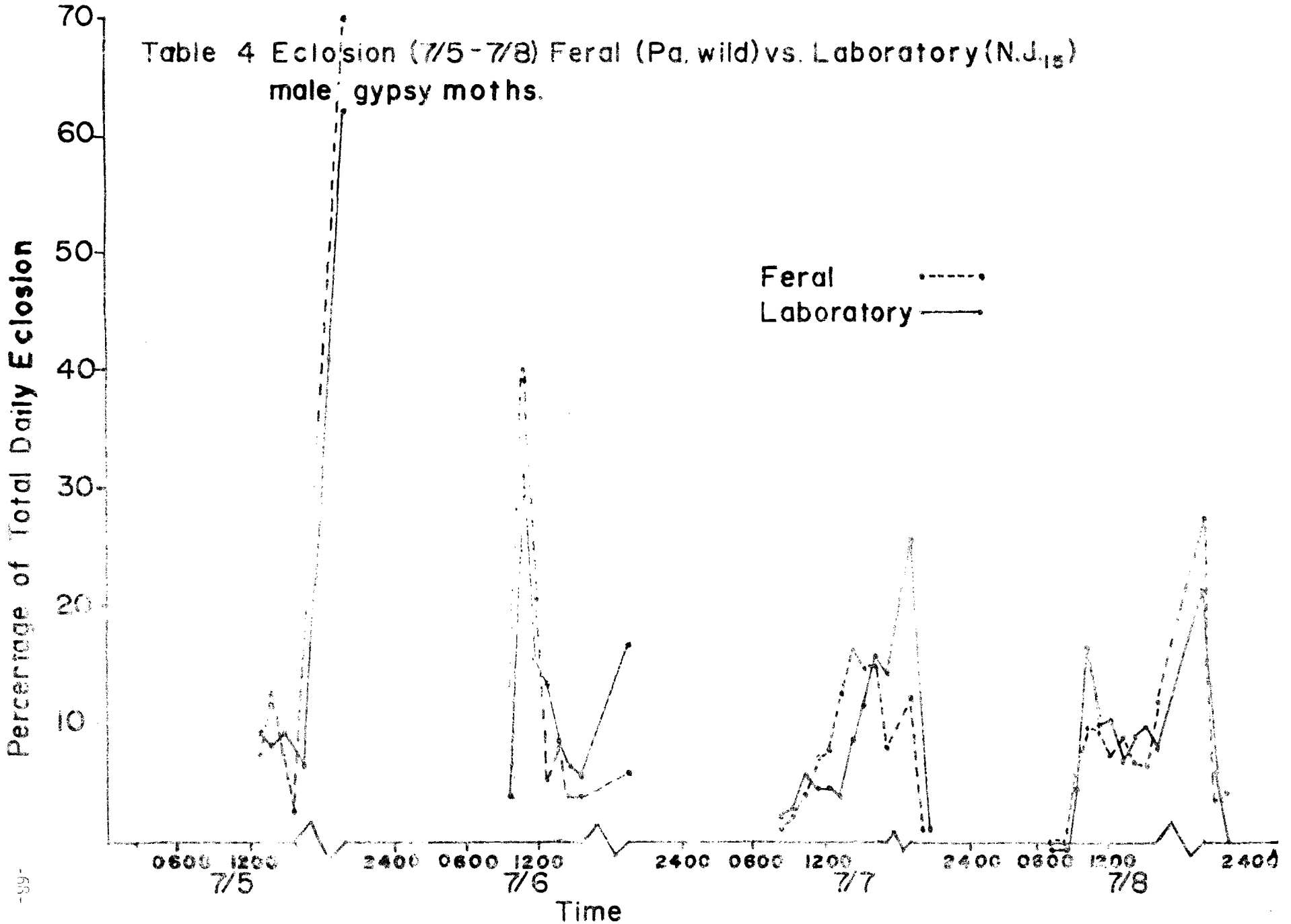


Table 4 Eclosion (7/5-7/8) Feral (Pa. wild) vs. Laboratory (N.J.15) male gypsy moths.
Percentage of Total Daily Eclosion
70
60
50
40
30
20
10
Feral
Laboratory
0600 1200 2400 0600 1200 2400 0600 1200 2400 0600 1200 2400
7/5
7/6
7/7
7/8
Time

Table 5 Eclosion (7/11 - 7/14) of Feral (Dighton, Ma.) vs. Laboratory (N.J.$_{15}$) male gypsy moths

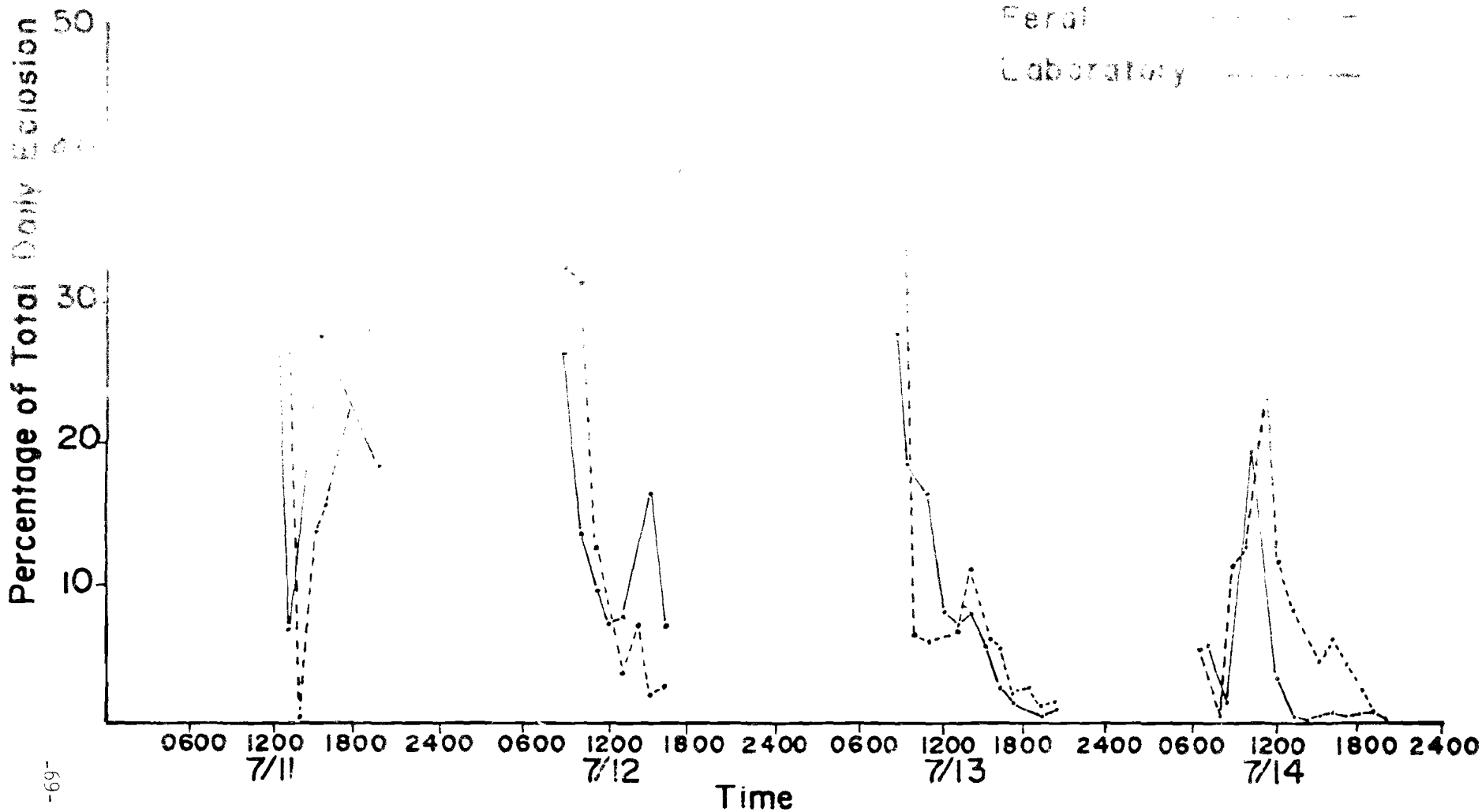


Table 6 - Eclosion (7/18 - 7/20) of Feral (Abington, Ma.) vs. Laboratory ($N.J._{15}$) male gypsy moths.

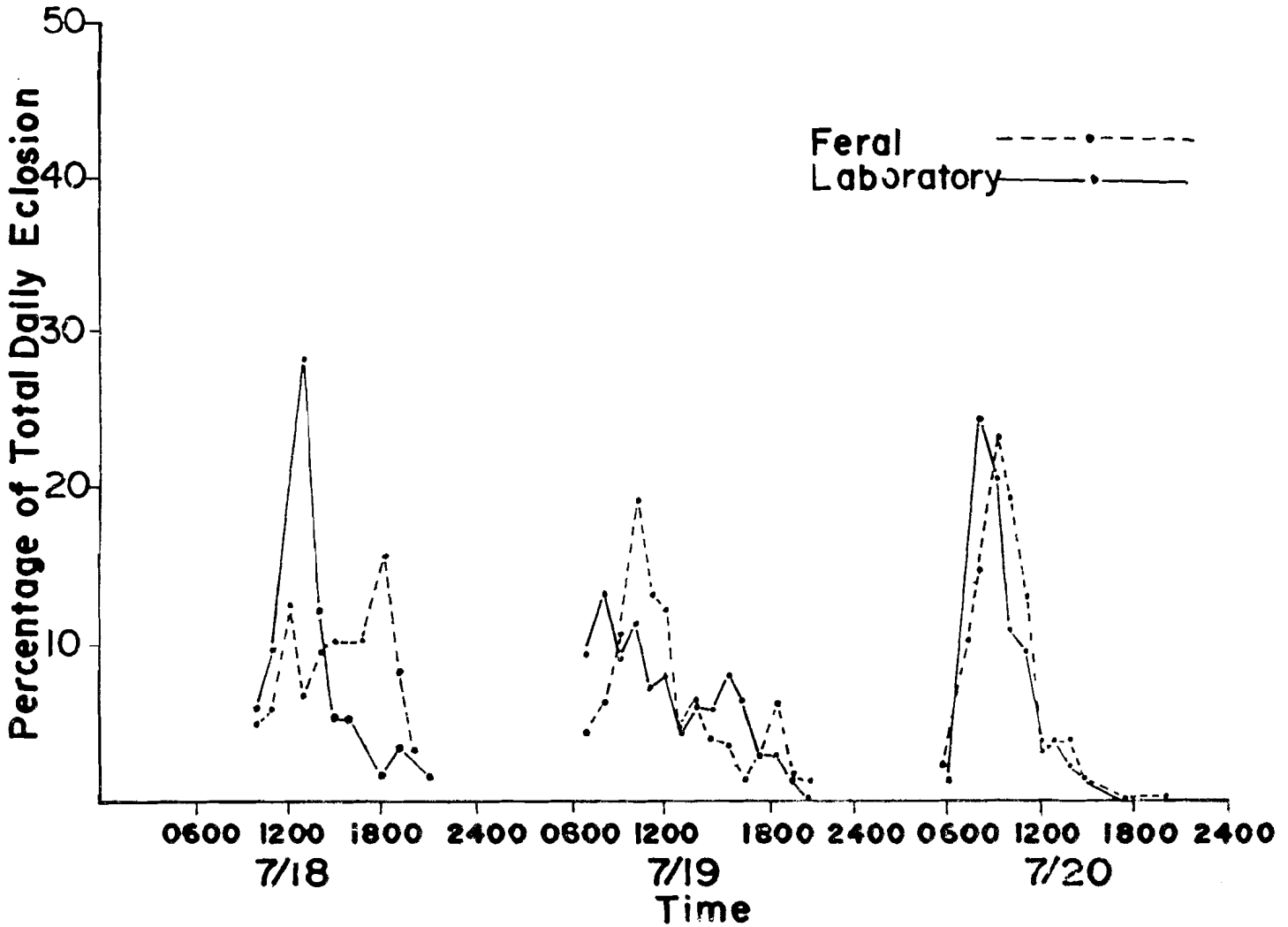
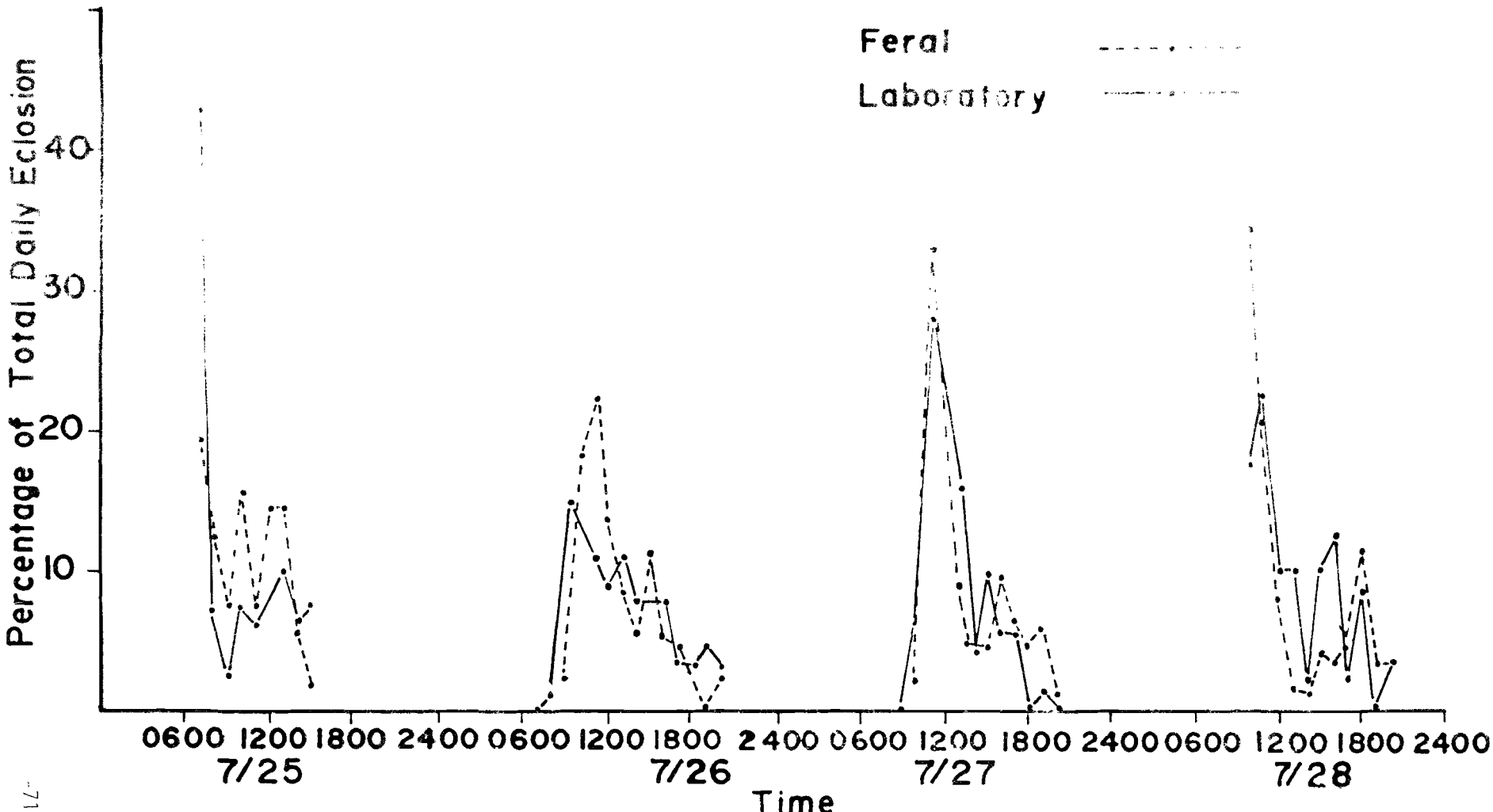


Table 7 Eclosion (7/25 - 7/28) of Feral (Fern Lake, N.Y.) vs. Laboratory ($N.J._{15}$) male gypsy moths.



