October 1, 1979 - September 30, 1980

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

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Summary

Japanese beetle (JB) testing in airports demonstrated the efficacy of the organophosphate insecticide, Amaze, in reducing larval populations. It has also been concluded that placement of traps (1 trap/acre) in airports sparsely infested with JB reduces regulatory hazard and may actually exert control on the population. Prototypes of novel, large capacity traps for use in this context were developed and tested.

Extensive evaluations of dust formulations of conventional insecticides have been conducted in an effort to develop effective treatments for recreational vehicles and regulated articles. Dylox, Diazinon, Sevin and Dimilin dust formulations were found to have very good contact effects on 2nd instar larvae walking on dusted surfaces. Rotenone and Malathion were essentially inactive. Other tests concerning ovicidal properties of candidate materials demonstrated that Sponto H-44C, Insecticidal Soap, Pine Scent, Top Job and light water may be useful in regulatory treatments. Thirty-nine ultraviolet opaque materials were screened for possible incorporation into formulations adversely affected by ultraviolet radiation (BT, Gypchek). OU-88, OU-26, DOBP, Escalol 507 and Parsol MCX were identified as excellent UV screening agents in Gypchek mixes.

Dosage response and weathering tests were conducted with UC-62644, Bendiocarb, DPX-5444, U-56295, Kryocide, diatomaceous earth, U-57770, Bolstar, Basic-H, Malathion, Marlate and Insecticidal Soap. Marlate (Methoxychlor) is frequently used by arborists for gypsy moth control. It was demonstrated to have poor activity and deposit retention characteristics.

Tests were conducted which demonstrated very poor weathering properties of BT formulations; this deficiency was remedied by addition of sticker to the mixes. Twenty-six stickers were screened and several were selected for additional study and development (RA-1645, Acrylocoat, Rhoplex B-15, Plantgard, Rhoplex A6-33). Other tests concerning feeding deterrency of various BT formulations demonstrated that this may be a significant factor in formulation efficacy; substantially less feeding occurred on treated foliage than on that untreated.

The herbicides DEF-6 and Ethrel were tested for defoliating red oak seedlings.

The growth regulators Dimilin and SIR 8514 and Thuricide 16B and 24B were evaluated for gypsy moth population suppression in mixed-hardwood forest. Reduction in egg mass density resulted from one application of SIR 8514 (0.03 lb AI/acre) at one-half gallon per acre. Eighty-two percent reduction in egg mass density was achieved with two applications of Thuricide 16B (8 BIU AI/acre) at one-half gallon per acre. The other Thuricide treatments failed to prevent population increases. This failure was partly attributed to rainfall which disrupted the spray schedule and possibly washed some deposited material from foliage. In contrast with previous field trials, Dimilin treatment coinciding with foliage budbreak did not effect control, suggesting inadequate coverage of expanding foliage.

A series of laboratory wind tunnel tests was conducted to determine the effect of rearing temperature on the pheromone mediated behavior of male moths. While pupal holding temperatures did not affect the ability of moths to perceive pheromone and fly in the wind tunnel plume, a number of differences among the temperature treatment groups (pupae held at 20, 25, 30°C and fluctuating temperature) were observed. Moths from pupae held at cooler temperatures were more responsive to pheromone earlier in the day than those reared at higher temperatures and males from fluctuating temperature holding conditions may be more prone to appetitive flight. Males held at 30°C required longer periods of wing fanning before taking flight, were less likely to take flight, displayed less sustained flight in the plume and did not show periodicity of responsiveness similar to that of other groups.

We have been adapting an actograph for monitoring male moth activity in the laboratory and have found that, if males from 2 treatments had different periodicities of recapture at phermone sources in the field, then they also had similarly different periodicities of activity in the actograph. Because of this, we are confident that actograph data can be used to predict male sexual periodicity. Further, the sexual periodicity of NJS males can be manipulated by adjusting the temperature at which they are held as pupae. We soon should be capable of producing sterile males whose sexual periodicity is in synchrony with the sexual periodicity of males from target populations.

Field tests designed to determine sterile male overflooding ratios necessary to effect control resulted in fewer sterile matings than expected, indicating decreased competitiveness of sterilized moths. These tests in simulated populations, however, demonstrated that released insects interacted measurably with the fertile population.

The sterile male technique was pilot tested in an isolated infestation in Berrien Co. Michigan. Sterile:fertile ratios were monitored throughout the season and were lower than desired during peak flight. While effects of the release will not be known until the 1981 field season, a number of important logistical and biological problems associated with the large scale application of the technique were identified.

Trapping studies were conducted in 104 compgrounds in an effort to relate regulatory risk to adult moth density. No relationships were found among number of moths captured, egg mass density and risk rating.

Tests were conducted to optimize the efficiency of large capacity traps for use in delimitation survey. Optimum trap size, entry port number and size, hood size and killing agent concentration were determined. An improved dispenser of (+) disparlure was also developed and field tested. Several experiments were conducted to assess the biological activity of various sources of (+) disparlure and only minor differences were observed.

Substantial reductions in trap catch and female mating incidence were observed in plots treated with 50 g disparlure/ha (Hercon and Albany Internation formulations). These reductionns were greatest at low population densities (2 egg masses/ha) and least at higher levels (200 egg masses/ha). Preliminary observations indicate, however, that populations in treated plots increased in density.

Mass trapping was tested in simulated populations of 2 ♀♀ /ha and 10 ♂♂ /ha. Trap density of .75 traps/ha reduced female mating 38% and 25 traps/ha caused 82% mating reduction.

Additional refinements have been made in procedures used for monitoring the quality of insects produced in the rearing facility. This system was used for evaluating various changes in rearing methods (stacking rearing containers, infesting with 2nd instar larvae, duration of egg storage, and diet ingredient substitution).

PESTICIDE TESTING SECTION

Project Number	Project Title		Report Type	Page
JB 9.1.1	Management of Japanese Beetle Populations in and Around Airports	McLane	Final	5
GM 6.1.5	Regulatory Treatments	McLane	Interim	8
GM 8.1.1	Evaluation of Ultraviolet Screening Agents for Pesticides and Microbials	McLane	Interim	14
GM 8.1.3	Laboratory Screening of Candidate Pesticides Against the Gypsy Moth	McLane	Interim	19
GM 9.1.2	1979 Field Tests of Bay SIR-8514, Dimilin and *Bacillus thuringiensis*	McLane	Final	44
GM 0.1.2	Field Studies with SIR-8514, *Bacillus thuringiensis* and Gypchek	McLane	Interim	53

PHEROMONE, BEHAVIOR AND ECOLOGY SECTION

Project Number	Project Title		Report Type	Page
GM 7.2.4A	Development of Laboratory Evaluation Techniques for Gypsy Moth Male Comparisons - The Effect of Temperature on the Pheromone-mediated Behavior of Laboratory-reared Male Gypsy Moths	Lance	Interim	68
GM 7.2.4B	Development of Laboratory Evaluation Techniques for Gypsy Moth Male Comparisons - Comparing the Pheromone-mediated Behaviors of Wild vs Laboratory-reared Males	Lance	Interim	85
GM 8.2.2	Radiological Sterilization of Male Gypsy Moths	Schwalbe	Interim	89
GM 8.2.3	Gypsy Moth Mating Behavior	Schwalbe	Final	90
GM 8.2.6	Large Capacity Milk Carton Traps - Design Improvements	Paszek	Interim	91
GM 9.2.2	Efficacy of Different Overflooding Ratios of Sterile Males in Simulated Populations	Mastro	Final	99
GM 9.2.3	Trial Sterile Male Field Test in a Simulated Sparse Population	Mastro	Final	107
GM 9.2.5	1979 Field Evaluation of Micro Dispersable Formulations of Disparlure for Mating Disruption	Schwalbe	Final	112
GM 9.2.6	Monitoring Populations of Gypsy Moth in Campgrounds with Disparlure-baited traps	Mastro	Final	113

PHEROMONE, BEHAVIOR AND ECOLOGY SECTION

Project Number	Project Title		Report Type	Page
GM 0.2.1	Whether Wicks Weather	Simser	Final	114
GM 0.2.2	Preseason Bioassay of Recently Synthesized (+) Disparlure	Lance	Final	117
GM 0.2.3	Evaluation of (+) Disparlure used in Detection and Delimitation Survey Programs	Simser	Final	122
GM 0.2.4	Comparative Evaluation of (+) and (±) Disparlure Formulations in Eliciting Male Trap Catch	Simser	Final	126
GM 0.2.5	1980 Field Evaluation of Hercon and Conrel Formulations of Disparlure for Disrupting Mating at Varying Population Densities	Schwalbe	Interim	128
GM 0.2.6	Development of Mass Marking Techniques for Gypsy Moth Male	Mastro	Final	130
GM 0.2.7	Sterile Male Trial Berrien County, Michigan	Mastro	Interim	132
GM 0.2.8A	Factors Affecting Sexual Periodicity in Male Gypsy Moths - Actograph Studies	Lance	Interim	141
GM 0.2.8B	Factors Affecting Sexual Periodicity in Male Gypsy Moths	Lance	Interim	152
GM 0.2.9	The Effect of Temperature on the Duration of the Pupal Stage	Mastro	Final	158
GM 0.2.10	Preseason Bioassay of Recently Synthesized (+) Disparlure	Mastro	Final	162
GM 0.2.11	Mass Trapping of Male Gypsy Moths	Simser	Final	166a

PHYSIOLOGY AND MASS REARING SECTION

Project Number	Project Title		Report Type	Page
GM 6.3.6	Establishing Standards	Tanner	Interim	167
GM 8.3.2	Rearing Technique and Production Costs	Forrester	Final	176
GM 8.3.4	Evaluating the Sampling Techniques used to Establish Standards and Monitor Production	Tanner	Interim	177
GM 9.3.2	Silk Removal from Gypsy Moth Pupae	Forrester	Final	182
GM 9.3.5	Comparison and Evaluation of Rearing Techniques	Forrester	Final	183
GM 9.3.6	Substituting Supro-610 for Casein in the Gypsy Moth Diet (B-4) Under Mass Rearing Conditions	Tanner	Interim	184

Project Number: JB 9.1.1
Project Title: Management of Japanese Beetle Populations in and Around Airports
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leader: Winfred H. McLane

Japanese beetles frequently infest a number of major airports in the eastern United States and in some cases populations are very heavy. They pose a threat to agricultural crops in the western part of the country because beetles may be transported there on aircraft.

During 1979, pilot tests of multi-component control strategies were conducted to determine the most effective means for reducing beetle populations at airports to non-hazardous levels. A number of techniques were tested: adult treatments with carbaryl, trap crops, granular treatments of Dasanit and Amaze for grub control and mass trapping. At hazardous airports, aircraft were treated with d-Phenothrin and mechanical barriers were used in some cases.

The end result of the 1979 work was a greatly reduced number of live beetles transported to the western part of the United States. Although airports treated with Dasanit and Amaze were ultimately regulated, approximately 80 percent of the grubs present were eliminated by the treatments, thus lessening risk.

It was demonstrated that, in some cases, mass trapping (at least 1 trap per acre) can be an effective tool for control. At Dulles we were able to hold a light infestation in check with traps, to the degree that the airport could have stayed in a non-regulated status.

One of the main problems associated with mass trapping programs is the frequency at which traps have to be emptied. During 1979, traps at Cincinnati were being emptied two times a day and each time around, most traps were running over. With a larger capacity trap more beetles would be captured and far less man power would be needed.

Mr. J.G.R. Tardif and Win McLane met with personnel at the Wooster, Ohio Japanese Beetle Laboratory and discussed trap design and looked at traps that have already been tested. Thereafter, we designed 6 high capacity trap styles: a galvanized baffled trap on a 5 gallon collecting container, a cut baffled galvanized trap on a 5 gallon collecting container, a regular standard yellow trap on a 5 gallon collecting container and each of the above fitted to a hole in the ground as a collecting container.

Because of our workload, traps were not placed in the field until July 24. They were picked up on September 2, 1980. The commercially available Bag-A-Bug, standard 0.5 gallon traps, blank traps and standard traps with granular lure only were used as standards. All traps were treated with pheromone and euginol liquid lure except the bag-a-bug traps, blanks and those with granular lure only. Additional granular lure was added to those traps every 2 weeks. Each trap design was replicated 5 times. Traps were placed in an open field at Otis Air Base on 5 parallel lines. A line consisted of 1 replication of each trap design plus standards. Lines and traps were 200 feet apart.

Table 1. Total number of Japa
july 24 - September 2, 1980.

Type of Trap	Number Beetles Captured
Standard 0.5 gallon	1,382
Standard 0.5 gallon (Granular)	749
Bag-A-Bug (Commercial)	1,226
Galvanized 5 gallon can	1,049
Cut galvanized 5 gallon can	783
Standard 5 gallon can	1,083
Galvanized hole	759
Cut galvanized hole	740
Standard hole	704
Blank	1

Only a small number of beetles were captured because of the late date traps were placed in the field and the light population at Otis Air Base.

Results indicate that, of those tested, the standard yellow, 0.5 gallon trap baited with pheromone and lure is the most effective. The Bag-A-Bug trap also gave excellent results. Five gallon container traps captured fairly good numbers of beetles and perhaps such traps are practical for field use. It is necessary to redesign somewhat before they are tested again. Traps placed over holes in the ground were not particularly effective. We know that some beetles escaped from the hole traps and this could easily be remedied.

Based upon 1979 tests, it appears that we could utilize large capacity traps at airports. However, those used this year and, perhaps, newly designed ones should be tested at an airport that has a heavy population of Japanese beetles.

Port Columbus airport was treated in September, 1979 by aircraft with granular Amaze. Post-treatment grub counts were made and the results are summarized below. Based on grub counts population reduction was 81.5 percent.

Table 2.. Post-treatment Japanese beetle grub counts at Port Columbus Airport, May 15, 1980.

Plot No.	Amaze	Control
1	0 1/	6
2	1	4
3	3	3
4	0	2
5	1	9
Total	5	27

1/ Number of grubs found per evaluation plot.

Project Number: GM 6.1.5
Project Title: Regulatory Treatments
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: W. H. McLane, J. A. Finney

The main objective of this work is the development of new and improved treatment techniques that can be used in addressing the movement of regulated items from regulated areas to non-infected locations. This project is primarily directed toward the development of treatments for recreational vehicles, mobile homes and lawn furniture.

At this time it appears that a dust application of insecticide at the point of origin is the most effective way to treat RV's and house trailers. In the past we found that some dust formulations are very effective against early instar gypsy moth larvae. Also, a dust, applied with the right equipment, would be able to penetrate hard to get places.

Using a laboratory duster mounted inside a spray chamber, a number of materials have been tested. Petri dishes were dusted with various amounts of formulations, insects were allowed to crawl on treated surface for given lengths of time and then transferred to artificial diet. Mortality readings were made daily following treatment for 8 days.

Table 1. Laboratory tests with Dylox Dust against 2nd instar gypsy moth larvae.

PERCENT DUST	GRAMS PER DISH	PERCENT MORTALITY AFTER 8 DAYS — TIME ON TREATED SURFACE										
		5S	10S	15S	20S	25S	30S	1M	2M	3M	4M	5M
25	0.1	78	86	88	96	88	84	94	96			
25	0.05		78		80		46	78	78	90		
25	0.02		30		32		26	30	42	48		
25	0.01		8		10		2	4	8	14		
20	0.1		66				38	34	50	62		
20	0.05		70		42		42	66	74	72		
15	0.1		64				64	62	58	66		
15	0.05		22		18		16	10	12	18		
15	0.02		2		0		2	0	16	4		
12.5	0.1		24				32	32	42	44		
10	0.1						54	58	54	46	48	24
10	0.05		14		4		10	12	16	20		
5	0.1						8	20	14	22	12	20
2.5	0.1						6	6	0	4	0	0
1.25	0.1									4	4	3
Control	Talc											0

Table 2. Laboratory tests with Diazinon Dust against 2nd instar gypsy moth larvae.

PERCENT DUST	GRAMS PER DISH	PERCENT MORTALITY AFTER 8 DAYS — TIME ON TREATED SURFACE						
		10S	30S	1M	2M	3M	4M	5M
4	0.05	62	76	82	56	70	90	98
2	0.05	44	94	34	92	88	92	78
1	0.05	54	94	84	100	96	100	100
Control	Talc							100[1/]

1/ Because of high mortality in control, test will be repeated.

Table 3. Laboratory tests with Sevin Dust against 2nd instar gypsy moth larvae.

PERCENT DUST	GRAMS PER DISH	PERCENT MORTALITY AFTER 8 DAYS — TIME ON TREATED SURFACE						
		10S	30S	1M	2M	3M	4M	5M
5	0.05	56	60	72	58	62	100	92
3	0.05	32	26	62	86	66	50	78
1	0.05	0	4	2	8	8	28	90
Control	Talc							0

Table 4. Laboratory tests with Dimilin 25WP against 2nd instar gypsy moth larvae.