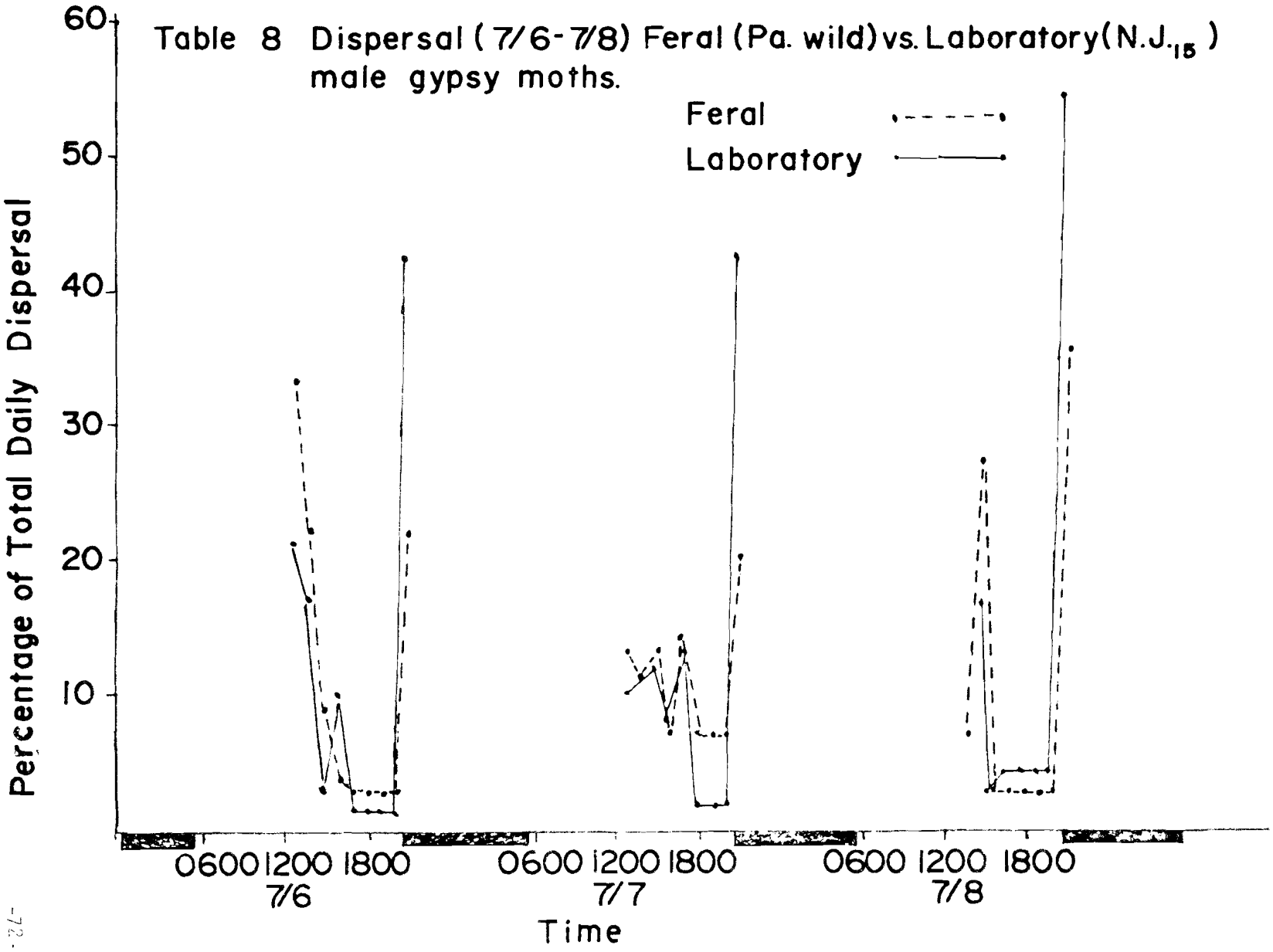


Table 8 Dispersal (7/6-7/8) Feral (Pa. wild) vs. Laboratory (N.J.15) male gypsy moths.
Feral
Laboratory
Percentage of Total Daily Dispersal
60
50
40
30
20
10
0600 1200 1800
7/6
0600 1200 1800
7/7
0600 1200 1800
7/8
Time

Table 9 Dispersal (7/12-7/14) Feral(Dighton, Ma.) vs. Laboratory($N.J._{15}$) male gypsy moths.

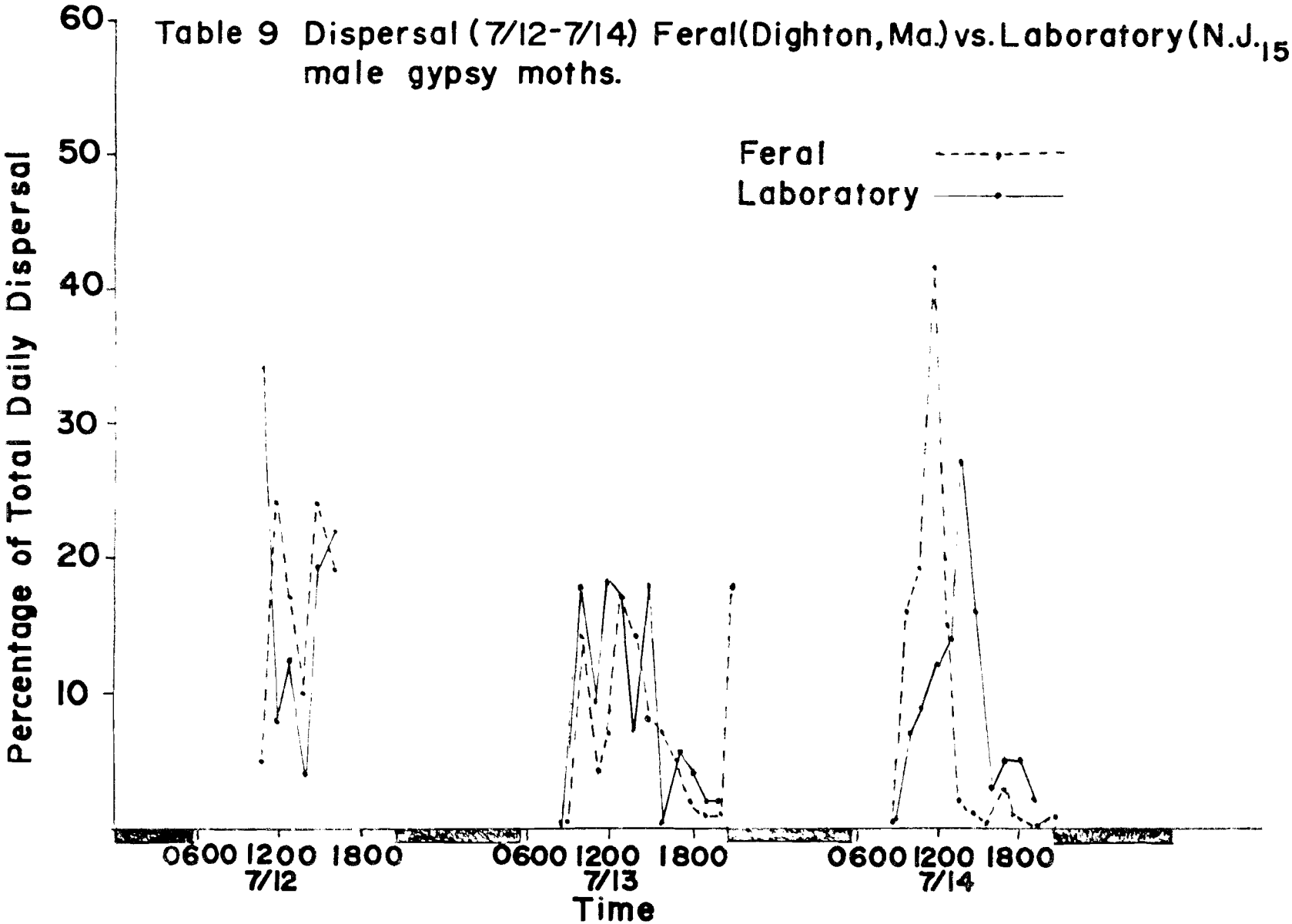


TABLE II.- Dispersal (7/25 - 7/27) of feral (Fern Lake, N.Y.) vs. laboratory ($N.J._{15}$) male gypsy moths.

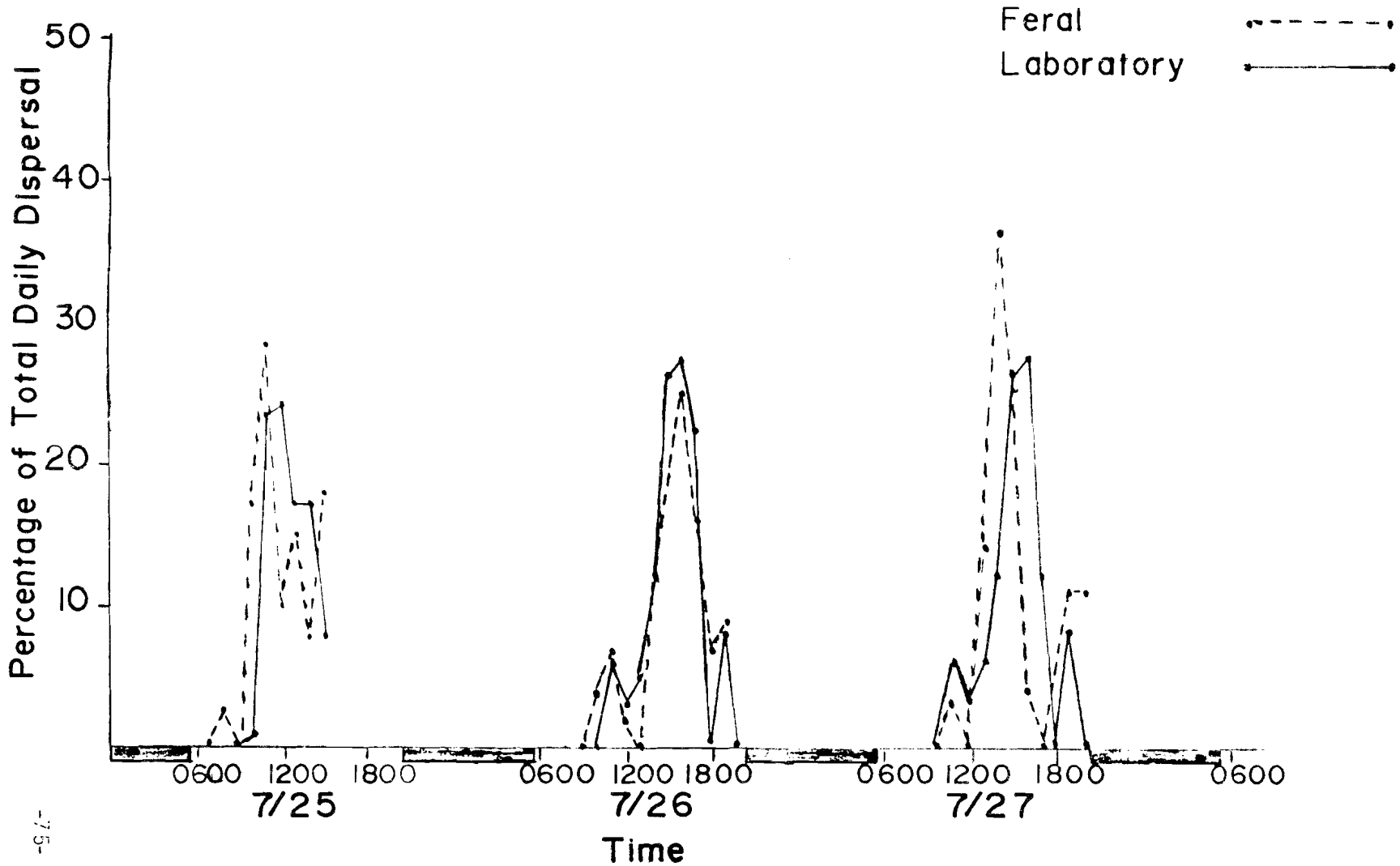


Table 10 Dispersal (7/18-7/20) of feral (Abington, Ma.) vs. Laboratory ($N.J._{15}$) male gypsy moths.