PERCENT DUST	GRAMS PER DISH	PERCENT MORTALITY AFTER 8 DAYS — TIME ON TREATED SURFACE						
		10S	30S	1M	2M	3M	4M	5M
25	0.5	100	100	100	100	100	100	100
25	0.01	100	100	100	100	100	100	100
25	0.005	100	100	100	100	100	100	100
20	0.5	100	100	100	100	100	100	100
20	0.01	100	100	100	100	100	100	100
20	0.005	100	100	100	100	100	100	100
15	0.5	100	100	100	100	100	100	100
15	0.01	88	100	100	100	100	100	100
15	0.005	96	100	100	100	100	100	100
15	0.003	86	78	88	94	90	96	100
15	0.001	52	56	54	76	92	98	66
15	0.0005	30	20	28	56	56	36	32
15	0.0003	0	6	6	2	6	6	6
15	0.0001	0	0	8	2	0	2	2
10	0.5	100	100	100	100	100	100	100
10	0.01	100	100	100	100	100	100	100
10	0.005	94	98	100	100	100	100	100
10	0.003	84	86	76	98	92	100	100
10	0.001	58	74	76	70	92	90	98
10	0.0005	64	60	64	66	66	62	34
10	0.0003	26	8	18	34	72	20	22
10	0.0001	6	4	2	2	2	6	24

Table 4. (cont.)

PERCENT DUST	GRAMS PER DISH	PERCENT MORTALITY AFTER 8 DAYS — TIME ON TREATED SURFACE						
		10S	30S	1M	2M	3M	4M	5M
5	0.5	100	100	100	100	100	100	100
5	0.01	96	98	96	100	100	100	100
5	0.005	94	92	94	100	86	100	96
5	0.003	78	96	94	98	94	96	94
5	0.001	52	74	72	88	96	80	62
5	0.0005	28	56	48	56	72	66	94
5	0.0003	36	18	36	36	32	46	38
5	0.0001	10	10	10	14	16	12	30
1	0.5	100	100	100	100	100	100	100
1	0.01	2	30	24	58	58	44	44
1	0.005	36	62	38	32	42	72	100
1	0.003	6	2	0	4	2	8	0
1	0.001	6	6	16	10	18	24	4
1	0.0005	0	10	14	12	14	30	2
1	0.0003	0	2	0	0	0	2	0
1	0.0001	0	0	0	0	0	0	0
Control	Talc							0

Table 5. Laboratory tests with Rotenone Dust against 2nd instar gypsy moth larvae.

PERCENT DUST	GRAMS PER DISH	PERCENT MORTALITY AFTER 8 DAYS — TIME ON TREATED SURFACE						
		10S	30S	1M	2M	3M	4M	5M
1	0.05	0	0	0	6	6	22	8
1	0.01	0	0	0	0	2	0	0
1	0.005	4	0	2	2	2	0	4
Control	Talc		0	0	0	0	0	0

Table 6. Laboratory tests with Malathion Dust against 2nd instar gypsy moth larvae.

PERCENT DUST	GRAMS PER DISH	PERCENT MORTALITY AFTER 8 DAYS — TIME ON TREATED SURFACE						
		10S	30S	1M	2M	3M	4M	5M
4	0.05	2	0	2	4	6	2	2
2	0.05	0	0	12	32	6	4	2
1	0.05	0	50	0	8	6	20	46
Control	Talc							0

Once effective materials are identified, tests will be conducted outdoors to establish residual.

A primary treatment with alternatives will be recommended within one year.

Table 7. Results of egg mass treatments using detergents and pesticides.

MATERIAL	PERCENT	PERCENT HATCH OF 3 EGG MASSES
Methoxychlor	25	95
	10	52
	5	64
Insecticide Soap + Methoxychlor	25	66
	10	46
	5	2
Dimilin 25W (Dust)	25	93
	10	82
	5	72
Bay Sir 8514	25	87
	10	89
	5	88
S-20546	25	79
	10	75
	5	89
Sponto AK 16-95	25	14
	10	5
	5	48
Sponto H-44C	25	0
	10	8
	5	86
Insecticide Soap	25	0
	10	60
	5	71
Pine Scent	25	9
	10	36
	5	85
Top Job	25	0
	10	4
	5	66
Wax Remover	25	85
	10	83
	5	78
Floor Wax	25	89
	10	79
	5	68
Dylox Dust	25	32
	10	82
	5	79

Table 7. (cont.)

MATERIAL	PERCENT	PERCENT HATCH OF 3 EGG MASSES
	25	0
Light Water	10	22
	5	73
	25	88
Dowgard	10	96
	5	94
	25	11
Dowgard + Light Water	10	49
	5	54

Dimilin, Dylox and SIR 8514 were applied to egg masses as dusts. All other applications were made in liquid form using water as a dilutent.

Project Number: GM 8.1.1
Project Title: Evaluation of Ultraviolet Screening Agents for Pesticides and Microbials
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: W. H. McLane, J. A. Finney

Laboratory tests have demonstrated that many pesticides and most microbials deteriorate rapidly when exposed to direct sunlight. Because of this, and a number of other problems, a number of materials have not found use in the gypsy moth program. Gypchek, the gypsy moth nucleopolyhedrosis virus is a classic example.

During the year a number of ultraviolet opaque materials were tested to identify the most effective sun screening adjuvants for incorporation into formulations adversely affected by ultraviolet light.

The following data were compiled on 39 ultraviolet absorbers.

Table 1. Bioassay results of gypsy moth virus formulated with various ultraviolet opaque additives.

MATERIAL (carrier in parenthesis)	PERCENT MORTALITY AFTER 20 DAYS							
	NPV + SOLV.		NPV + H_2O		NPV + UV Screen + SOLV.			
	NO UV	UV	NO UV	UV	NO UV	1%	5%	10%
OS-49 (H_2O)	-	-	84	35	85	53	55	67
Cyasorb UV-248 (H_2O)	-	-	84	35	61	35	23	26
OU-100 (H_2O)	-	-	93	36	32	34	47	3
OU-101 (H_2O)	-	-	93	36	6	33	36	2
GIV TAN F (1:2 Acetone:H_2O)	47	43	-	-	71	60	82	59
GIV TAN F (Acetone)	87	48	91	60	81	56	79	79
GIV-820042 (1:3 Acetone:H_2O)	18	6	-	-	20	57	30	15
GIV-820042 (Acetone)	93	83	89	45	59	72	63	66
OU-105 (1:5 Acetone:H_2O)	30	21	84	18	43	36	45	30
OU-105 (Acetone)	97	71	99	81	72	78	64	85
OU-94 (1:3 Acetone:H_2O)	33	16	81	36	20	37	30	20
EUSOLEX 4360 (1:2 Acetone:H_2O)	33	14	80	19	18	38	31	15
Parsol MCX (Acetone)	61	36	73	43	77	66	68	67
OU-103 (Acetone)	65	37	76	32	64	56	52	61

Table 1. (cont.)

MATERIAL (carrier in parenthesis)	PERCENT MORTALITY AFTER 20 DAYS							
	NPV + SOLV.		NPV + H_2O		NPV + UV Screen + SOLV.			
	NO UV	UV	NO UV	UV	NO UV	1%	5%	10%
OU-87 (Acetone)	68	46	93	62	49	60	70	46
OU-86 (Acetone)	68	46	93	62	81	82	69	79
OU-88 (Acetone)	83	83	89	46	78	71	74	94
OU-98 (Acetone)	85	57	87	28	70	71	84	49
OU-107 (1:1 Acetone:H_2O)	61	9	-	-	53	40	35	26
OU-91 (1:1 Acetone:H_2O)	61	9	-	-	24	29	30	26
OU-89 (1:2 Acetone:H_2O)	16	1	-	-	11	7	14	17
OU-92 (1:2 Acetone:H_2O)	16	1	-	-	13	5	1	3
OU-92 (Acetone)	80	28	97	72	66	77	66	64
OU-96 (1:1 Acetone:H_2O)	84	38	-	-	27	17	18	13
OU-97 (1:2 Acetone:H_2O)	33	17	-	-	28	28	26	30
OU-97 (Acetone)	80	48	94	57	83	81	79	85
OU-99 (1:2 Acetone:H_2O)	33	17	-	-	27	9	33	19
UVINUL N-35 (Acetone)	59	50	-	-	34	58	49	43
Eastman RMB (Acetone)	84	56	94	65	73	49	61	77

Table 1. (cont.)

MATERIAL (carrier in parenthesis)	PERCENT MORTALITY AFTER 20 DAYS							
	NPV + SOLV.		NPV + H_2O		NPV + UV Screen + SOLV.			
	NO UV	UV	NO UV	UV	NO UV	1%	5%	10%
Cyasorb UV 531 (Acetone)	84	56	94	65	86	91	78	56
OU-102 (Acetone)	88	59	97	51	81	70	66	53
OU-104 (Acetone)	88	59	97	51	81	76	46	70
OU-93 (Acetone)	89	29	76	69	77	68	69	63
DOBP (Acetone)	87	48	91	60	81	93	66	73
OU-108 (Acetone)	85	76	94	49	75	63	76	81
OU-95 (Acetone)	84	57	87	28	67	42	53	50
Cyasorb 5411 (Acetone)	70	28	98	56	80	46	69	67
Escalol 507 (Acetone)	70	28	98	56	74	71	52	85
Cyasorb 1988 (1:1 Acetone:H_2O)	79	26	-	-	56	55	56	49
GIV 82-2154 (1:2 Acetone:H_2O)	48	21	-	-	38	66	30	36
OU-106 (1:1 Acetone:H_2O)	61	9	-	-	73	50	52	51
OU-109 (Acetone)	88	53	94	56	97	59	62	81
OU-110 (Acetone)	80	40	91	48	85	54	64	45
Carstab 700 (Acetone)	70	28	98	56	84	67	90	59

All UV treatments were exposed for 60 minutes to a standard blacklight + sunlight set up. These test identified a number of very effective ultraviolet screening agents. The 5 most effective materials were OU-88, OU-86, DOBP, Escalol 507 and Parsol MCX. We found that most materials were not soluble in water and, therefore, acetone was used as the carrier in most cases.

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides Against the Gypsy Moth
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

The main objective of this laboratory screening project is to collect and evaluate mortality data on experimental and registered compounds potentially useful for gypsy moth control. Selection of materials for field study and development is based on data from this project. New materials that are active against the gypsy moth are identified and formulations of registered products are modified to increase effectiveness.

Unless otherwise stated, all tests have been conducted using the standard oak seedling technique. All test insects are laboratory rearaed on artificial diet.

Table 1. Mortality of second instar gypsy moth larvae exposed to experimental insecticide UC-62644 (1 gal/ac).

Dosage (lb/ac)	Rain	Percent Mortality after 8 days
1.0	-	65
1.0	1"	86
1.0	3"	79
0.5	-	69
0.5	1"	86
0.5	3"	80
0.25	-	69
0.25	1"	82
0.25	3"	91
0.12	-	71
0.12	1"	88
0.12	3"	88
0.06	-	74
0.06	1"	87
0.06	3"	81
0.03	-	70
0.01	-	74
Check		9

Table 2. Mortality of second instar gypsy moth larvae exposed to Bendiocarb (Fisons Corporation) (1 gal/ac).

Dosage (lb/ac)	Rain	Sticker	Mortality after 5 days
1.0	-		100
1.0	1"		51
1.0	3"		11
0.5	-		100
0.5	1"		11
0.5	1"		36
0.5	1"	3% Acrylocoat	99
0.5	1"	3% RA-1645	100
0.5	1"	3% Chevron	95
0.5	1"	3% Nufilm 17	93
0.5	1"	3% Rhoplex B-15	100
0.5	1'	3% Bond	100
0.5	3"		25
0.5	3"	3% Acrylocoat	75
0.5	3"	3% RA-1645	94
0.5	3"	3% Chevron	62
0.5	3"	3% Nufilm 17	26
0.5	3"	3% Rhoplex B-15	98
0.5	3"	3% Bond	78
0.25			100
0.12			100
0.06			100
0.03			94
0.015			68
0.007			46
0.003			22
0.001			13
0.0009			3
0.0004			1
Control			0

Table 3. Tests with experimental material DPX-5444 (DuPont) (1 gal/ac).

Dosage (lb/ac)	Rain	Percent Mortality after 6 days
0.5	-	100
0.5	1"	98
0.5	3"	82
0.25	-	100
0.25	1"	99
0.25	3"	96
0.12	-	100
0.12	1"	89
0.12	3"	87
0.03	-	100
0.03	1"	49
0.03	3"	52
0.01	-	74
0.01	1"	20
0.01	3"	12
Check		0

Table 4. Tests with experimental material U-56295 (UpJohn) (1 gal/ac)

Dosage (1 lb/ac)	Rain	Percent Mortality after 3 days
1.0		100
0.5		96
0.25		97
0.12		93
0.06		93
0.06	1"	25
0.03		36
0.03	1"	19
0.01		14
0.01	1"	1
0.007		9
0.007	1"	0
0.003		9
0.003	1"	0

In working with a laboratory strain of test insects it is important that the test insect be no more resistant or susceptible than the field strain that will be treated. After a number of laboratory generations it is possible for a strain to respond differently than a wild strain. For this reason we check our laboratory strain against wild insects on a yearly basis.

Table 5. Tests comparing the susceptibility of wild and laboratory gypsy moth to various registered insecticides.

Material	Dosage (lb/ac)	GM Strain	Percent Mortality 24H	48H	72H	96H	168H	216H	240H
Dimilin	0.03	Lab	0	12	45	54	70	88	93
Dimilin	0.03	Wild	0	4	25	33	65	84	92
Sevin 4-oil	1.0	Lab	26	65	97	99	100		
Sevin 4-oil	1.0	Wild	14	41	77	89	100		
Orthene	1.0	Lab	34	86	98	98	100		
Orthene	1.0	Wild	35	82	99	100			
Dylox	1.0	Lab	51	99	100				
Dylox	1.0	Wild	40	98	100				
Sevin 80S	1.0	Lab	35	89	98	100			
Sevin 80S	1.0	Wild	30	86	96	100			
Check		Lab	0	0	0	0	0	0	1
		Wild	0	0	0	0	0	0	1

Table 6. Seedling tests of low dosages of registered materials against second instar gypsy moth larvae.

Material	Dosage (lb/ac)	Percent Mortality 24H	48H	72H	96H	144H	192H	216H
Orthene 75S	0.062	29	62	84	95	100		
Orthene 75S	0.031	22	29	46	79	98	100	
Orthene 75S	0.015	9	21	41	73	90	99	100
Orthene 75S	0.007	2	7	24	45	78	87	89
Orthene 75S	0.003	0	0	3	5	8	8	8
Sevin 4-oil	0.12	19	49	73	91	99	99	100
Sevin 4-oil	0.06	9	27	48	69	85	99	99
Sevin 4-oil	0.03	4	10	18	23	41	83	88
Sevin 4-oil	0.01	1	5	11	11	20	39	75
Sevin 4-oil	0.007	4	4	6	7	10	10	10
Check		0	0	0	0	0	0	0

Table 7. Seedling tests with Kryocide (Pennwalt).

Dosage (lb/ac)	Percent Mortality After 4 Days
20 1/	1
15 1/	0
10 1/	0
8 1/	0
6 1/	1
4 1/	0
2	1
1	0
0.5	0
0.25	0
0.12	0
Check	0

1/ Applied at the rate of 3 gallons per acre. All other applications applied at 1 gallon per acre.

Table 8. Seedling tests with diatomaceous earth.

Dosage (lb/ac)	Formulation	Percent Mortality After 7 Days
4	Liquid Spray	3
2	Liquid Spray	0
1	Liquid Spray	0
0.5	Liquid Spray	0
0.25	Liquid Spray	2
0.12	Liquid Spray	0
100%	Dust	31
50%	Dust	6
Check		0

Table 9. Seedling tests with Dia-Pro (International Diatoms Industries Ltd.).

Dosage (lb/ac)	Formulation	Percent Mortality After 7 Days
4	Liquid Spray	5
2	Liquid Spray	0
1	Liquid Spray	0
0.5	Liquid Spray	0
0.25	Liquid Spray	0
0.12	Liquid Spray	1
100%	Dust	100
75%	Dust	100
50%	Dust	93
25%	Dust	51
10%	Dust	9
5%	Dust	1
Check	Dust	2

Table 10. Results of topical tests with U-57770,U-56295 and BOLSTAR against second instar gypsy moth larvae.

Material	Dosage (ug/ul)	Percent Mortality After 48 Hours
U-57770	20	82
U-57770	10	88
U-57770	5	85
U-57770	2.5	76
U-57770	1.25	80
U-57770	0.62	83
U-57770	0.31	69
U-56295	20	85
U-56295	10	89
U-56295	5	80
U-56295	2.5	77
U-56295	1.25	67
U-56295	0.62	66
U-56295	0.31	61
BOLSTAR	20	100
BOLSTAR	10	99
BOLSTAR	5	99
BOLSTAR	2.5	97
BOLSTAR	1.25	92
BOLSTAR	0.62	95
BOLSTAR	0.31	83
Check		0

A test was conducted to determine how long the first instars of the gypsy moth will survive without food.

Table 11. Results of starvation tests with gypsy moth larvae.