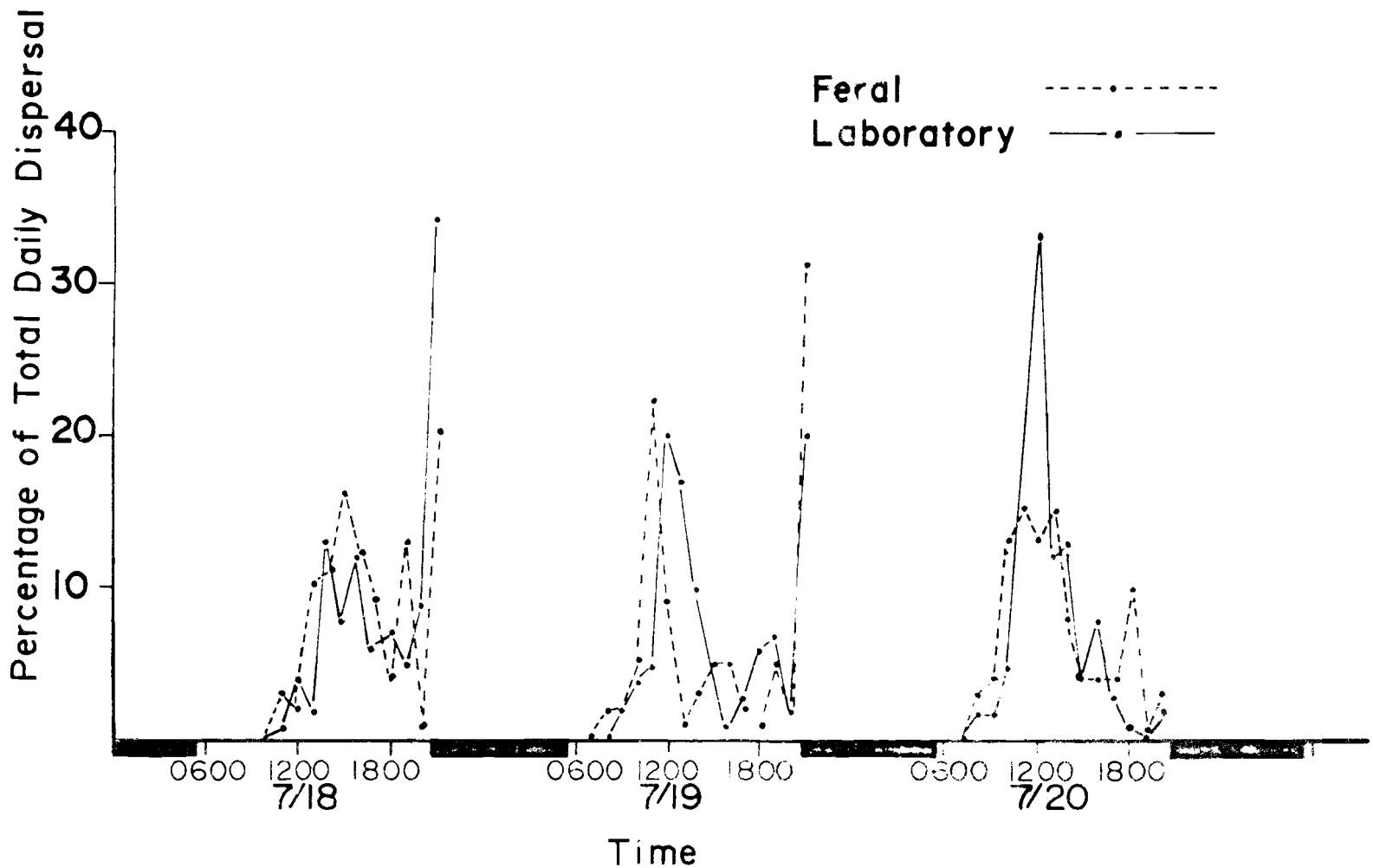
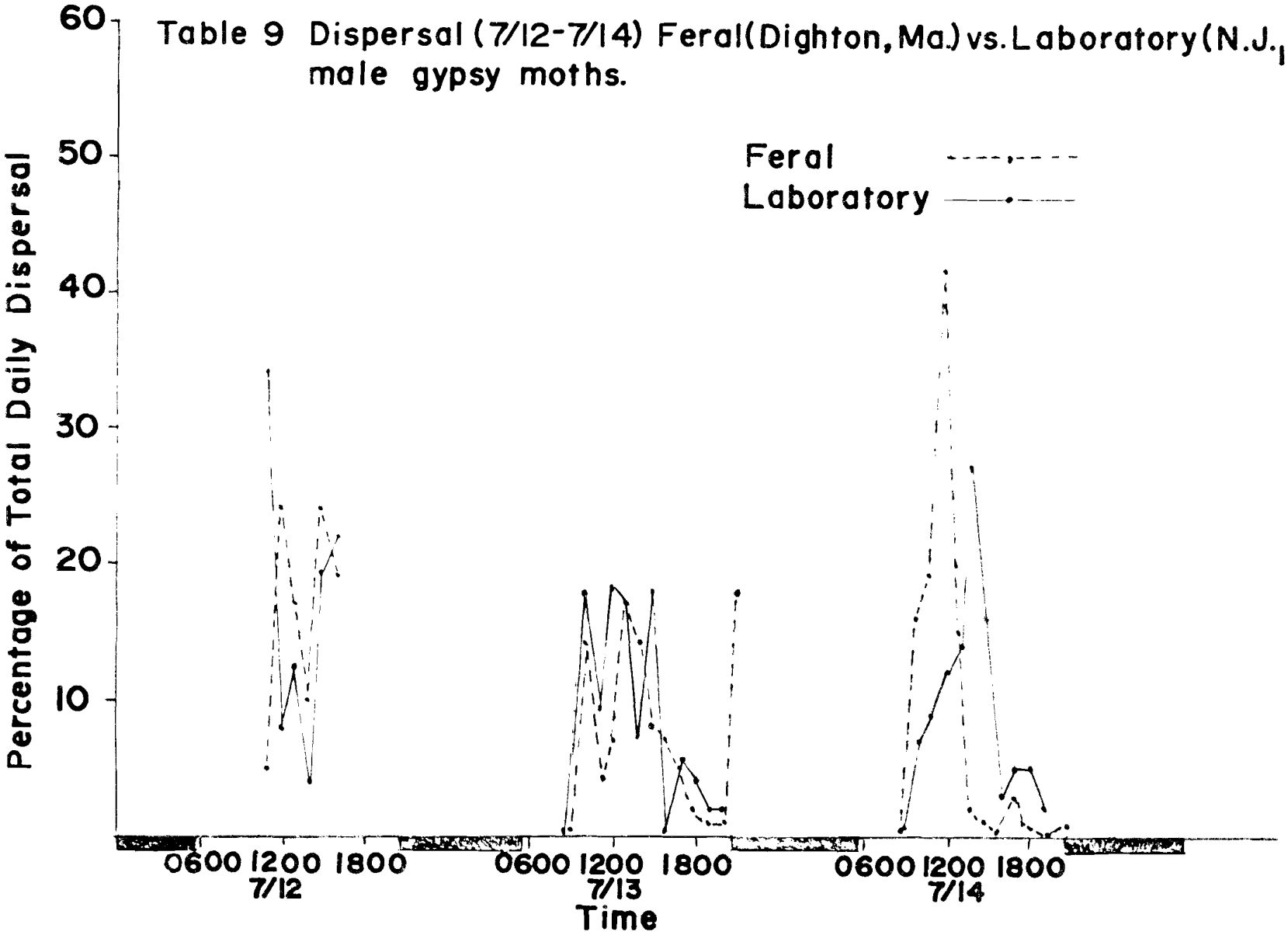


Table 9 Dispersal (7/12-7/14) Feral(Dighton, Ma.) vs. Laboratory($N.J._{15}$) male gypsy moths.



Time

Table 8 Dispersal (7/6-7/8) Feral (Pa. wild) vs. Laboratory ($N.J._{15}$) male gypsy moths.

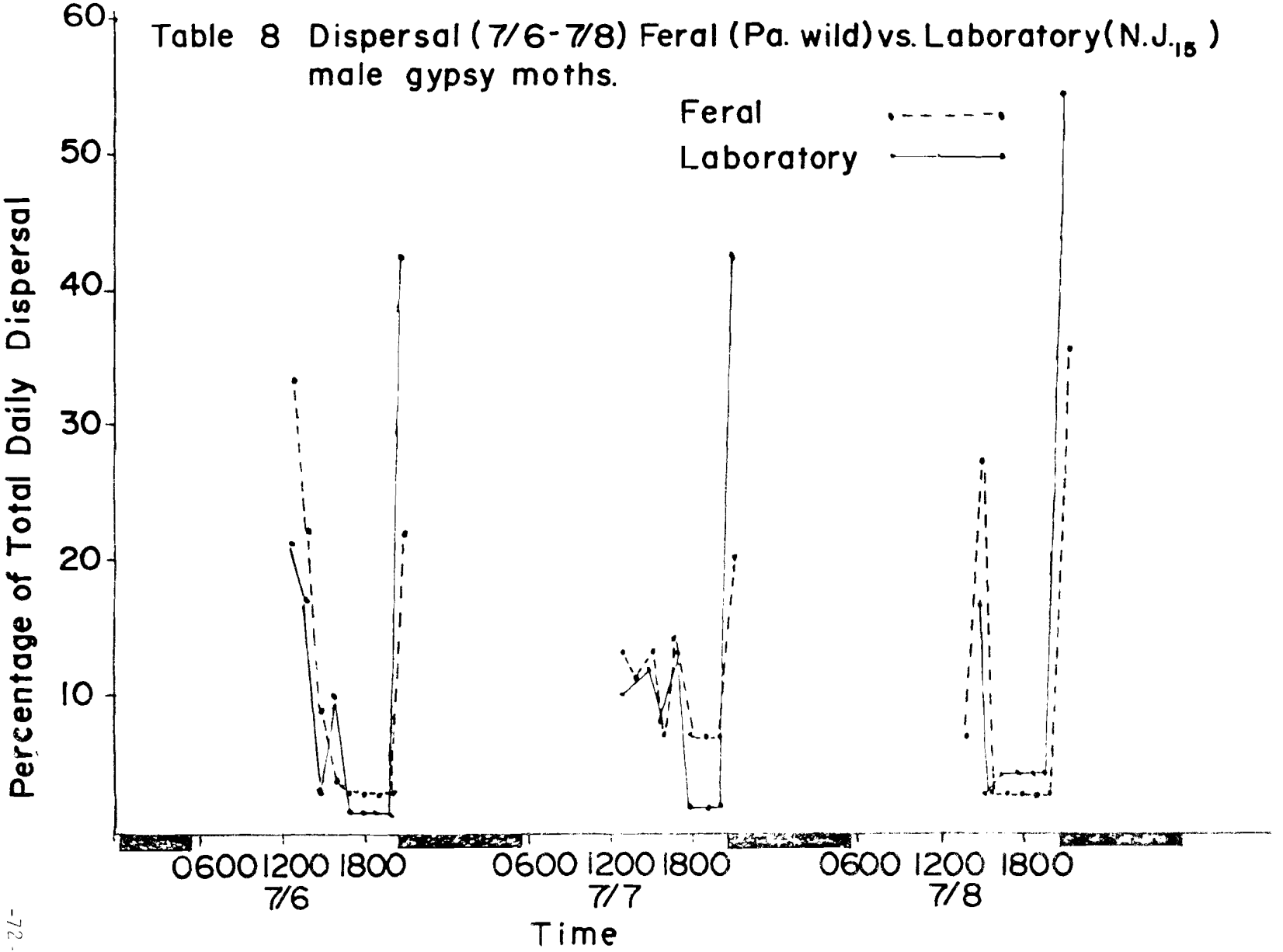


Table 6 - Eclosion (7/18 - 7/20) of Feral (Abington, Ma.) vs. Laboratory ($N.J._{15}$) male gypsy moths.

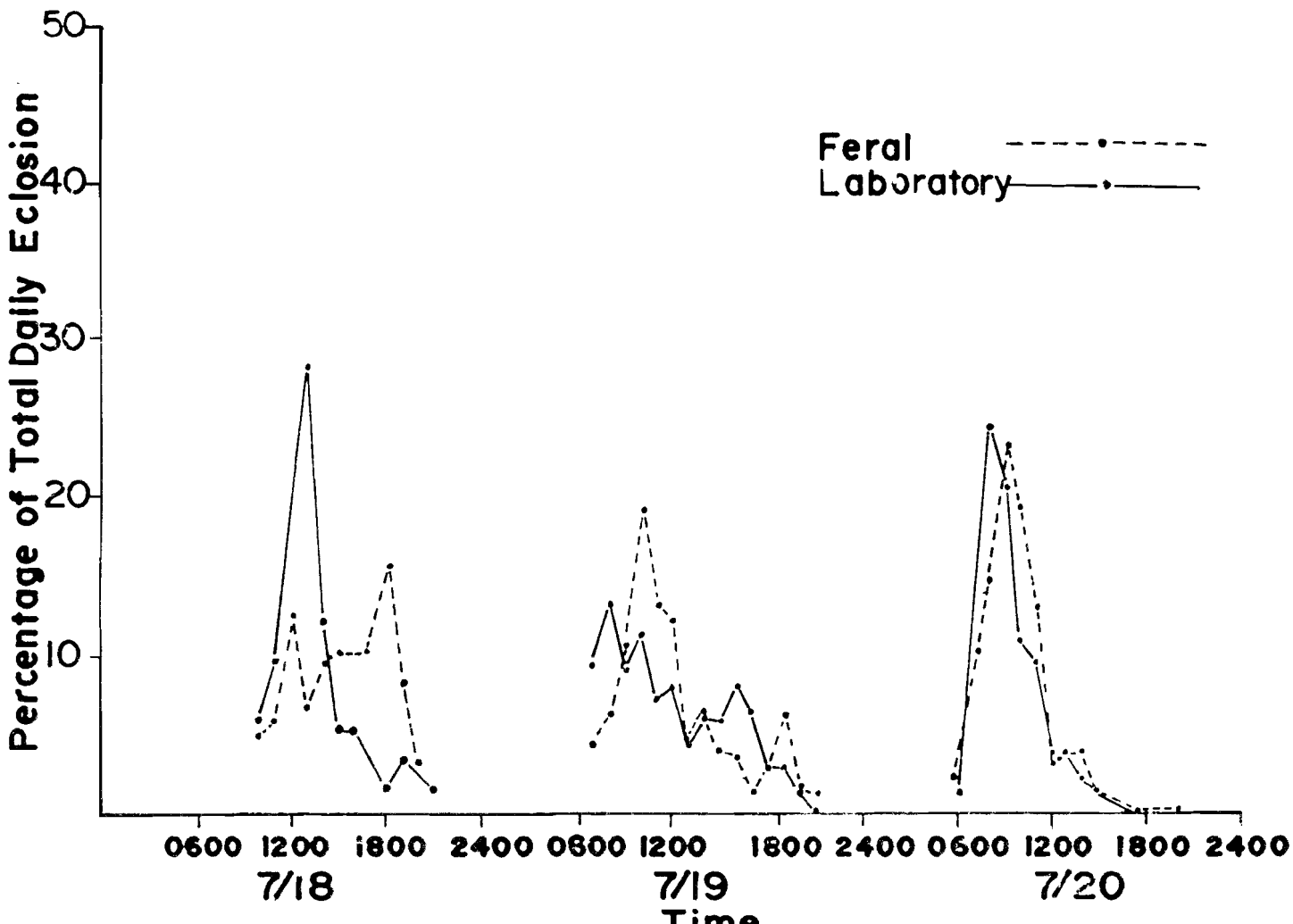
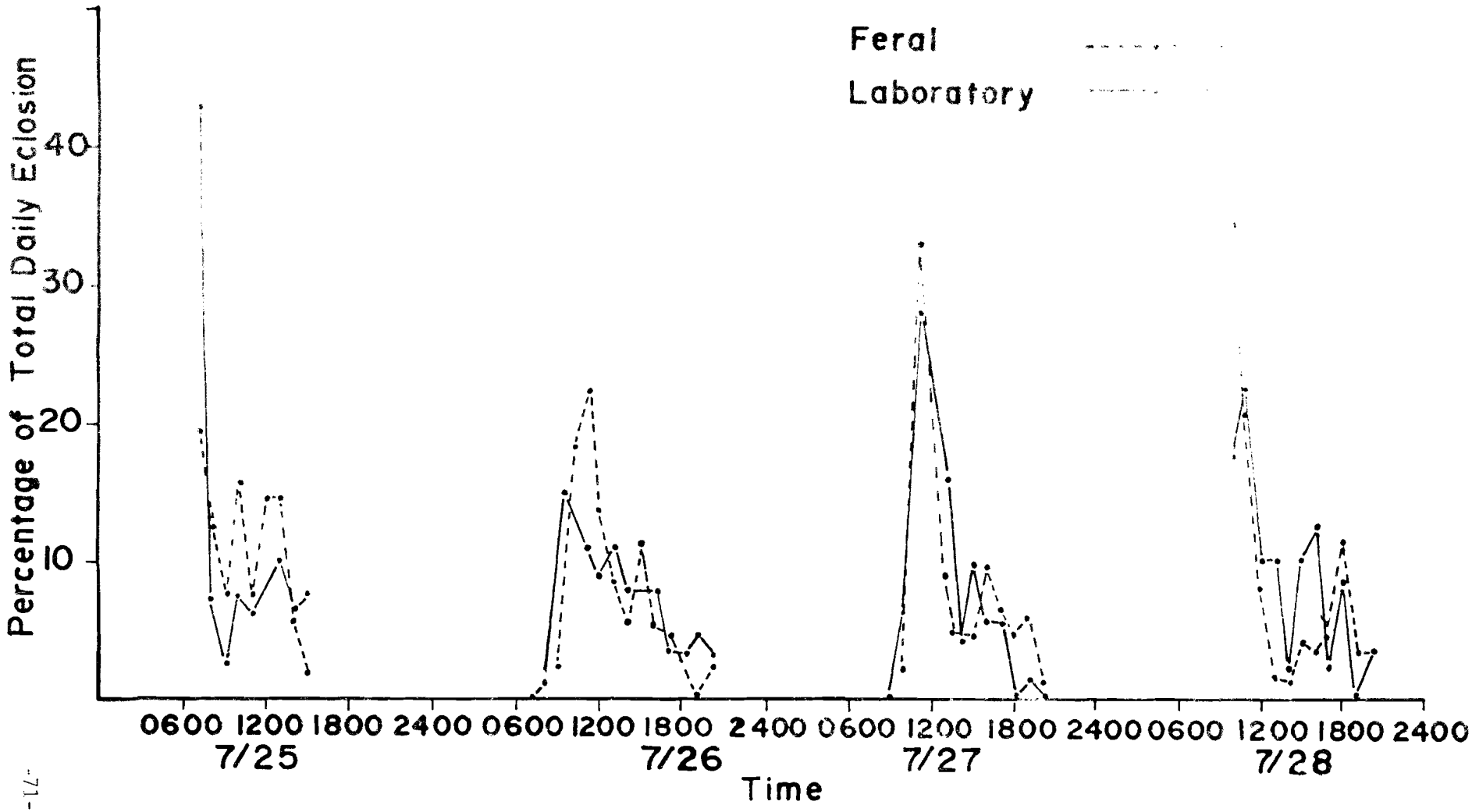


Table 7 Eclosion (7/25 - 7/28) of Feral (Fern Lake, N.Y.) vs. Laboratory ($N.J._{15}$) male gypsy moths.