Instar	Days of Starvation: No Diet	Days of Starvation: Before Back on Diet	Percent Mortality No Diet: 2D	4D	6D	8D	Percent Mortality Days Back on Diet: 4D	6D	8D	10D
I	X		1.5	19	78	99				
II	X		0	1.5	56	95				
III	X		0	0	26	86+				
IV	X		0	1	3	60				
I		1					3	3	4	5
I		4					27	28	28	28
I		6					76	76	76	76
I		8					100	100	100	100
I		10					99	99	99	99
II		1					0	0	0	0
II		4					9	9	9	9
II		6					62	63	65	
II		8					98	98	98	98
II		10					100			
III		1					0	1	1	1
III		4					15	16	18	0
III		6					95	95		
III		8					100			
III		10					100			
IV		1					0	0	0	0
IV		4					19	19	20	
IV		6					69	77		
IV		8					83	85		
IV		10					95			

Table 12. Test results of Basic - H, a cleansing agent (seedling test method).

Percent of Actual Material Used	Percent Mortality: 1 Day	2 Days	3 Days	4 Days	5 Days
100	0	0	0	2	3
75	0	0	0	0	0
50	0	0	0	0	0
25	0	0	0	0	1
Check	0	0	0	0	0

A series of tests was conducted with 4 formulations of Sevin to test their shelf life after mixing. Sevin 4-oil (Old), Sevin 4-oil (New), UCSF-4 and UCSF-1 (XLR) were mixed and held in beakers at 18° C. Small amounts of Triton X-190 (1 - 3%) and water were mixed with one new and one old Sevin 4-oil formulation. This was also done with the UCSF-4 formulation. A new sample of Sevin 4-oil was also mixed with kerosene. All applications were made at 1.0 lbs AI/Acre.

The various mixes were held for up to 9 weeks, after which they were bioassayed (standard seedling test). In all cases >95% mortality was noted within 6 days of introduction of larvae, indicating long shelf life following mixing.

The Canadian Forestry Service and Plant Products and Quarantine Division have conveyed much interest in a product called "Safer Insecticide Soap". In Canada, limited testing has demonstrated that the material is somewhat active as a gypsy moth larvacide.

When foliage and first and second instar larvae were sprayed with various amounts of Safer Soap and Methoxychlor mortality occurred. However, the material was reported to be ineffective in the field.

Table 13. Canadian tests results with Safer Soap and Methoxychlor combinations.

	Mortality (%)	
Solution	1st Instar	2nd Instar
1% Safer soap	52.0	51.3
2% Safer soap	not tested	48.7
1% Safer + 0.1% AI methoxychlor	100	86.6
2% Safer + 0.1% AI methoxychlor	not tested	99.0
Controls	0	0

A series of seedling and topical tests was conducted with Safer Soap alone and in combination with Marlate (Methoxychlor).

Safer Soap (75 and 100%) demonstrated good contact toxicity against 1st and 2nd instar gypsy moth larvae. At full concentration it showed better effect against 3rd instar larvae. Little or no activity was observed on 2nd instar larvae in seedling tests. When a 25% solution was applied to egg masses Safer Soap prevented hatch. A second test with 25% Safer Soap and 1% Marlate gave little reduction in hatch, however.

Table 14. Contact toxicity of Marlate 50WP and Insecticidal Soap against the first 3 instars of laboratory reared gypsy moth larvae.

	PERCENT MORTALITY AFTER 48 HOURS								
	Insecticidal Soap			Marlate 50WP			Insecticidal Soap + Marlate 50WP		
Concentration	I[1/]	II	III	I	II	III	I	II	III
100%	98	86	25				97[2/]	92	55
75	94	68	7				99[3/]	76	23
50	90	52	3				100[4/]	59	10
25	56	13	0	93	40	10	99[5/]	42	10
15	40	9	0	91	47	18	99[6/]	47	23
10	22	5	0	79	35	18	98[7/]	48	24
5	15	5	0	81	16	0	97[8/]	41	22
3	16	6	0	85	11	0	91[9/]	36	15
1	6	4	0	59	5	0	89[10/]	30	7
0.5	4	7	0	49	2	0	64[11/]	14	2
Control	0	0	0	0	0	0	0	0	0

1/ Instar of test insects
2/ 25% Marlate in 100% Insecticidal Soap
3/ 25% Marlate in 75% Insecticidal Soap
4/ 25% Marlate in 50% Insecticidal Soap
5/ 25% Marlate in 25% Insecticidal Soap
6/ 15% Marlate in 15% Insecticidal Soap
7/ 10% Marlate in 10% Insecticidal Soap
8/ 5% Marlate in 5% Insecticidal Soap
9/ 3% Marlate in 3% Insecticidal Soap
10/ 1% Marlate in 1% Insecticidal Soap
11/ 0.5% Marlate in 0.5% Insecticidal Soap

Water was used as a solvent.

Table 15. Seedling test results with Marlate and Insecticidal Soap. All rates were 1 gal/acre.

Formulation		Percent Mortality				
Marlate	Soap	24 Hr	48 Hr	120 Hr	168 Hr	192 Hr
1 lb		0	12	74	89	96
	100%	0	0	0	4	6
1%	1%	0	0	1	5	5
5%	5%	0	0	1	4	5
10%	10%	0	2	19	42	44
25%	25%	1	5	78	97	100
35%	35%	0	3	62	87	95
Control		0	0	0	0	0

Table 16. Laboratory test results of Insecticidal Soap applied to egg masses.

Concentration	No. of E/M Treated	Average Percent Hatch
25%	5	0
10	5	60
5	5	71
Control	5	85

Table 17. Laboratory test results of Marlate and Insecticidal Soap mixtures applied to egg masses.

Formulation			
Marlate	Soap	No. of EM Treated	Average Percent Hatch
1%	25%	5	66
1%	10%	5	46
1%	5%	5	2
Control		5	85

During this reporting period, a series of laboratory tests was conducted with formulations of methoxychlor and malathion frequently used by PCOs for backyard gypsy moth control. The tank mix consists of 1 pint of malathion and 1.5 quarts of methoxychlor per 100 gallons of usable spray (approximately 1.012 pounds of active methoxychlor and 0.9125 pounds of active malathion per 100 gallons). This formulation is applied with hydraulic equipment to residential properties for control of a number of insects (including gypsy moth). Trees are treated to the point of run-off and, on the average, 400 gallons of formulation are used per property, or about 400 gallons per acre.

Table 18. Toxicity of Marlate 50WP + 25EC, Malathion 50EC to 2nd instar gypsy moth larvae (Seedling Test Method).

Material	Dosage (lb/acre)	Percent Larval Mortality After 24H	48H	72H	96H	120H	144H	168H	192H
Marlate 50WP	2	0	1	13		71			
	1	0	4	13		65			
	.5	1	1	2		21			
	.25	0	0	0		4			
	.125	0	0	0		1			
Marlate 25EC	2	0	6	12	27	60*	72	75	75
	1	1	5	10	22	30	56	60	60
	.5	0	2	6	12	23	49	49	49
	.25	0	0	6	8	13	30	31	31
	.125	0	0	2	4	4	14	15	15
Malathion 50EC	2	40	83	97	99	100			
	1	29	67	84	96	100			
	.5	10	22	60	82	100			
	.25	10	19	36	59	97			
	.125	13	18	30	47	92			

* Larvae transferred to diet

Table 19. Toxicity of Marlate and Malathion, individually and in combination.

Material	Dosage (lb/acre)	Rain	Percent Mortality 1 Day	2 Day	3 Day	4 Day
	1.0	-	8	29	79	96
Marlate +	1.0	1"	0	1	4	6
Malathion	0.5	-	8	19	86	88
	0.5	1"	0	0	0	0
	1.0	-	29	41	85	92
	1.0	1"	0	1	1	1
Malathion	0.5	-	20	33	73	94
	0.5	1"	0	0	0	0
	1.0	-	0	7	14	25
	1.0	1"	1	3	6	10
Marlate	0.5	-	1	5	17	32
	0.5	1"	0	2	3	5
Check			0	0	0	0

Table 20. Results of Malathion and Methoxychlor tests (1gal/acre).

Material	Dosage (lb/acre)	Percent Mortality 24 Hr	48 Hr	96 Hr	120 Hr	144 Hr
	7.3	90	100			
	3.6	75	99	100		
Malathion	1.8	62	90	96	100	
	.9	40	46	83	97	100
	.4	7	15	58	93	97
	2.6	8	19	37	59	73
	1.3	1	8	14	20	31
Methoxy-	.65	0	1	3	7	15
chlor	.32	0	0	0	2	9
	.16	0	0	0	1	1
Control	-	0	0	0	0	0

Table 21. Test results of Malathion and Methoxychlor mixes.

Dosage (lb/acre)	Rate[1/] (gal/acre)	Aging[2/] Days	Percent Mortality 24H	48H	72H	96H	120H	144H
0.47	25	-	6	16	32	76	99	99
0.37	20	-	6	26	53	93	99	100
0.28	15	-	3	23	48	81	95	98
0.18	10	-	2	15	35	75	92	99
0.09	5	-	1	9	17	25	28	33
0.01	1	-	0	0	0	0	0	0
Control		-	0	0	0	0	0	0
0.47	25	3	0	3	11	21	37	47
0.37	20	3	0	3	29	45	57	68
0.28	15	3	0	10	27	42	61	65
0.18	10	3	0	0	3	17	27	30
0.09	5	3	0	0	0	0	0	0
0.01	1	3	0	0	0	0	0	0
Control		3	0	0	0	0	0	1
0.47	25	7	0	0	0	1	2	2
0.37	20	7	0	0	0	0	0	0
0.28	15	7	1	1	1	7	12	13
0.18	10	7	0	0	0	0	2	3
0.09	5	7	0	0	0	0	0	5
0.01	5	7	0	2	2	2	2	2
Control		7	0	0	0	0	0	0

1/ It is not practical to exceed 25 gallons per acre in the laboratory spray chamber.
2/ Number of days plants were aged outside before bioassay.

These laboratory tests showed little activity from methoxychlor and good activity from malathion when applied at 1 gallon per acre. In combination, 1 pint of malathion and 1.5 quarts of methoxychlor per 100 gallons of water, and applied at 10 or more gallons of formulation per acre, results were very good. However, once this formulation was aged for 3 days results were poor at all rates. Little if any mortality occurred after plants were aged for 7 days.

A number of tests were conducted with Bacillus thuringiensis, namely, Thuricide 16B, Thuricide 24B, Thuricide 32B, Dipel 4L and 3 new strains of BT. We found that all formulations of BT are effective against laboratory reared 2nd instar gypsy moth larvae in laboratory tests. We also found that most formulations washed off foliage when exposed to rainfall. Because of this a number of stickers were tested with the 4L formulation. We also found that formulations of Thuricide containing no xylene were as effective as those with this solvent. Tests also indicated that we may be able to use two appliccations of 4 BIU/acre instead of the standard 8 BIU/acre.

Table 22. Laboratory test results with Thuricide 16B, Thuricide 32B and Abbott 32B with no added sticker (1 gal/acre).

Material	Dosage (lb/ac)	Drying Time	Rain	Percent Mortality 24H	48H	72H	96H	120H	144H
	1.0	-	-	2	19	55	83	93	96
Abbott	1.0	1.5 H	3	0	1	1	3	12	22
32B	1.0	72.0 H	3	4	5	10	13	30	71
	0.5	72.0 H	3	0	2	7	18	33	49
Thuricide	1.0	-	-	4	29	51	79	96	99
16B	1.0	1.5 H	3	0	14	19	29	51	65
	1.0	-	-	2	27	57	80	94	99
Thuricide	1.0	1.5 H	3	0	0	0	19	26	34
32B	1.0	72.0 H	3	0	3	19	43	65	86
	0.5	72.0H	3	0	5	12	41	66	86
Control	-	-	-	0	0	0	0	37	62

Control mortality on days 5-6 was due to foliage being completely eaten by end of day 2.

Table 23. Laboratory evaluation of Abbott's 32 BIU formulation of Bacillus thuringiensis with a number of stickers. Spray deposit was dried 2 hours before being exposed to 1" of rainfall. The material was applied at 1 lb AI/ac. Amount of sticker used was 3% of total formulation.

Sticker	Percent Larval Mortality					
	24 Hr	48 Hr	72 Hr	96 Hr	120 Hr	144 Hr
Acrylocoat	10	24	43	78	89	97
Adsee 775	0	0	0	4	13	32
Bond	0	5	15	26	50	73
Chevron	0	0	1	3	30	71
Maywood	0	0	1	4	21	58
Nufilm 17	0	0	0	11	44	79
Plantgard	1	7	22	38	59	79
Plyac	1	1	4	12	30	53
Polyamide resin BC-1104	0	0	0	0	18	71
Polyco 2631	0	2	3	12	24	86
Polyco 2719	1	12	32	61	89	97
Polytrap	0	0	0	0	12	79
RA-1645	4	16	33	60	84	90
Rhoplex AC 33	0	6	15	32	65	85
Rhoplex B-15	5	7	17	40	70	86
Rhoplex B60A	0	5	14	32	60	80
Super Target NL	1	1	3	9	22	64
Ucar Paper Coating Binder-40	0	0	24	53	86	98
Ucar-Pigment Binder-180	0	0	0	1	36	67
UCAR-30298	0	3	3	6	29	59
UCAR-32068	1	11	18	33	61	84
UCAR-WC-130	0	1	2	7	34	60
Vapergard	0	0	0	2	22	48
WEX	0	3	7	16	42	75
Control	0	0	0	6	9	14

Samples of Dipel WP and Thuricide 16B stored at Moosic PA, were bioassayed.

Table 24. Laboratory tests using 5 studies in two formulations of Bacillus thuringiensis (8 BIU/Ac).

		Percent Mortality After 4 Days					
Material	Rain	Sticker=	1645	Acrylocoat	Rhoplek-B15	Plantgard	Rhoplex A6-33
Thuricide 32B	3	5%	92	81	68	52	76
Dipel 4L	3	5%	73	38	85	40	40
Thuricide 32B	3	4%	86	76	92	54	65
Dipel 4L	3	4%	64	57	44	17	27
Thuricide 32B	3	3%	94	83	86	50	61
Dipel 4L	3	3%	52	67	34	29	28
Thuricide 32B	3	2%	72	83	66	20	65
Dipel 4L	3	2%	48	60	35	21	15
Thuricide 32B	3	1%	82	74	73	36	14
Dipel 4L	3	1%	59	18	16	12	40
Thuricide 32B	3	0.5%	73	56	78	30	13
Dipel 4L	3	0.5%	32	14	16	8	15
Thuricide 32B		0%	91*	92*	77*	85*	80*
Dipel 4L		0%	89*	92*	81*	72*	82*
Thuricide 32B	3	0%	51*	69*	38*	21*	39*
Dipel 4L	3	0%	6*	1*	5*	0*	2*
Check			0	0	0	0	0

* Note: No sticker was included in these formulations.

Table 25. Bioassay results of tests conducted with Moosic samples of Dipel WP and Thuricide 16B.

Material	Dosage	Percent Mortality 2 Days	4 Days	6 Days
Dipel WP (Moosic)	8 BIU/Acre	32	96	100
Dipel WP (Moosic)	4 BIU/Acre	29	81	95
Thuricide 16B (Moosic)	8 BIU/ACRE	33	84	98
Thuricide 16B (Moosic)	4 BIU/Acre	31	69	87
Thuricide 16B (Lab Sample)	8 BIU/Acre	24	60	83
Thuricide 16B (Lab Sample)	4 BIU/Acre	19	61	87
Check		0	1	14

Sandoz, Inc. has formulated samples of Thuricide without Xylene. Old formulations contained 3% Xylene added as a preservative. Samples without Xylene are identified by "BMP" and "BDL". A serie 1" 86
1.0 s of seedling and feeding tests were conducted to see how they compare to the standard Xylene formulations.

Table 26. Laboratory tests using Xylene free Thuricide samples and Xylene samples.

Material	Rain	Percent Mortality 2 ay	4 Day	6 Day	8 Day
Thuricide 16B		23	92	99	100
Thuricide 16B	1.0	1	16	49	71
Thuricide 16B (BMP)		24	91	100	
Thuricide 16B (BMP)	1.0	0	118	54	78
Thuricide 16B (BDL)		11	83	99	99
Thuricide 16B (BDL)	1.0	0	9	48	79
Thuricide 24B (BMP)		6	61	82	97
Thuricide 24B (BMP)	1.0	3	52	93	99
Thuricide 24B (BDL)		14	87	97	100
Thuricide 24B (BDL)	1.0	1	38	74	93
Thuricide 32B		19	78	100	
Thuricide 32B	1.0	1	29	78	99
Thuricide 32B (BMP)		12	72	83	93
Thuricide 32B (BMP)	1.0	0	46	85	90
Thuricide 32B (BDL)		10	60	96	100
Thuricide 32B (BDL)	1.0	1	29	91	94
Check		0	0	4	4

One inch dia. discs of oak foliage were used to determine possible repellency of Thuricide to gypsy moth larvae. Using 9 formulations of Thuricide, one series of discs was sprayed with the test materials and the material was brushed onto the other series. Wet filter paper lined petri dishes were used to hold paired discs, and 10 second instar larvae were introduced into each dish for 48 hours. Test insects selected the treated or untreated disc to feed on. Percent feeding was determined after 48 hours. Results indicated much less feeding on treated foliage. There was little difference in feeding between the individual formulations.

Table 27. Feeding tests to check feeding repellency of Thuricide formulations.