Time

Table 8 Dispersal (7/6-7/8) Feral (Pa. wild) vs. Laboratory ($N.J._{15}$) male gypsy moths.

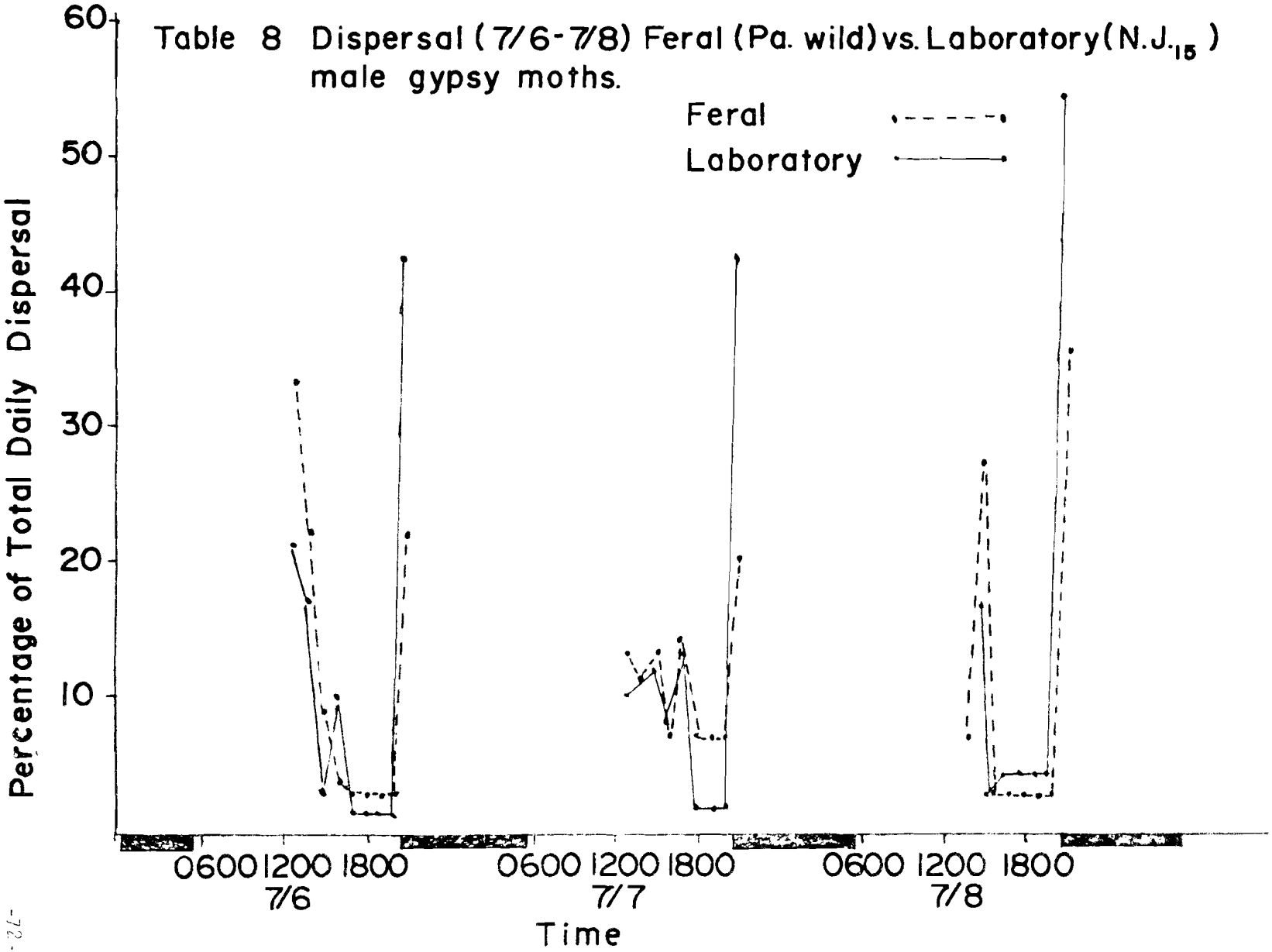
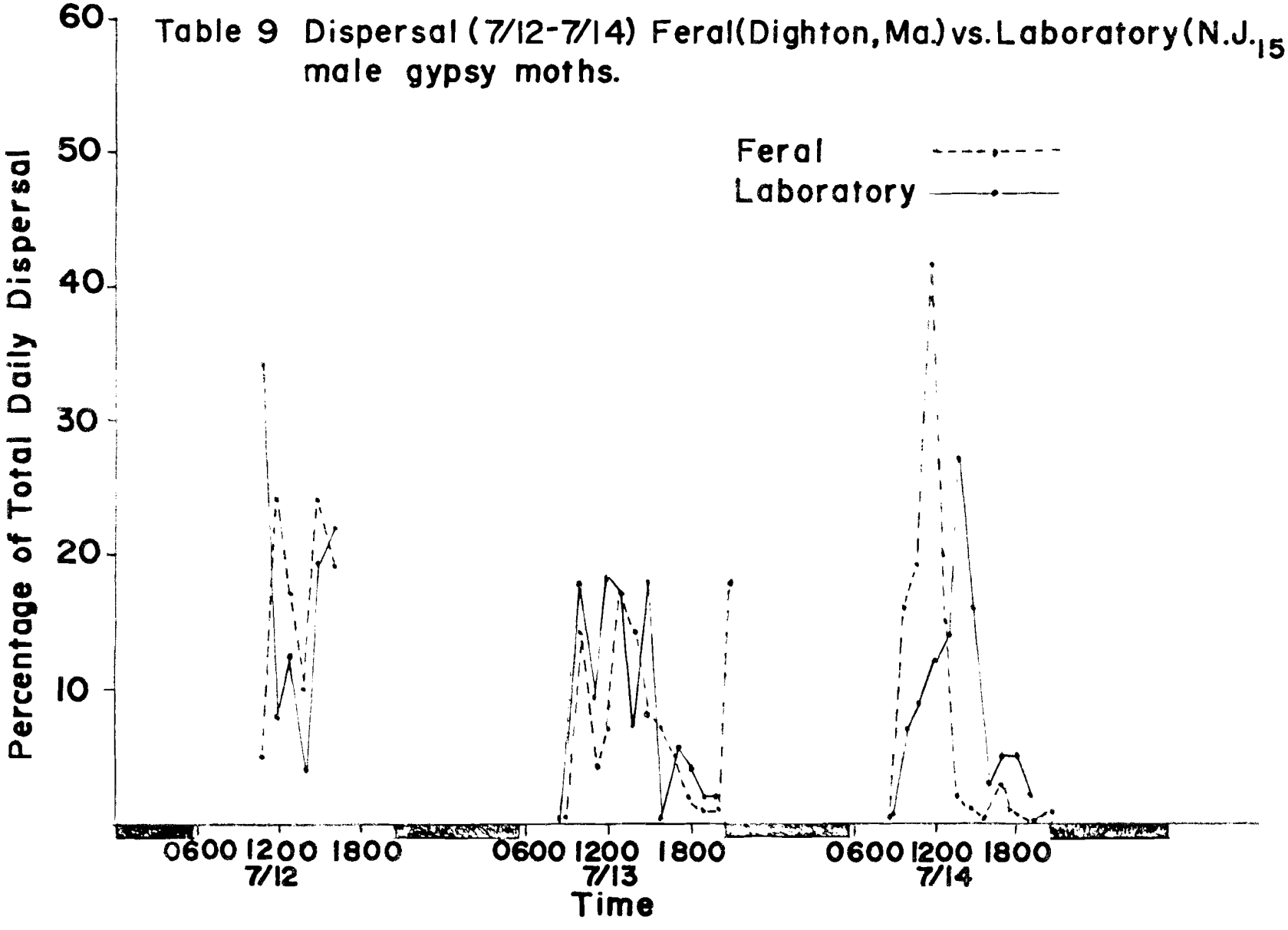


Table 9 Dispersal (7/12-7/14) Feral(Dighton, Ma.) vs. Laboratory($N.J._{15}$) male gypsy moths.



Time

Table 10 Dispersal (7/18-7/20) of feral (Abington, Ma.) vs. Laboratory ($N.J._{15}$) male gypsy moths.

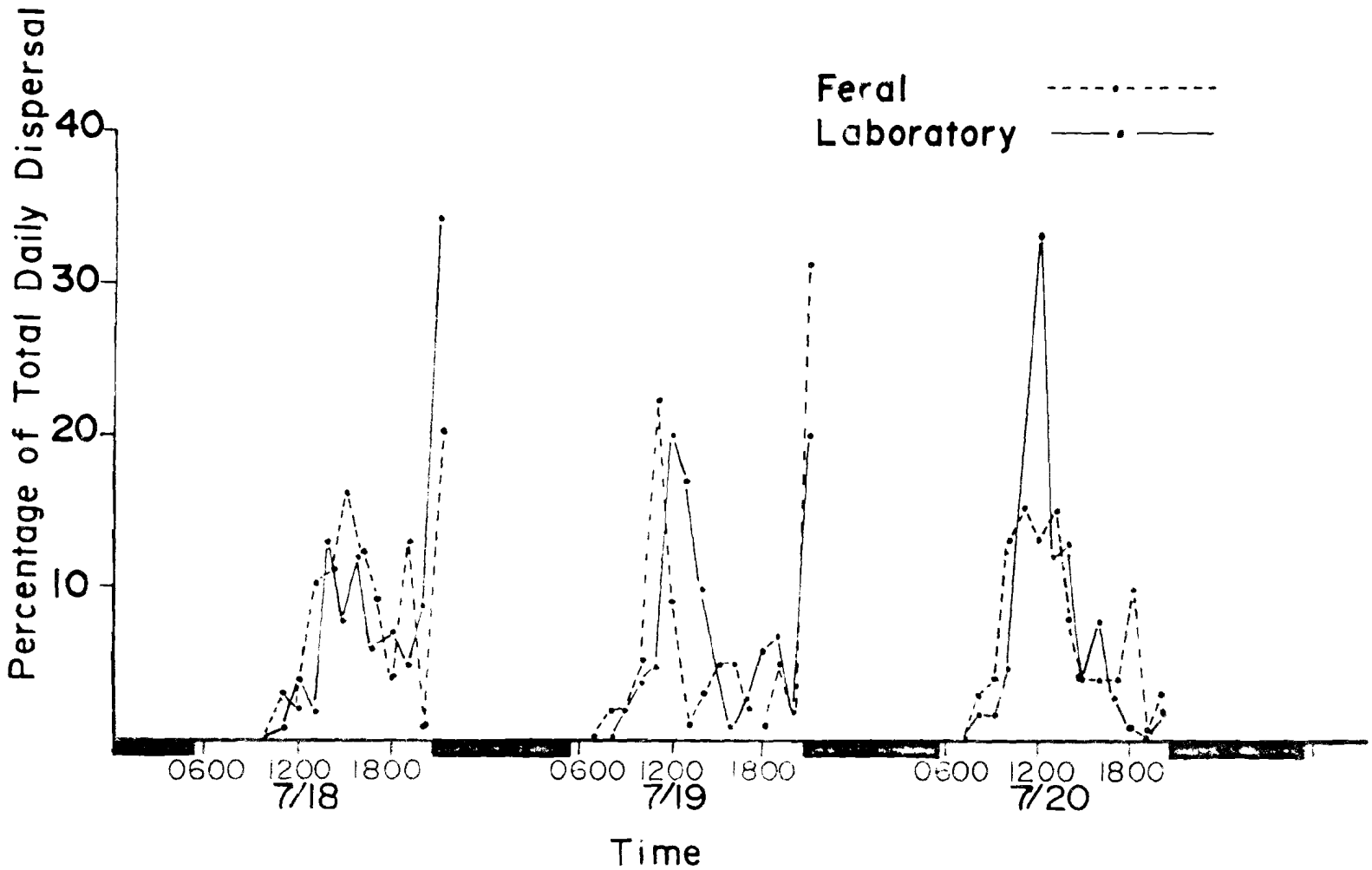
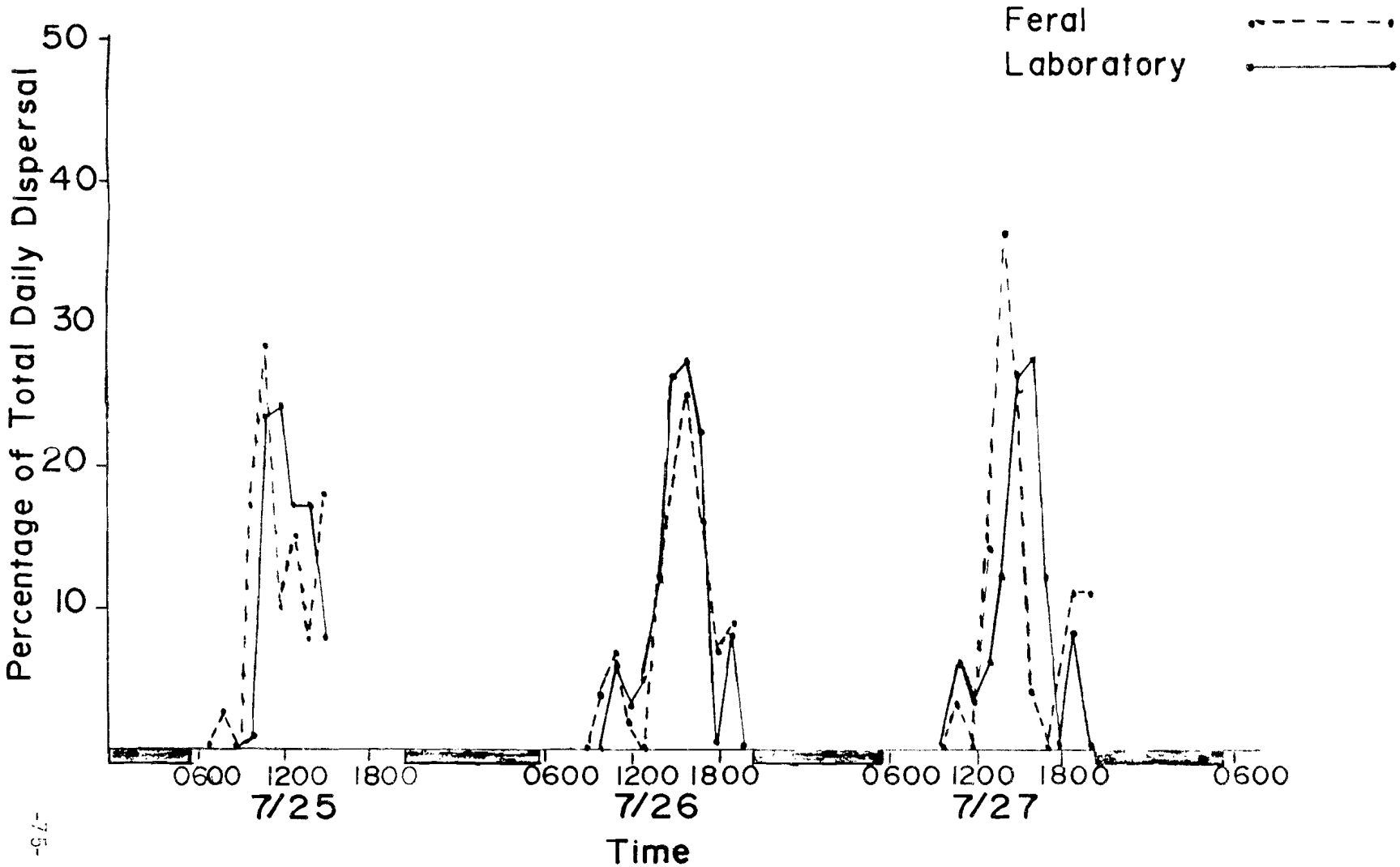


TABLE II.- Dispersal (7/25 - 7/27) of feral (Fern Lake, N.Y.) vs. laboratory ($N.J._{15}$) male gypsy moths.



Project Number: GM 7.2.6
Project Title: Emission of Disparlure from Cotton Wicks
Report Period: April 1, 1977 - September 30, 1977
Report Type: Final
Project Leaders: Charles P. Schwalbe, D. H. Simser, L. T. Mundy

In the last laboratory report, emission of disparlure from cotton wicks was described. However, erratic results were obtained with wicks loaded with 10 μg disparlure. That section of the experiment was repeated according to procedures previously reported:

	μg Disparlure Remaining	
Days Aged	Disparlure	Disparlure + Trioctanoin
0	9.67	10.75
1	6.76	10.43
3	5.25	8.90
5	4.07	8.60
10	1.85	6.80
16	1.18	3.12

Wicks containing 10 μg disparlure + trioctanoin released pheromone uniformly over the 16 day period at ca. .43 μg/day. The release from wicks without trioctanoin was constant from days 1-10 when ca. .55 μg/day was released.

Project Number: GM 6.3.5
Project Title: Development of Diet and Efficient Rearing Techniques
Report Period: April 1, 1977 - September 30, 1977
Report Type: Interim
Project Leaders: Robert A. Bell, M. Shapiro, O. T. Forrester

Several tests involving various aspects of dietary improvement and enhanced rearing efficiency were carried out over the past 6 months. However, due to the poor quality of a large shipment of wheat germ utilized during this period, a high incidence of malformed pupae was observed in all of our tests as well as in the colony. Generally, larval development was not affected and male pupae were normal. Thus, most of the tests are being repeated with fresh, good quality wheat germ. Apparently, the poor quality wheat germ was old and had undergone considerable auto-oxidation when it was shipped. Thus, quality control tests are being devised to determine oil content and peroxide formation in future shipments of wheat germ to avoid the problems that occurred this past summer.

Effect of cooking time on nutritional quality of diet:

In a mass rearing situation where large volume kettles are employed, and especially if carageenans are used as the gelling agent, the diet may have to be held for periods of up to 2 hours at nearly 80°C during processing and dispensing. Thus, an experiment was conducted to determine the effect of diets that had been held at 80°C for 30, 60, 90 and 120 minutes on gypsy moth development. In the control, diet was prepared and dispensed from a Waring Blender in the conventional manner and was exposed to 80°C for about 5 minutes. The diets tested included agar vs. Gelcarin (HWG) as gelling agents.

Judging from the weight and developmental time data (Table 1) there was little difference between the various treatments. Thus, it appears that with the hi-wheat germ diet, there is not a critical loss of nutrients even when diet is held at 80°C for up to 2 hours. However, egg hatch data from this test had not yet been taken.

Method of Mixing:

Diets prepared in a Breddo ("Likwifier") food mixer (18 gallon test model) were compared with that prepared in a Waring Blender (1 gallon). Agar was introduced into water at boiling, allowed to cool to 80°C and all remaining ingredients were added. Time to mix the ingredients in the Likwifier was about the same as in the Waring Blender, i.e. only a few minutes. In the blender, the blades are rotated at up to 17,500 rpm and result in considerable shearing of dietary ingredients. In the Likwifier, the mixing blades are rotated at 2500 rpm that results in a very rapid mixing of the ingredients but little shearing action. Thus, the blender reduces the ingredients to a very fine particle size whereas the Likwifier does not.

There was no difference in development of the gypsy moth reared on the Likwifier vs. blender mixed diet (Table 2).

Effects of holding containers and diet change:

It has been observed many times that type of rearing container and age of the diet seems to influence the incidence of malformed pupae. We refer to this particular malformation as the "sunken thorax syndrome". Larvae generally develop normally, pupation occurs on schedule and pupal weights are normal to excessive. However, sclerotization of the female pupae is not completed or occurs slowly in the thoracic region resulting in excessive water loss through the soft cuticle causing this part of the anatomy to collapse.

Apparently the main cause of this particular abnormality is diet that has undergone excessive deterioration. The problem is generally not observed when diets are prepared using good quality wheat germ. Using diets prepared with old, deteriorated wheat germ, we were able to show that the type of rearing container can influence the incidence of pupal malformations (Table 3). Also, if insects were transferred to freshly prepared diet at 14 days post-infestation, the incidence of pupal abnormality was reduced.

Of the plastic containers tested, the m-trene cups yielded the lowest incidence of abnormal pupae. The m-trene containers are less permeable to air than the other containers and are transparent rather than translucent as are the other plastic containers. Thus, part of the deterioration of the diet leading to pupal abnormalities is probably excessive oxidation which, because of differences in permeability to air, is greater in some types of containers than in others. Transfer of insects to fresh diet reduces the problem since the amount of oxidative and other changes in the diet is a function of time.

It was also of interest that when gypsy moths are reared in the conventional containers (treatment 7, 8, 9), i.e. 16 ounce paper Dixie cans, the kind of containers used to hold the diet within the can may effect the quality of the diet. In this case, plastic cups were preferable to paper cups with the L04 plastic cup showing the lowest incidence of malformed pupae.

For routine mass rearing, insects are being reared at 15 larvae/6 ounce fluted cup from infestation to 21 days of age and then transferred to 16 ounce cans (15 L/can) containing 1 L04 (4 ounce) cup of diet. No additional food change is necessary and pupal abnormalities are rarely observed since receiving a new shipment of good quality wheat germ. To rear insects throughout larval feeding without a change of diet and still obtain good quality adults is possible if we can prevent excessive oxidation.

Anti-microbial agents:

Several anti-microbial agents were screened to determine their relative effectiveness. Effects on virus production and insect reproduction will be reported later. An effort was made to find a concentration of a single anti-microbial agent that would prevent any microbial contamination of the diet. The test diet was the hi-wheat germ diet; the wheat germ used was old and heavily loaded with microbial contaminants. Diet was prepared in the conventional manner with a Waring Blender and infested at 10 larvae/6 ounce fluted cup. After 7 days at

$25^{o}C$, the surface of the diet was examined for microbial growth. Each treatment involved 30 cups. Results (Table 4) showed that sorbic acid, methyl paraben and 10% formalin were effective in preventing any contamination when used at a concentration of 0.4%. Even at 0.2%, sorbic acid and formalin (10%) were about 97% effective. These compounds will be used singly in further tests at 0.2, 0.3 and 0.4% to determine effects on development and reproduction of the insect. Other tests which included aureomycin in addition to the antimicrobial agents (Table 4) will be completed shortly.

Quality control:

Quality control measurements were performed with the NJF_{15} over the past few months and results are compared with the PA wild strain (Table 5). Unfortunately, the wild eggs had been in cold storage for nearly 6 months after they were collected (January 1977) and the hatch was low. The only significant difference in the various biological attributes measured to date was the duration of hatch (being shorter in the wild strain) and duration of larval development (a few days shorter in the NJF_{15} strain). At present, evaluations are being made on all colonized strains; NJF_{15}, Ludlow F_{15}, PA F_7, and PA F_1. The results of these measurements will be included in the next report.

Table 1. Effect of cooking time during diet processing on development of gypsy moths (NJF_{15} strain).

	COOKING TIME AT 80°C (Min)				
Diet = Hi-Wheat Germ - Agar	Control	30	60	90	120
Larval wt (G), Day 26, M	0.93	0.94	0.90	0.93	0.93
Larval wt (G), Day 26, F	3.52	3.76	3.75	3.83	3.86
Pupal wt (G), Male	0.67	0.66	0.62	0.66	0.69
Pupal wt (G), Female	2.55	2.24	2.36	2.77	2.84
Avg days to pupation, M	29.8	28.8	29.0	29.1	28.6
Avg days to pupation, F	34.9	32.9	33.0	33.4	32.8
Avg days to adult, M	42.8	41.4	42.0	42.0	41.4
Avg days to adult, F	44.0	44.5	44.0	43.6	43.2
Diet = Hi-Wheat Germ - HWG					
Larval wt (G), Day 26, M	0.92	0.92	0.89	0.86	0.86
Larval wt (G), Day 26, F	3.36	3.86	3.69	3.77	3.79
Pupal wt (G), M	0.64	0.62	0.62	0.62	0.70
Pupal wt (G), F	2.63	2.39	2.34	2.47	2.86
Avg days to pupation, M	30.9	29.0	29.2	29.4	29.1
Avg days to pupation, F	36.4	33.5	32.9	32.9	33.4
Avg days to adult, M	44.6	42.2	42.6	42.6	42.2
Avg days to adult, F	46.0	44.3	44.8	45.3	45.3

Table 2. Development of gypsy moths on diet mixed with the Breddo Likwifier vs. Waring Blender.

	Method of Mixing	
	Blender	Likwifier
Avg Larval Wt, Day 26, M	0.99 G	0.87 G
Avg Larval Wt, Day 26, F	2.60	2.65
Avg Pupal Wt, M	0.67	0.63
Avg Pupal Wt, F	2.13	2.32
Avg Days to Pupation, M	30.2	29.7
Avg Days to Pupation, F	33.3	34.0
Avg Days to Adult, M	42.9	41.9
Avg Days to Adult, F	44.9	45.1

Table 3. Effect of type of holding container and changing of food on incidence of malformed gypsy moth pupae.

Treatment	Holding Container[1/]	Food[2/] Change (days after infest)	% Malformed Pupae Males	Females
1	SH-mtrene, 4 oz. (4L/cup)	-	6.9	47.3
2	"	14	2.0	27.9
3	SH Fluted, 6 oz. (5L/cup)	-	5.0	80.9
4	"	14	6.9	55.1
5	Lily, 6 oz. (5L/cup)	-	33.3	95.0
6	"	14	10.0	80.0
7	Dixie can, 16 oz. with 1 (3oz.) solo cup (12L/can)	14	6.5	64.1
8	Dixie can, 16 oz. with 2 (1-3/4 oz.) Dixie cups (12L/can)	14	1.6	89.4
9	Dixie can, 16 oz. with 1 (4 oz.) L04 cup (12L/can)	14	1.9	35.2

Holding containers, mtrene 4 oz. (ME-3R) and fluted 6 oz. (XE-6) are obtained from Sweetheart Plastics; Lily 6 oz. (A6074) from Lily Cup Division, Owens, IL; 16 oz. Dixie cans (paper - 2186 SE), 1-3/4 oz. Dixie cups (waxed paper, 23 ST) and 4 oz. L04 cups (plastic - LD4-11) are obtained from Dixie Cups of American Can Company; Solo cup (P300) from Solo Cup Company.

Table 4. Relative effectiveness of various antimicrobial agents in preventing contamination of hi-wheat germ diet for rearing gypsy moths.

Anti-microbial Agent	Concentration (o/o)	% Rearing Cups w/o Contamination
Sorbic Acid	0.4	100
Methyl Paraben	0.4	100
Formalin (10%)	0.4	100
Sorbic Acid	0.2	96.7
Formalin	0.2	96.7
Potassium Sorbate	0.4	93.3
Glacial Acetic Acid (10%)	0.4	90.0
Sodium Propionate	0.4	86.7
Sodium Benzoate	0.4	83.3
Formalin (10%)	0.1	83.3
Glacial Acetic Acid (10%)	0.2	80.0
Sodium Benzoate	0.2	73.3
Calcium Propionate	0.4	70.0
Sodium Benzoate	0.1	50.0
Sorbic Acid	0.1	33.3
Formalin (10%)	0.05	30.0
Potassium Sorbate	0.2	26.7
Calcium Propionate	0.2	26.7

Containers examined for contamination at 1 week after larvae were infested.

Table 5. Performance of NJF_{15} colony and a PA (wild) strain of gypsy moths[1/]

	NJF_{15}	PA (wild)
Eggs laid/female	831 (165)	876 (211)
Duration of hatch (days)	6.1 (.96)	4.3 (.64)
% hatch	59 (8.9)	41 (8.7)
Pupal weight, males (G)	.65 (.05)	.57 (.03)
Pupal weight, females (G)	2.16 (.14)	1.95 (.28)
% mating pairs	92.5 (8.2)	93.6 (4.7)
Longevity of males (days)	7.3 (1.2)	7.2 (1.7)
% adults recovered ($\bar{x}$ and range)	72 (41-94)	66 (46-85)

1/ Data taken on wild strain that had been collected in midwinter and eggs chilled for an additional 6 months at $5^{o}C$. Standard deviation in parentheses.

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: April 1, 1977 - September 30, 1977
Report Type: Interim
Project Leaders: Charles D. Owens, J. Tardif, R. A. Bell

Clean Air Filters:

Clean air (HEPA) filters are being tested for effectiveness in removing insect setae (hairs and scales) from work areas. The efficiency of various filters in removing airborne particles over 0.3 microns in diameter ranges from 60 to 99.97%. However, the higher the efficiency rating, the more costly the filter. Also, the pressure drop across the filter increases with an increase in efficiency. Because of this increased resistance to air passage, additional blower horsepower is required to force a given amount of air through the highly efficient filters thus increasing the operating cost. A small scale test chamber was constructed to determine the optimal filter for removing undesired airborne contaminants including insect setae, viruses and microbial spores. Filters to be tested have efficiency ratings of 60, 80, 95 and 99.97%.

Clean Air Vacuum System for Handling Larvae:

Although the rooms within the rearing facility are equipped with HEPA filters to prevent an unacceptable load of viable and non-viable contaminants, workers are still exposed to urticating insect material when handling or transferring the insects. To minimize such exposure, a vacuum system was constructed directly over the work areas. Thus, the air is drawn away from the worker by use of an exhaust fan and into a small drum. The air is returned to the work area through a HEPA filter. The vacuum system is not sufficient to remove intact insects - only the dislodged, hair scales and cast skins.