Thuricide Combinations	Spray Tower Petri Dish Method % Feeding 48 H		Brush - Petri Dish Method % Feeding 48 H	
T-32B:Control	37	92	30	61
T-24B:Control	52	96	9	65
T-16B:Control	34	79	25	67
T-32BDL:Control	60	96	32	62
T-24BDL:Control	49	75	27	41
T-16BDL:Control	29	82		
T-32BMP:Control	43	95	25	99
T-24BMP:Control	53	86	31	89
T-16BMP:Control	63	88	17	47
T-32B:T-24B	35	23	12	5
T-32B:T16B	43	27	10	6
T-24B:T-16B	27	19	8	6
T-32BDL:T-16BDL	48	29	20	11
T-32BDL:T-24BDL	48	40	17	12
T-24BDL:T-16BDL	38	29	4	10
T-32BMP-T-16BMP	53	48	9	10
T-32BMP:T-24BMP	61	49	24	19
T-24BMP:T-16BMP	43	34	13	3
T-32B:T-32BDL	29	41	14	11
T-24B:T-24BDL	31	54	10	7
T-16B:T16BDL	22	31	4	7
T-32B:T-32BMP	39	30	15	8
T-24B:T-24BMP	39	28	5	12
T-16B:T-26BMP	19	48	8	3
T-32BDL:T-32BMP	35	45	7	16
T-24BDL:T-24BHP	46	39	7	10
T-16BDL:T-16BMP	28	41	8	6

Table 28. Tests with Dipel 4L and three new strains of *Bacillus thuringiensis*.

Material	Dosage (BIU/ac)	2 Day	4 Day	6 Day
Dipel 4L	8	14	67	99
ABG 6106	8	5	41	81
ABG 6107	8	9	62	77
ABG 6111	8	8	39	94
Dipel 4L	4	7	34	92
ABG 6107	4	7	25	75
ABG 6107	4	2	44	92
ABG 611	4	1	11	22
Dipel 4L	2	0	36	91
ABG 6106	2	7	27	66
ABG 6107	2	6	50	91
ABG 6111	2	0	2	3
Dipel 4L	1	0	4	36
ABG 6106	1	6	30	63
ABG 6107	1	1	16	50
ABG 6111	1	0	2	3
Check		0	0	0

Table 29. Test results using Dipel 4L, Thuricide 16B and Thuricide 32B at various dosages.

Material	BIU/acre	Percent Mortality after 6 days
Dipel 4L	8	86
	6	77
	4	65
	2	34
	1	4
Thuricide 32B	8	99
	6	97
	4	99
	2	90
	1	81
Thuricide 16B	8	100
	6	100
	4	100
	2	94
	1	86
Check		0

Table 30. Outdoor aging and weathering test with Dipel 4L, Thuricide 32B and Thuricide 16B.

Formulation	Aging[1/]	Rain[2/]	Mortality 6 days after introduction of larvae				
			8 BIU	6 BIU	4 BIu	2 BIU	1 BIU
	-	-	100	100	100	100	99
	4	.25	95	76	67	54	29
	6	.35	93	84	52	57	12
Dipel 4L	8	.65	73	22	18	13	7
	11	.65	12	8	0	0	1
	13	.65	22	8	0	4	1
	15	.65	17	4	4	2	0
	-	-	100	100	100	100	100
	4	.25	73	100	99	59	22
	6	.35	87	100	89	43	12
Thuricide 32B	8	.65	38	92	27	8	3
	11	.65	3	4	14	1	1
	13	.65	2	1	0	0	0
	15	.65	16	2	2	1	2
	-	-	100	100	100	100	97
	3	.4	98	100	100	100	93
Thuricide 16B	6	.4	46	20	80	29	12
	8	.4	37	7	59	15	6
	15	.65	16	18	7	5	6

1/ Length of time treated plants were aged outdoors before bioassay.
2/ Natural rainfall

Table 31. Outdoor aging tests with Dipel 4L and Thuricide 32B with 0-5% Acrylocoat added. Values are percent mortality 6 days after larvae placed on test plants.

Aging (days)	Rain (inches)	8 BIU Dipel 4L + Acrylocoat - (%) Acrylocoat Concentration					8 BIU Thuricide 32B + Acrylocoat - (%) Acrylocoat Concentration				
		0	1	2	3	5	0	1	2	3	5
2-3	.4	100	100	100	100	100	99	100	97	99	99
6-7	1.55	75	97	85	95	90	99	100	100	98	90
8-9	1.55	84	87	94	80	95	94	99	96	96	73
11-13	1.55	79	81	85	68	96	23	29	25	24	16
14-15	1.55	44	57	52	34	76	10	36	40	15	39
16	1.55	49	87	73	8	61					
17-18	1.75	7	5	15	31	65	9	31	26	34	15
20-21	1.75	1	5	8	14	55	39	31	25	35	40
22-23	1.85	4	4	15	14	25	24	12	10	28	31
24-25	1.85	5	10	59	24	38	10	5	7	10	11
27-28	1.85	4	27	22	44	32	1	1	0	2	3
29	1.85						5	6	5	6	7

Table 32. Aging tests with Dipel 4L using 8 BIU/acre.

Aging [1/]	Rain [2/]	Sticker	PERCENT MORTALITY 2 Day	4 Day	6 Day
2 day	0.4"		4	79	100
2 day	0.4"	Acrylocoat 1%	3	70	100
2 day	0.4"	Acrylocoat 2%	4	82	100
2 day	0.4"	Acrylocoat 3%	5	74	100
2 day	0.4"	Acrylocoat 5%	5	26	100
Check			1	5	6
7 days	1.55"		0	30	75
7 days	1.55"	Acrylocoat 1%	2	48	97
7 days	1.55"	Acrylocoat 2%	1	28	85
7 days	1.55"	Acrylocoat 3%	1	44	95
7 days	1.55"	Acrylocoat 5%	2	31	90
Check			0	3	30
9 days	1.55"		2	19	84
9 days	1.55"	Acrylocoat 1%	1	45	87
9 days	1.55"	Acrylocoat 2%	6	45	94
9 days	1.55"	Acrylocoat 3%	2	22	80
9 days	1.55"	Acrylocoat 5%	2	24	95
Check			0	0	4
11 day	1.55"		5	-	79
11 day	1.55"	Acrylocoat 1%	3	62	81
11 day	1.55"	Acrylocoat 2%	3	68	85
11 day	1.55"	Acrylocoat 3%	3	37	68
11 day	1.55"	Acrylocoat 5%	5	80	96
Check			0	1	9
14 day	1.55"		3	13	44
14 day	1.55"	Acrylocoat 1%	1	29	57
14 day	1.55"	Acrylocoat 2%	7	37	52
14 day	1.55"	Acrylocoat 3%	2	20	34
14 day	1.55"	Acrylocoat 5%	11	56	76
Check			0	1	10
16 day	1.55"		0	9	49
16 day	1.55"	Acrylocoat 1%	0	14	87
16 day	1.55"	Acrylocoat 2%	1	8	73
16 day	1.55"	Acrylocoat 3%	0	2	8
16 day	1.55"	Acrylocoat 5%	1	15	61
Check			5	5	5
18 day	1.75"		0	3	7
18 day	1.75"	Acrylocoat 1%	0	1	5
18 day	1.75"	Acrylocoat 2%	0	7	15
18 day	1.75"	Acrylocoat 3%	0	21	31
18 day	1.75"	Acrylocoat 5%	0	44	65
Check			0	6	21

Table 32. Continued

21 day	1.75"		0	1	1
21 day	1.75"	Acrylocoat 1%	0	1	5
21 day	1.75"	Acrylocoat 2%	0	3	8
21 day	1.75"	Acrylocoat 3%	0	6	14
21 day	1.75"	Acrylocoat 5%	1	36	55
Check			0	0	14
23 day	1.85"		0	0	4
23 day	1.85"	Acrylocoat 1%	0	1	4
23 day	1.85"	Acrylocoat 2%	0	2	15
23 day	1.85"	Acrylocoat 3%	0	0	14
23 day	1.85"	Acrylocoat 5%	0	6	25
Check			0	2	7
25 day	1.85"		0	1	5
25 day	1.85"	Acrylocoat 1%	0	0	10
25 day	1.85"	Acrylocoat 2%	0	9	59
25 day	1.85"	Acrylocoat 3%	0	4	24
25 day	1.85"	Acrylocoat 5%	0	4	38
Check			0	1	1
28 day	1.85"		0	2	4
28 day	1.85"	Acrylocoat 2%	0	3	27
28 day	1.85"	Acrylocoat 3%	0	4	22
28 day	1.85"	Acrylocoat 3%	1	4	44
28 day	1.85"	Acrylocoat 5%	1	10	32
Check			0	14	17

1/ All plants were aged outside
2/ Natural rainfall - (accumulative)

Table 33. Aging tests with Thuricide 32B using 8 BIU/acre.