Dust Collector in Adult Holding Room:

Although somewhat noisy, the system devised for removing moths and scales after mating and egg collection generally works satisfactorily (see previous semi-annual report). We have noticed a problem of buildup of insect parts on the fan blade of the cyclone separator. The accumulation results in an unbalanced fan which then leads to a shutdown of the unit so the fan can be removed and cleaned. In accordance with the recommendation of the manufacturer, we have purchased a plastic coated fan which should eliminate the problem.

Portable Work Table with Clean Air Hood:

A special work table, clean air hood mounted on wheels, was constructed for protection of workers in the virus production facility involved in the in-

ɔculation and harvesting of caterpillars. A plexiglass, sloping type hood was mounted on a table. The air is drawn away from the worker through elongated slots in the rear of the table top. A fan mounted within a plenum underneath the table forces the air through industrial grade (60%) HEPA filters and expels the cleaned air back into the room. The work station (ca. 8x2.5') will accomodate about 3 workers. If this prototype unit functions satisfactorily, 4 to 6 man stations will be constructed for use in the final rearing facility.

Virus Production Facility:

To protect workers in the virus production facility during harvest and processing of diseased cadavers, a hood was constructed above the sink area and adjacent counter top. Air from this work area is drawn away from the workers and cleaned by HEPA filters (99.97%) before returning it to the room. Also, to further protect the workers from airborne contaminants and to prevent viral contamination of the adjacent rearing facility, all air within the virus production facility is drawn through absolute HEPA filters installed in the main ducts. In addition, the facility is designed to be maintained under constant negative pressure by exhausting 150 cfm to outdoors after passing it through absolute HEPA filters.

The air within the virus production facility will be monitored to determine levels of airborne viable and non-viable contaminants.

Monitoring Levels of Airborne Contaminants:

Recently a portable air particle counter (Coulter clean air monitor, model 550; Coulter Electronics, Inc.) was demonstrated at the Otis laboratory. Samples of air in various locations were taken and incidence of airborne particles in size categories were counted electronically. The results are shown in Table 1. The kinds of particles in the various locations are yet to be determined but the relative amounts indicate the effectiveness of HEPA filters and air flow design in reducing airborne particles.

There seems to be a relationship between the incidence of airborne particles and microbial contamination. For example, frequent contamination of certain diets held in the environmental chambers (high particle counts) was observed during the summer months whereas contamination was rare in the colony rearing trailer and particularly in the prototype trailer. If further studies reveal a precise correlation between incidence of microbial contamination and gross counts of airborne particles, monitoring with particle counters alone may indicate the probability of microbial contamination.

The adult moth room may need to be modified depending upon the results of direct microscopic examination of the airborne particles. However, it is believed that the combination dust collector-absolute HEPA filter is effective in removing all wing scales from the air. Measurements made before, during and after dumping of large quantities of wing scales into the outgoing air stream did not show an increase in particle counts in the air returned to the rooms. Some revamping of the moth room may be required to further reduce the level of airborne particles.

Table 1. Levels of particles in air sampled in various locations within the laboratory and rearing facility (11-9-77).

Location	$\bar{x}$ particles / ft^3 air / minute	
	0.5 to 5 microns	5 microns+
Library - Conference Room	57,833	269
Environmental Chamber w/o HEPA Filter	62,954	56
Environmental Chamber (with portable HEPA filter)	19,660	46
Rearing Trailer, Colony (vertical air flow, HEPA filter, 95%)	533	42
Rearing Trailer, Prototype (horizontal laminar flow, HEPA filter, 95%)	91	2
Adult Moth Room	1,332	26
Adult Moth Room (during mass dumping of scales)	1,316	-

Project Number: GM 6.3.9
Project Title: Evaluation and Development of Automated Procedures for Rearing Gypsy Moths
Report Period: April 1, 1977 - September 30, 1977
Report Type: Interim
Project Leaders: Charles D. Owens, J. Tardif, R. A. Bell

Of the various operations involved in rearing the gypsy moth (Table 1), processing and dispensing of diet, infesting larvae and harvesting and sexing of pupae require by far the most labor. Therefore, emphasis has been placed on devising methods for mechanizing these operations.

Several units for mixing diet have been tested over the past several months. Of these, the so-called "Likwifier" (manufactured by Breddo Food Products, Kansas City, MO) was the most efficient. The unit is a combination steam-jacketed kettle-mixer. With high pressure steam (40 psi), the combined time to heat water to boiling, mix in the agar and remaining nutrients and pump to an adjacent holding kettle for dispensing should require 10-15 minutes. The 18 gallon unit should handle 72 gallons/hour. The advantage of this machine is that as little as 3-4 gallons can be prepared for test purposes or, if necessary, up to 250 to 300 gallons of diet could be prepared in about 4 hours, i.e. sufficient to rear 100,000 insects/day. The cost of the "Likwifier" is about $6000.00.

Studies this past summer showed that it was possible to infest eggs and obtain yields of pupae of better than 80%. An automatic device for infesting eggs is being obtained from ARS agricultural engineers in Tifton, GA and will be tested for infesting gypsy moth eggs.

Table 1. Operations and worker-hours involved in rearing and handling gypsy moths.

	OPERATIONS	WORKER HRS/10,000 INSECTS
1.	Diet-weighing, processing & dispensing	6*
2.	Infesting larvae	10*
3.	Larval transfer to fresh diet	10*
4.	Sexing & transfer to adult emergence containers	30*
5.	Mating of adults	0.1
6.	Harvesting eggs	0.1
7.	Eggs transferred to chilling	-
8.	Chilled eggs (clean up & disinfection)	0.2
	TOTAL	60 hours

*Operations 1, 3 and 4 may be mechanized resulting in a 75% decrease in cost of labor. Operation 3 may be omitted altogether. Thus, worker-hours/10,000 insects may be reduced from 60 to 12-15.

Size differences between male and female pupae are sufficient to allow separation by a sieving device. This principle will be tested in the near future.

Egg Storage and Chilling Room:

A refrigerated walk-in chamber (10x15') is currently being modified to provide efficient handling and uniform chilling of gypsy moth egg masses. To accomplish this, each day's production of eggs, after pre-diapause development, will be transferred to the cold chamber and placed on a conveyor. Each day the conveyor will be activated by a time clock and rotate the eggs to a new position within the chamber. At 180 days, the eggs will have advanced to the end of the conveyor where they will be removed each day. Therefore, despite some variations in temperature and airflow within the chamber, because of rotation through the chamber during chilling, all eggs should experience similar temperature conditions for the same duration of time.

Mass Rearing Facility:

A facility was designed to handle 122,000 insects per day based on the system that is now being used to mass rear the gypsy moth. The rooms required are as follows: kitchen, 670 square feet; infest and transfer area, 756; diet storage, weighing and container area, 490;colony adult, 162; egg holding, 128; rearing-4 units each with 638; harvesting of pupae for sterile male, 514 square feet for a total of 5272 square feet. Construction of the entire facility was based on using modular panels made of 4 inches of rigid urethane foam with aluminum on each side. The same kind of structure is used in fabrication of frozen food storage warehouses. The wall partitions are mounted on the existing concrete floor of the building. All rooms are to be equipped with temperature control and clean air filtration. The diet storage room will be held at 45°F and 40 to 50% RH to reduce deterioration of wheat germ, vitamins and other critical material during storage. All rooms used to hold insects will be humidity and temperature controlled with air flow designed to give maximum temperature uniformity. The final adult room will be equipped with an air vacuuming and filtering system used in the prototype facility. The proposed facility would cost between $12 and $16 per square foot using the temperature controllin equipment in the prototype facility. The total cost should be between $20 to $25 per square foot.

If a container system was developed to rear more insects per unit of space (especially in the later stages), the overall space requirements could be reduced proportionally. It was estimated that 24 workers would be required to handle 120,000 insects per day with the present system. By eliminating hand infesting of larvae and re-feeding, it should be possible to do the work with 6 workers, 2 of whom would prepare diet and handle infesting of eggs.

Virus Production Facility:

A facility designed to handle up to 12,000 insects per day for 12 days is almost completed. The facility includes a 12x18' rearing room, about 500 square feet of work area, lavatories, office, 8x8' steam room and 8x12 walk-in deep freeze. Workers will enter and leave the facility through an air

Project Number: GM 6.3.15
Project Title: Development of Efficient Methods for Gypsy Moth Virus Production and a Pilot Plant Facility
Report Period: April 1, 1988 - September 30, 1977
Report Type: Interim
Project Leaders: Martin Shapiro, R. A. Bell, J. A. Tanner

In the last report (October, 1976 - March, 1977; pp. 74-79), several factors affecting in vivo virus (NPV) production were identified for further evaluation. The results of these evaluations are presented here.

The primary objective of the research was to define optimal conditions for NPV production. Initially, however, certain standards had to be established involving the test insect, the diet and the virus inoculum. Thus, the colonized NJF_{15} standard strain was chosen as the insect; modified hornworm diet was selected as the standard diet; and the Hamden isolate of NPV was the virus standard.

The sequence of operations involved in NPV production is summarized in Table 1. The scheme involved a single transfer of 14 day old larvae to new diet. It would be desirable, from a cost efficiency standpoint, if larvae were maintained in the same containers throughout the production period and preliminary data indicate the feasibility of this approach.

Table 1. Sequence of steps involved in production of NPV-infected larval gypsy moths (processing excluded).

1. Infested newly hatched larvae (10/1-3/4 oz. cup)
2. Larvae held from Day 1-14 @ 26°C.
3. At day 14, (14 day old, 4th instar) larvae transferred to 6 oz. cup (10 larvae/cup) with 90 ml diet; surface of diet previously coated with 1 ml of aqueous suspension of virus inoculum ($5x10^6$ PIBs/ml).
4. Larvae on inoculated media incubated @ 29°C for 10 days.
5. At day 24 (10 days post inoculation), containers with larvae placed in freezer @ -20°F; virus induced mortality at day 24 = ca. 30%.
6. At day 25, frozen NPV infected larvae harvested and returned to freezer until required for processing.

The results from the large scale production run are summarized in Table 2. The interesting set of figures to keep in mind is the yield of virus from males vs. female larvae. Because they attain a larger size, females produce greater yields of NPV; their utilization would result in a more efficient system.

Table 2. Yield of gypsy moth virus from pilot scale production run.

Total larvae inoculated	=	502,500
Total estimated PIBs recovered	=	1.07×10^{15}
Yield (PIBs/larva)	=	2.04×10^{9}
Range (PIBs/larva)	=	$1.29 - 2.90 \times 10^{9}$
Yield/male larva	=	$\bar{x} = 1.57 \times 10^{9}$
range	=	$8.13 \times 10^{8} - 2.80 \times 10^{9}$
Yield/female larva	=	$\bar{x} = 3.99 \times 10^{9}$
range	=	$2.74 \times 10^{9} - 6.55 \times 10^{9}$

Method of Inoculation:

The virus inoculum is presented to the insect in association with the food. Thus, the route of uptake of the virus is via virus contaminated diet. The virus inoculum may either be incorporated into the diet during its preparation or added later to the surface of the prepared diet. The effectiveness of these two methods for transmitting the inoculum to the host is compared in Table 3. The mortality obtained at 10 days post-inoculation and the yield of virus/larva were much greater when the inoculum was applied to the surface rather than via incorporation within the diet. Incorporation within the diet may have resulted in some heat inactivation during diet preparation; also, less virus is ingested per unit of diet. From a practical viewpoint, much less inoculum is required to obtain a given level of virus yield when applied on a surface treatment.

Table 3. Mortality and NPV yield when inoculum was applied to the surface vs. incorporation in the diet.

Treatment	Mortality [1/] (%)	Yield PIBs/larva
Surface	28.0	1.49×10^{9}
Incorporation	2.8	4.86×10^{8}

[1/] Virus induced mortality at 10 days post-inoculation; T - 29°C.

Table 4 Activity of virus obtained from larvae reared on diet inoculated by surface treatment vs. incorporation method 1/

Treatment	PIBs Required to Obtain LD_{50}
Surface	6.5×10^4
Incorporation	2.5×10^6

1/ Bioassay conducted on 2nd instar larvae at 26°C.

Timing of Inoculation:

The objective of the time of inoculation experiment was to obtain the maximum yield of virus from the minimum dosage in the shortest time. The results of this experiment showed larvae inoculated in the later instars produced more virus than those inoculated in the earlier instars. To obtain a minimum of 1×10^9 PIB/larva, it was necessary to inoculate larvae in the 4th or later instars. When 5th or 6th instars were inoculated, it was possible to obtain greater NPV yields than with 4th instar larvae. The greatest yields were obtained with females, which undergo 6 larval instars as compared to only 5 instars for the male. A system wherein only females, or predominantly females, were reared and inoculated would allow a 2-4x increase in NPV yield with little additional cost input. This prospect is being further investigated.

Table 5. NPV yield in relation to stage when larvae were inoculated 1/

Instar	PIB/larva
2nd	3.96×10^8
3rd	5.63×10^8
4th	1.40×10^9
5th	2.86×10^9
6th (females only)	4.97×10^9

1/ Yields were calculated from virus killed larvae only; T = 29°C.

Temperature:

For routine rearing, gypsy moth larvae are maintained at 25-26°C. To determine the optimal temperature for NPV production, tests were carried out in which larvae were reared, after inoculation, at 23, 26, 29 and 32°C. The effect of temperature was evaluated with respect to mortality, NPV yield, activity and time. It was found that NPV yields and activity from larvae reared at 23, 26 and 29°C were similar (Table 6). Lower yields were obtained from larvae reared at 32°C. Thus, 29°C seemed to be nearly optimal since highest yields could be obtained in the shortest time (Table 7).

Table 6. Effect of post-inoculation holding temperature on NPV yield and activity 1/

Temperature	Yield (PIB/larva)	PIBs to Obtain LD_{50} 2/
23	1.14×10^9	5.0×10^3
26	0.97×10^9	7.0×10^3
29	1.13×10^9	4.0×10^3
32	3.48×10^8	8.5×10^4

1/ Larvae inoculated in late 3rd instar; yields based only on virus killed larvae.

2/ Bioassay conducted on 2nd instar larvae.

Table 7. Effect of temperature on rate of NPV induced mortality and incidence of mortality and yield @ 10 days post-inoculation 1/

Temperature	Time to LD_{50} (days)	Mortality (%) at 10 days	Yield/Larva at 10 days
23°C	22	8	7×10^8 PIB
26°C	19	18	1.30×10^9
29°C	∿10	44	2.76×10^9
32°C	<10	54	4.76×10^8

1/ Inoculated at 5th instar; yields calculated from virus killed larvae only.

Data presented in Table 7 confirm the previous results. As the temperature was increased, the LT_{50}, i.e. the time to kill 50% of test larvae, decreased. When virus was harvested at day 10, the maximum yield was obtained at 29°C. Again, a higher temperature (32°C) resulted in a lower virus yield.

Optimal Inoculum Dose:

In the production run, an inoculum dose of 5×10^6 PIB/ml/cup was utilized. This dosage was sufficient to inoculate 10 or more larvae and still permit the desired growth and virus yield. As the concentration increased to 1×10^6/ml/cup, the yield of virus/larva increased (Table 8). A greater initial virus concentration did not necessarily result in greater virus yields, however, in the majority of tests, the maximum virus yield was obtained using 5×10^6/ml/cup.

Table 8. Relationship between inoculum dosage, NPV induced mortality and yield 1/

Inoculum Dosage	Mortality (%)	Yield (PIB/larva)
$1x10^5$	3	$5.99x10^8$
$5x10^5$	10	$9.41x10^8$
$1x10^6$	17	$1.44x10^9$
$5x10^6$	24	$1.46x10^9$
$1x10^7$	35	$1.77x10^9$
$5x10^7$	63	$1.44x10^9$

1/ Inoculation in early 4th instar; mortality and yield based on counts obtained at 10 days post-inoculation; T = 29°C.

Density of Larvae:

For virus production, 10 fourth instar larvae were maintained in a 6 oz. cup. This number was chosen because growth and development was normal and crowding was not considered excessive. When larval density was related to virus yield/larva (Table 9), the results were roughly density-dependent. In the majority of tests, the maximum yield was obtained when larvae were reared singly. Ten insects/6 oz. cup provided a good virus yield, and this number has been routinely used as the standard larval density.

Table 9. Relationship between larval density and NPV yield 1/

Larvae/Container	Container Volume	Yield (PIB/larva)
1	50 mm^3	$2.11x10^9$
5	180 mm^3	$2.07x10^9$
10	180 mm^3	$1.94x10^9$
15	180 mm^3	$1.71x10^9$
20	180 mm^3	$1.41x10^9$

1/ Inoculated in 4th instar (day 14) and held @ 29°C.

Harvest - Time of Harvest vs. Recovery vs. Yield:

In the standard NPV production schedule, larvae are harvested at day 10, i.e., the time of ca. 30% mortality. Larvae were harvested at this time in order to (1) minimize wilting of virus-killed larvae and loss of recoverable virus and (2) minimize the bacterial load per larva. The virus yield from day 10 harvested insects was comparable to that from larvae harvested on subsequent days (Table 10). At present we are investigating a possible "maturation"effect, e.g., as the virus kill increases, the activity per viral inclusion body increases. Preliminary data indicate that viral activity increases (up to 5 fold) as the incidence of NPV induced mortality progresses from 0 to 100%. Further testing, however, is needed to confirm this important observation.

Table 10. Incidence of NPV-induced mortality and yield in relation to time of harvest after inoculation.

Time (in days) after inoculation	Mortality (%)	Yield PIB/larva
6	0	1.30×10^8
8	1.4	1.05×10^9
10	31.7	1.75×10^9
12	81.9	2.07×10^9
14	97.8	1.53×10^9
16	100.0	2.07×10^9

Processing:

For the initial production run, infected larvae were shipped in a frozen state to Boyce Thompson Institute, Yonkers, NY for processing to yield the final air dried powder. The major steps in the processing involved blending, centrifugation and drying. At Otis much work has been carried out to develop an effective and efficient processing procedure.

Blending:

For all processing, the standard procedure is to blend 1 gm of larval weight in 10 ml of water for 60 seconds. Following filtration through coarse cheesecloth, ca. 95% of the PIBs can be recovered from infected larvae.

Initial tests compared the relationship between dilution (gm larvae/ml H_2O) and PIB recovery. At dilutions of 1:1 to 1:5, less than 40% of all PIBs were recovered after a single blend. At a dilution of 1:10, however, recovery was increased to 94%. At greater dilutions recovery was only slightly increased.

Blending time can be very important as inadequate blending time will result in low yields. At a larval weight/H_2O dilution of 1:10, a 5 second blend resulted in a recovery of 75% of the total available PIBs. When the blend time was increased to 15 seconds, recovery was increased to 98%. Additional blending time resulted in little additional recovery.

Centrifugation:

In general, centrifugation has been used to concentrate the virus prior to air drying. Centrifugation, if it is to be economical, cannot be differential but must be carried out in a single run. At our laboratory, the filtrate with virus was centrifuged at ca. 7000 g for 20 minutes. Thereafter, the pellets were removed and air dried. In addition to concentrating the virus, centrifuging allows one to remove most of the water incorporated during the blending process.

The question naturally arises as to whether or not centrifugation is necessary to obtain the desired virus product. Certain preliminary tests involving elimination of the centrifugation step indicate that a suitable virus product may be obtained by direct air drying of the filtrate. This approach is being given further study.

Drying:

The objective of our research is not only to maximize virus yield/larva and to produce highly active virus but also to minimize the bacterial "load"/larva. To meet this objective we chose to harvest larvae on day 10 post-inoculation. From a bacteriological point of view, the bacterial population per larva should increase as the number of virus killed larvae increases. Data from Table 11 indicate that this premise is true.

Table 11. Bacterial loads in living and infected and virus-killed *Lymantria dispar* larvae.

Days after Inoculation	Living, Infected	Aerobes/Larva Virus Killed	Total (L+D)/# of larvae
6	6.785×10^5	-	6.785×10^5
8	6.178×10^5	1.511×10^7	9.338×10^5
10	4.925×10^5	3.831×10^7	13.61×10^5
12	3.973×10^6	3.761×10^7	302.8×10^5

It should be noted that these counts were made prior to processing and drying, i.e., they represent the inherent bacterial "loads".

Bacterial Load of Water Source:

It may be important to determine the bacterial levels present in H_2O used in blending. Unfortunately, significant differences may exist which would contribute to the "pre-drying" bacterial load.

In a series of tests, tap water, distilled water and sterile distilled water were compared as diluents. Data from these tests indicate that bacterial levels in tap water were quite variable on a day-to-day basis. On some days, the levels were high. Distilled water levels were high, only after prolonged storage in a holding tank. Data from Table 12 indicate the variability of Otis tap water.

Table 12. Bacterial loads in *L. dispar* larvae after blending in different water sources a/

Water Source	Aerobes/gm I (8/5/77)	Aerobes/gm II (8/8/77)
Sterile Distilled	1.710×10^7	9.377×10^6
Distilled	1.740×10^7	2.821×10^7
Tap	1.669×10^7	2.325×10^9

a/ Larvae harvested at day 10 post-inoculation.

Subsequently, distilled water was used routinely as the diluent in processing tests.

Method of Drying vs. Bacterial Content:

At present, the gypsy moth virus product to be registered is specified as an air-dried powder. In the production run, Boyce Thompson Institute utilized a clean air vertical laminar flow hood and air dried the centrifuged viral pellet under prevailing ambient conditions.

In tests here at Otis, we compared the efficiency of a horizontal laminar flow chamber (16"Lx13"Wx11"H) equipped with a dehumidifier vs. a vertical laminar flow hood (73"Lx25"Wx28"H) under ambient conditions. Both were equipped with HEPA filters (99.97%). Typical tests are summarized in Table 13.

Table 13. Bacterial loads in NPV product before and after air drying in laminar flow (clean air) hoods.

Pre-dried Product (aerobes/gm)	Post-dried Product (aerobes/gm) Horizontal with Dehumidifier	Post-dried Product (aerobes/gm) Vertical
2.611×10^7	3.449×10^8	1.325×10^9
8.531×10^7	4.143×10^9	-
8.654×10^7	-	6.107×10^9

In both cases, the post-dried bacterial load was higher when dried in the vertical-flow hood. In general, this increase resulted from slower drying under ambient conditions. In all subsequent tests, the horizontal flow chamber was used.

Dehumidification:

The horizontal flow chamber was adapted so that the chamber could be dehumidified with a Bry-air dehumidifier. During full dehumdification, the temperature in the chamber increased from 75° to 100°F, while the relative humidity decreased from 40% to 0%. When the dehumidifier was on 50% of the time, the temperature increased to 90°F, while the relative humidity dropped to 20%. The product can be dried much faster in dehumidified air thus avoiding the buildup of bacteria that occurs with conventional air-drying.

Volume vs. Bacterial Load:

The volume of material affecting the ultimate bacterial load to be air dried is a critical factor. As the volume increases, the time for drying increases and the final bacterial load in the product increases (Table 14).

Table 14. Bacterial content before and after drying in relation to volume of NPV suspension and method of drying.

Treatment and Volume of NPV Suspensions	Aerobes/gm Pre-Dried Preparation	Aerobes/gm Post-Dried Product
Air-Dry		
100 ml	4.49×10^7	1.01×10^8
250 ml	3.95×10^7	8.78×10^8
500 ml	4.66×10^7	172.8×10^8
Freeze-Dry		
210 ml	4.526×10^7	1.81×10^7

Freeze-drying resulted in a decrease in total aerobes/gm and these data were repeated consistently.

Bacterial Density:

The lower the original bacterial sample in the product, the lower will be the final bacterial load (Table 15).

Table 15. Relationship of initial bacterial load to final bacterial load in air dried product.

Initial ($x10^6$)	Aerobes/200 ml Sample Final ($x10^6$)
103.2	1335.5
49.8	558.8
27.1	25.9
12.14	8.3
7.8	2.8
3.32	0.8

These data indicate the importance of the initial bacterial load using air drying (with dehumdification) as the drying system. If freeze drying were used, the final bacterial load would be no greater (and probably less) than the initial bacterial load.

At present, a series of tests is being completed involving: (1) use of an air conditioner to reduce the drying chamber temperature and (2) use of both horizontal and vertical circulation within the drying chamber.

A good deal of effort is being made to utilize freeze drying as a feasible system. Instead of the standard blending, followed by drying, we are investigating the following system: intact harvested---freeze---freeze dry---dehair larvae.

Following dehairing, the larvae are blended and sieved. Thus, the resultant virus powder should contain a minimal amount of urticarious and allergenic setae and a maximal amount of virus.

Allergenicity tests will be conducted at the U. S. Forestry Lab, Hamden, CT.

Storage of Virus:

Tests are underway to determine the effect of (1) freezing-thawing and (2) long term storage upon the gypsy moth NPV.

Table 16. Effect of freezing and thawing on NPV virus activity.

Treatment	LD_{50} (PIB/ml)
Control (refrigerated)	
1	$3.6x10^3$
2	$2.8x10^3$
Frozen and thawed inoculum # of times frozen & thawed	
1	$2.4x10^3$
4	$3.0x10^3$
7	$3.0x10^3$
9	$3.2x10^3$

Samples of concentrated (1.4×10^8 PIB/ml) viral suspensions were frozen and thawed up to 10 times. Following treatments, the suspensions were bioassayed against early 3rd instar larvae. Freezing and subsequent thawing did not have any detrimental effect upon virus activity. Long term storage tests are underway comparing the efficacy of various diluents, i.e., water, glycols, glycerin, Dimethyl sulfoxide (DMSO). Preliminary data indicate that (after 1 month storage) virus diluted in polyethylene glycol (10%), ethylene glycol, monoethyl ether (10%), and glycerin (10%) is not as active as virus diluted in water, DMSO (10%) or diethylene glycol (10%). These tests will be continued for at least 6 months.

Males vs. Females for NPV Production:

Previous production data (Table 2) indicated that a greater yield was obtained from female larvae. Moreover, preliminary data show that, for a given virus dose, females die at a faster rate than males. Results from a test are summarized in Table 17.

Table 17. NPV induced mortality and yield in male and female *L. dispar* larvae[1/]

Days Post-Inoculation	Mortality (%)		PIB/larva x 10^8	
	Male	Female	Male	Female
12	34	56	8.5	30.8

1/ Inoculated 5th instar larvae; 5×10^6 PIB/ml/cup.

When females only are harvested after 100% mortality the yield/larva can increase to as much as 7.6×10^9. Much emphasis and study are being placed on the use of females for virus production.

Recently Initiated Research:

At present, we are investigating (1) the effect(s) of juvenile hormone compounds upon viral replication and (2) enhancement of viral activity by means of biological serial passage.

Virus Production Facility (Prototype):

The virus production research facility is in its final phases of construction. When completed, we will be capable of studying all facets of virus production in an isolated facility. This facility will also serve as a prototype facility.

Project Number: GM 7.3.1

Project Title: Gypsy Moth Mass Rearing: Hatch Rates of Laboratory and Wild Strains of Gypsy Moth Eggs as Affected by Duration and Temperature of Chilling

Report Period: April 1, 1977 - September 30, 1977

Report Type: Final

Project Leaders: John Allen Tanner, R. A. Bell, C. D. Owens, M. Shapiro, O. T. Forrester, J. J. Baker

This experiment has been suspended until reliable test equipment is procured.

Project Number: GM 7.3.3
Project Title: Weight Changes in Gypsy Moth Egg Masses During Pre-Diapause, Diapause and Post-Diapause Development
Report Period: April 1, 1977 - September 30, 1977
Report Type: Interim
Project Leaders: 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 is designed to quantify weight changes in newly deposited egg masses.

Laboratory reared New Jersey F_{15} and wild gypsy moths were mated and allowed to oviposit on craft paper. The resulting egg masses were collected 48 hours after mating. The excess paper was trimmed to about 5 mm of each mass to maintain the mass and to facilitate handling. Wild male and female pupae were collected, eclosed and mated in containers to eliminate weight losses caused by the egg parasitoid, *Ooencyrtus kuwanai*.

Pre-diapause development periods used were natural conditions, 14, 21 and 28 days. Ten egg masses each of NJF_{15} and wild were used for each treatment. The egg masses were weighed daily during the pre-diapause development and at seven and 14 day intervals for 84 days. Mean weight changes for each treatment are given in Figures 1 through 4. The percent weight loss of the egg masses is given in Table 1.

The incidence and duration of hatch from each treatment will be observed and optimum pre-diapause development period determined.

Table 1.

Pre-Diapause Developmental Period	Strain and Percent Wt. Loss NJF_{15}	Wild
Natural*	5.6	5.2
14 days (26°C)	4.8	5.7
21 days (26°C)	8.0	7.6
28 days (26°C)	8.7	9.2

* Natural % weight loss determined at 28 days.

Project Number: GM 7.3.1

Project Title: Gypsy Moth Mass Rearing: Hatch Rates of Laboratory and Wild Strains of Gypsy Moth Eggs as Affected by Duration and Temperature of Chilling

Report Period: April 1, 1977 - September 30, 1977

Report Type: Final

Project Leaders: John Allen Tanner, R. A. Bell, C. D. Owens, M. Shapiro, O. T. Forrester, J. J. Baker

This experiment has been suspended until reliable test equipment is procured.

Figure 1.

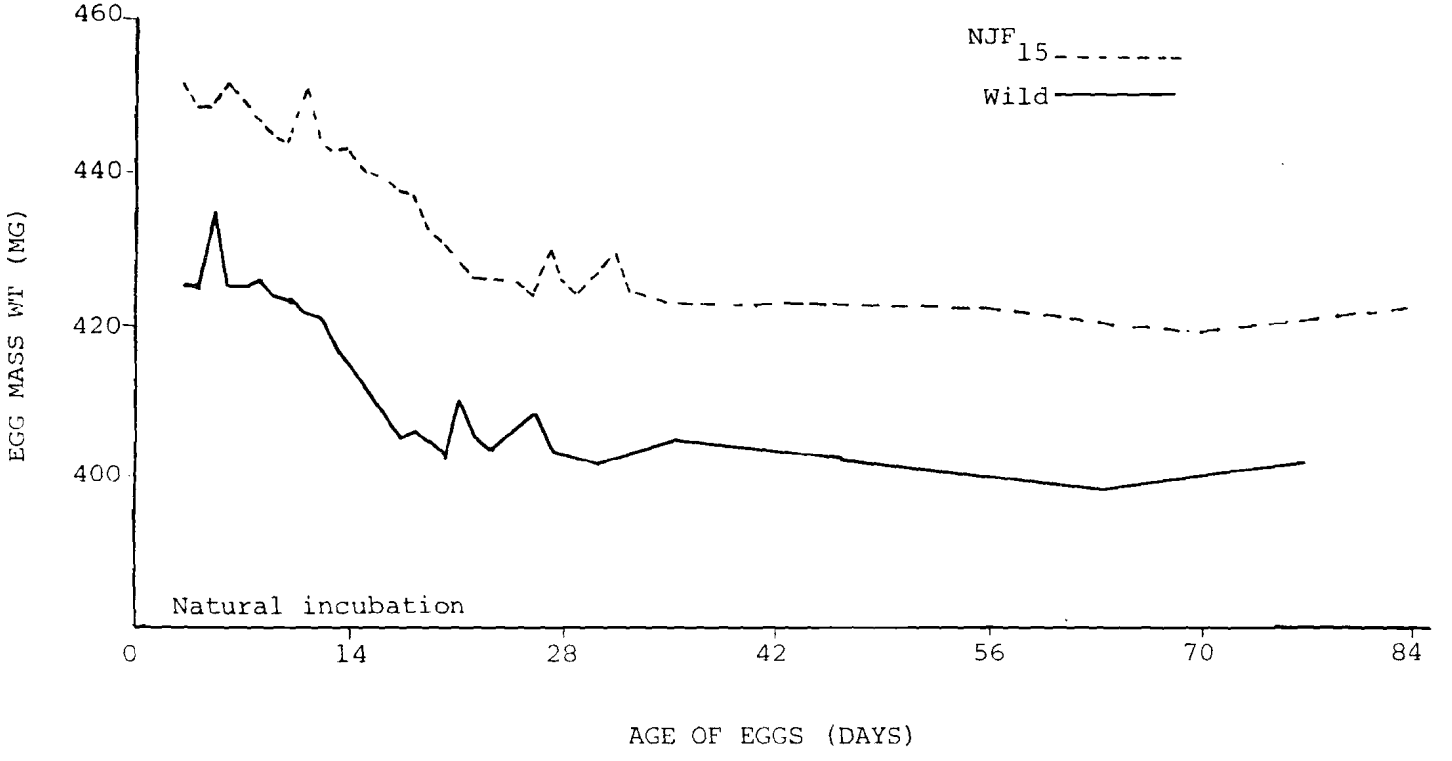

460
440
420
400
EGG MASS WT (MG)
NJF15
Wild
Natural incubation
0
14
28
42
56
70
84
AGE OF EGGS (DAYS)

Figure 2.

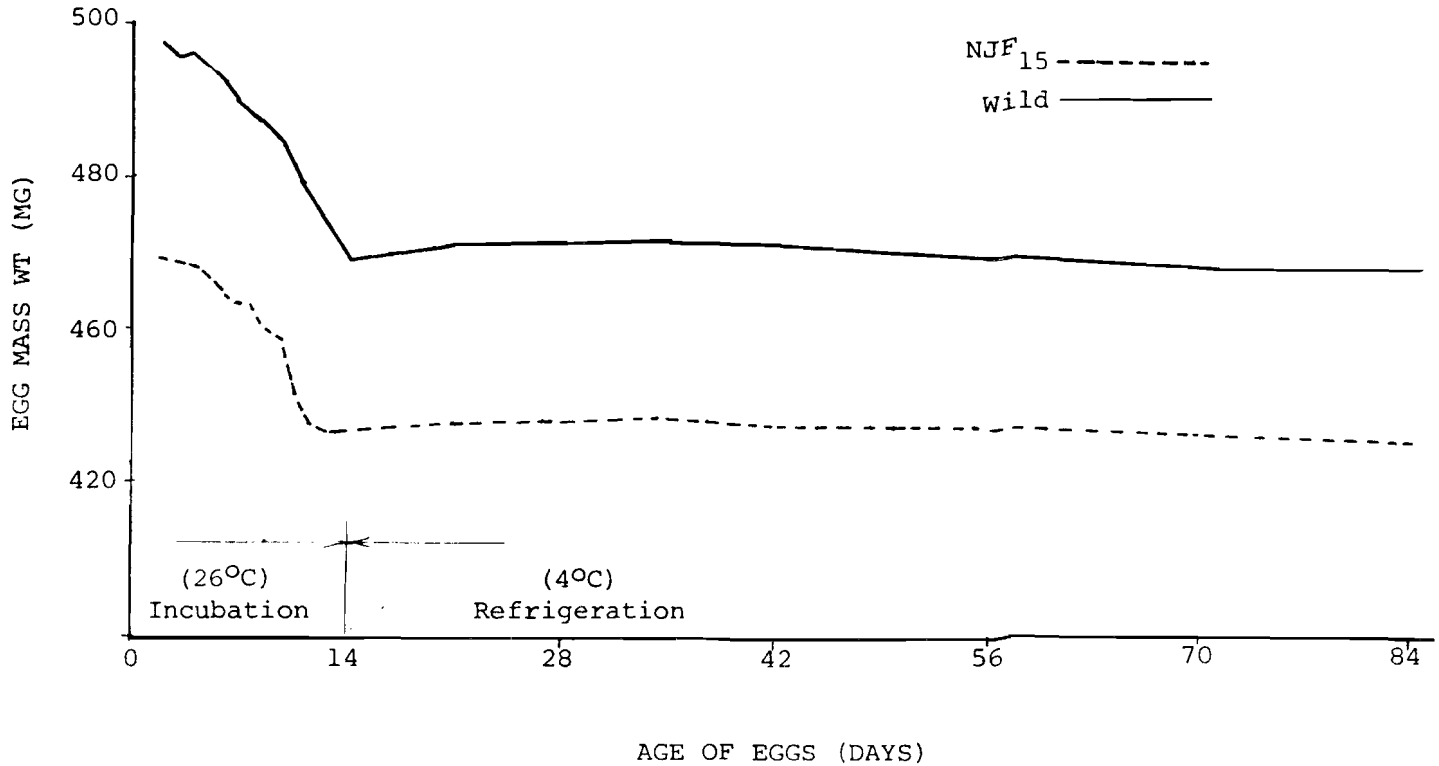

500
480
460
420
EGG MASS WT (MG)
NJF15
Wild
(26°C)
Incubation
(4°C)
Refrigeration
0
14
28
42
56
70
84
AGE OF EGGS (DAYS)

Figure 3.

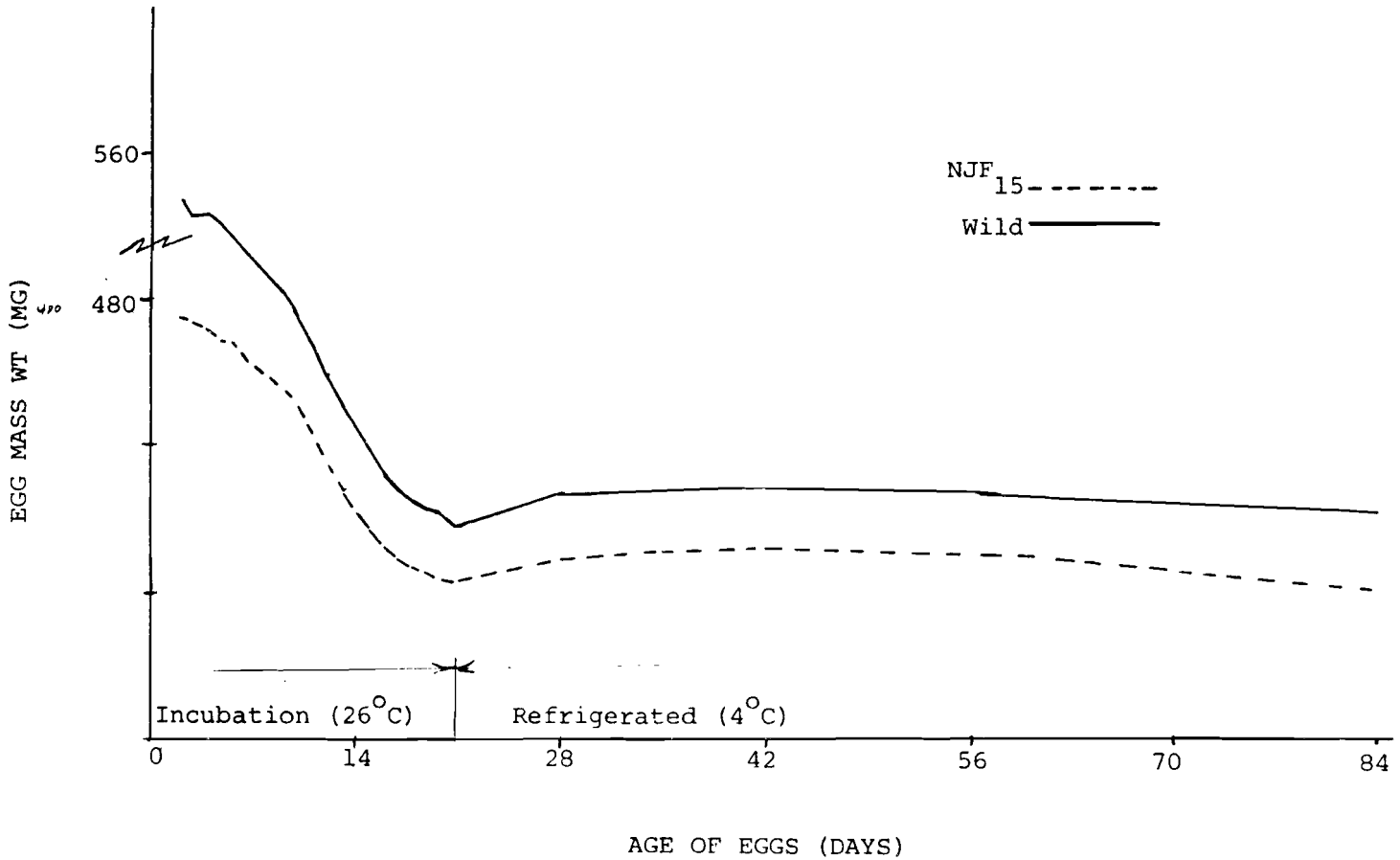
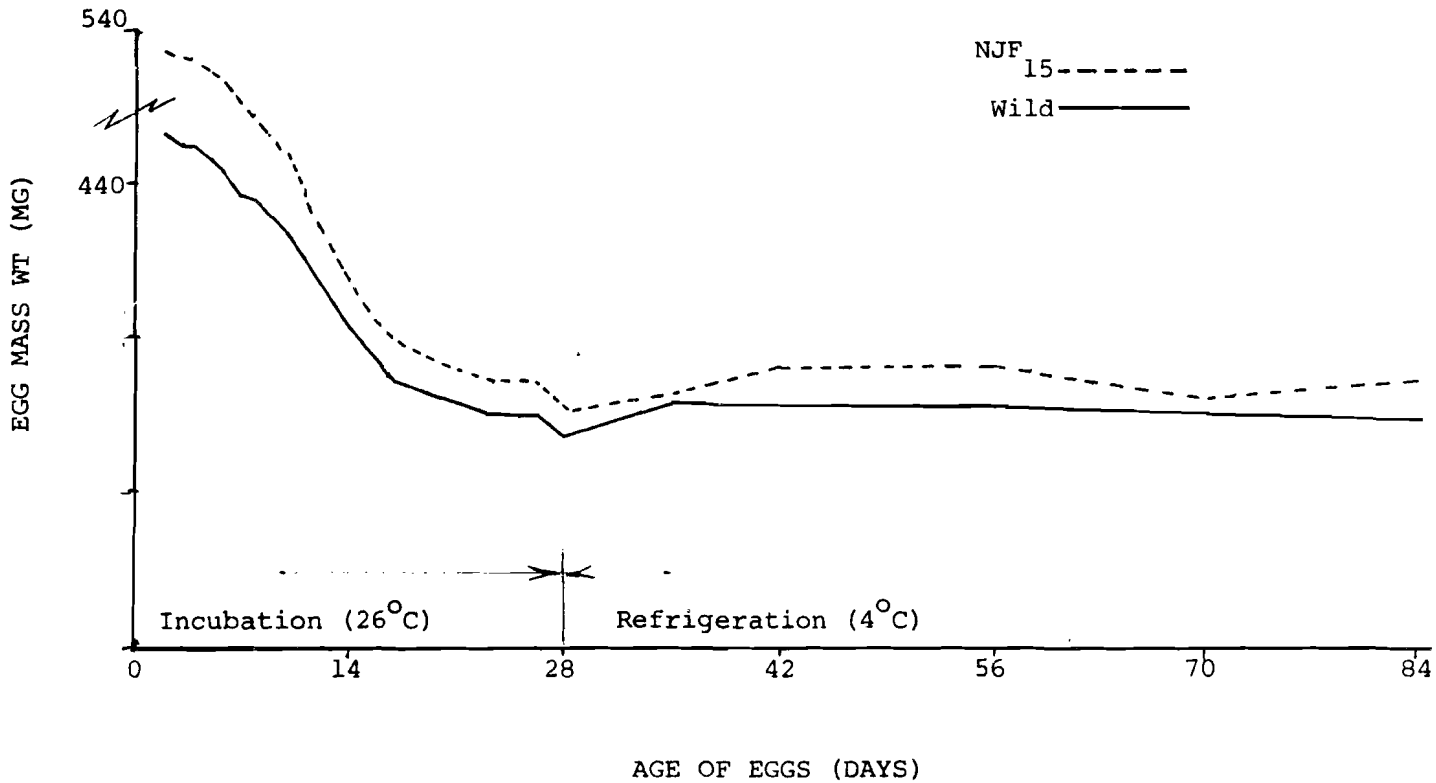

560
480
EGG MASS WT (MG)
NJF15
Wild
Incubation (26°C)
Refrigerated (4°C)
0
14
28
42
56
70
84
AGE OF EGGS (DAYS)

Figure 4.


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)

Project Number: GM 7.3.4
Project Title: Insect Production and Distribution
Report Period: April 1, 1977 - September 30, 1977
Report Type: Interim
Project Leaders: Leonard F. Kennedy, J. J. Baker, O. T. Forrester

The primary objective of the rearing facility of this laboratory is to produce sufficient quantities of all phases of the gypsy moth life cycle in support of research projects at this laboratory, several universities and experiment stations in the United States and foreign countries.

Following is a summary of production and distribution of such material.

Production:

Infested	904,100 neonates
Refrigerated	10,678 egg masses

Distribution:

Methods Development and ARS, Otis

Insecticide Screening

1st instar	10,300
2nd instar	39,190
3rd instar	12,225
4th instar	5,635
5th instar	2,585
Egg Masses	800

Ovicide Tests

Adult Females	300
Adult Males	300

Pheromones

Adult Males	7,400
Adult Females	600
Diet & Container Testing	25,000

Virus Studies

1st instar	1,000
2nd instar	68,998
3rd & 4th instar	12,100
5th instar	2,200

Cooperators

Michigan State University - R. Carde'

Male pupae	30,400
Female pupae	2,500

University of Massachusetts - P. Barbosa

3rd & 4th instar	2,400
Female pupae	2,400

Connecticut Agricultural Experiment Station - J. Anderson

Female pupae	2,800

University of Maine - D. Leonard

Female pupae	300

New York Agricultural Experiment Station - J. Miller

Male pupae	1,600

Forest Service, Hamden, CT - H. Smith (predators)

4th & 5th instar	19,720
Female pupae	10,700
Male pupae	14,475

Forest Service, Hamden, CT (parasites)

5th & 6th instar	40,520

Bio-Serv, NJ

Egg masses	2,300

Pennsylvania State University - Mumma

Egg masses	100

Agricultural Research Service, Beltsville, MD - R. Webb

Female pupae	1,000

Agricultural Research Service, Beltsville, MD - Addler

Female pupae	25

Department of Environmental Resources, Middletown, PA - R. Fusco

Egg masses	100