Aging [1/]	Rain [2/]	Sticker	PERCENT MORTALITY 2 Day	4 Day	6 Day
3 day	0.4"		2	33	99
3 day	0.4"	Acrylocoat 1%	1	35	100
3 day	0.4"	Acrylocoat 2%	3	38	97
3 day	0.4"	Acrylocoat 3%	3	58	99
3 day	0.4"	Acrylocoat 5%	3	43	99
Check			1	5	6
6 day	1.55"		2	85	99
6 day	1.55"	Acrylocoat 1%	2	74	100
6 day	1.55"	Acrylocoat 2%	0	65	100
6 day	1.55"	Acrylocoat 3%	3	42	98
6 day	1.55"	Acrylocoat 5%	1	35	90
Check			0	3	30
8 day	1.55"		1	43	94
8 day	1.55"	Acrylocoat 1%	1	62	99
8 day	1.55"	Acrylocoat 2%	1	77	96
8 day	1.55"	Acrylocoat 3%	0	37	96
8 day	1.55"	Acrylocoat 5%	0	23	73
Check					
13 day	1.55"		0	5	23
13 day	1.55"	Acrylocoat 1%	0	4	29
13 day	1.55"	Acrylocoat 2%	0	2	25
13 day	1.55"	Acrylocoat 3%	1	9	24
13 day	1.55"	Acrylocoat 5%	2	5	16
Check			0	0	4
15 day	1.55"		0	1	10
15 day	1.55"	Acrylocoat 1%	1	7	36
15 day	1.55"	Acrylocoat 2%	1	13	40
15 day	1.55"	Acrylocoat 3%	0	4	15
15 day	1.55"	Acrylocoat 5%	0	1	39
Check			5	5	5
17 day	1.75"		2	6	9
17 day	1.75"	Acrylocoat 1%	2	13	31
17 day	1.75"	Acrylocoat 2%	14	19	26
17 day	1.75"	Acrylocoat 3%	9	29	34
17 day	1.75"	Acrylocoat 5%	2	11	15
Check			0	6	21
20 day	1.75"		2	9	39
20 day	1.75"	Acrylocoat 1%	1	7	31
20 day	1.75"	Acrylocoat 2%	1	5	25
20 day	1.75"	Acrylocoat 3%	4	17	35
20 day	1.75"	Acrylocoat 5%	1	17	40
Check			0	0	14

22 day	1.85"		0	0	24
22 day	1.85"	Acryloocoat 1%	0	1	12
22 day	1.85"	Acrylocoat 2%	0	2	10
22 day	1.85"	Acrylocoat 3%	0	1	28
22 day	1.85"	Acrylocoat 5%	0	2	31
Check			0	2	7
24 day	1.85"		0	6	10
24 day	1.85"	Acrylocoat 1%	0	0	5
24 day	1.85"	Acrylocoat 2%	0	3	7
24 day	1.85"	Acrylocoat 3%	0	5	10
24 day	1.85"	Acrylocoat 5%	0	1	11
Check			0	1	1
27 day	1.85"		0	1	1
27 day	1.85"	Acrylocoat 1%	0	1	1
27 day	1.85"	Acrylocoat 2%	0	0	0
27 day	1.85"	Acrylocoat 3%	0	1	2
27 day	1.85"	Acrylocoat 5%	1	3	3
Check			0	14	17
29 day	1.85"		1	1	5
29 day	1.85"	Acrylocoat 1%	0	0	6
29 day	1.85"	Acrylocoat 2%	0	0	5
29 day	1.85"	Acrylocoat 3%	0	0	6
29 day	1.85"	Acrylocoat 5%	0	0	7
Check			0	14	17

1/ All plants were aged outside
2/ Natural rainfall - (accumulative)

In the control of isolated infestations of gypsy moth, it may be possible, in some cases, to make use of selected herbicides to defoliate an area at the time of first instar development and cause starvation of larvae. There is also the possibility of prolonging the dormant period of host tree species until after the gypsy moth larvae have hatched and starved.

Dr. John Starrett, SEA, has tested materials that will break down abcission. He found that 75 PPM of Ethrel mixed with 1,000 PPM of Endothall acid would givee 85 percent foliage drop of pine oak foliage anytime during the first 27 days of development. He also found that the trees refoliated within 10 weeks. Little damage occurred to the trees and the following year treated trees could not be singled out from the checks. He applied his mix to the point of run-off and found foliage would not drop unless a good application was applied to all foliage.

During this reporting period we did very limited work with "DEF 6" and Ethrel on tender northern red oak seedlings. Using a treatment of 1-2 pounds of active material we were able to defoliate all treated plants in each test. After a 30 day period 25 percent of defoliated plants refoliated. No additional work has been done at this time.

Forty-one shipments of materials were made to the laboratory from chemical companies during the year. Fourteen chemical representatives and a number of state and private parties visited the section during the year.

Project Number: GM 9.1.2
Project Title: 1979 Field Tests of Bay SIR-8514, Dimilin and *Bacillus thuringiensis*
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: W.H. McLane, J. A. Finney

ABSTRACT

The growth regulators Dimilin and SIR 8514 and two commercial formulations of *Bacillus thuringiensis* Berliner (Thuricide 16B and 24B) were evaluated for gypsy moth population suppression in mixed-hardwood forest. Reduction in egg mass density resulted from one application of SIR 8514 (0.03 lb AI/acre) at one-half gallon per acre. Eighty-two percent reduction in egg mass density was achieved with two applications of Thuricide 16B (8 BIU AI/acre) at one-half gallon per acre. The other Thuricide treatments failed to prevent population increases. This failure was partly attributed to rainfall which disrupted the spray schedule and possibly washed some deposited matereial from foliage. In contrast with previous field trials, Dimilin treatment coinciding with foliage budbreak did not effect control, suggesting inadequate coverage of expanding foliage.

Introduction

The evaluation of chemical and microbial insecticides is an important activity within a larger effort to develop economically and environmentally practical methods for managing gypsy moth populations. The Gypsy Moth Methods Development Center (Otis Air Base, MA) maintains an insecticide screening and field evaluation program to test the large number of compounds receiving consideration. This paper reports the findings of 1979 field trials conducted in the Pequannock Watershed Region of Northern New Jersey, in which two chemical insecticides and two commercial preparations of *Bacillus thuringiensis* Berliner (BT) were evaluated for gypsy moth population suppression and foliage protection.

Both chemical compounds evaluated were growth regulators (interfering with ecdysis by inhibiting chitin synthesis). Dimilin (Thompson-Hayward Chemical Co. Kansas City, KS) was selected for application at the onset of foliage expansion based on previous field trials which suggested that early Dimilin treatment effected foliage protection. SIR 8514 (Mobay Chemical Co., Kansas City, MO) was selected for field evaluation based on favorable results of laboratory tests.

The BT formulations included in these trials were Thuricide 16B and Thuricide 24B (Sandoz, Inc., Homestead, FL). Thuricide 16B tests were designed to compare the performance of two different rates of application and to explore the influence of application timing on population reduction. Thuricide 24B (currently nearing registration) was compared with Thuricide 16B at a standard dosage and rate of application. In addition, an inexpensive, non-centrifuged, preparation of Thuricide 24B was compared with a preparation which had been "super-centrifuged" prior to shipment.

Methods and Materials

Spray Plots - In order to make preliminary estimates of egg mass density in prospective test areas, a "five minute count" procedure was implemented. By this technique, an observer walked in a given direction for five minutes (avoiding atypical stands, trails, clearings), counting all visible current season egg masses. These counts were converted to egg masses/acre by the formula: EM/A = (EM/5 min.walk X 20) + 20. Using this procedure it was found that a tract of 36,000 acres in the Pequannock Watershed of New Jersey and managed by the Newark Watershed Conservation and Development Corporation harbored suitable infestations for the tests. Populations in this general area were estimated at ca. 50 egg masses per acre in April, 1979.

Twenty-seven square 50 acre plots were positioned in the aboved designated area. There were 3 replicates for each of seven treatments and 6 non-treated control plots. Boundry lines and plot corners were outlined with surveyor tape. At least one corner of each plot was readily accessible by road and adjacent plots were separated by at least 500 ft. The 15 plots scheduled to receive BT treatment and 3 control plots were positioned in areas which contained ca. 50-100 egg masses/acre. This relatively low density range was selected in order to determine the efficacy of BT at low population levels. Chemical treatment plots (6) and the remaining 3 control plots were placed in areas of higher density.

To monitor gypsy moth population levels throughout the test, a 4x 5 grid of 20 equidistant prism points (each separated by 150 ft) was established within the central ten acres of each plot.

Prior to treatment and again in the following autumn, egg mass counts were made at each prism point in all plots to assess egg mass density changes. The variable-radius and fixed-radius egg mass sampling techniques were used. A wedge prism of Basal Area Factor 20 was used to identify those live, overstory trees included in variable-radius counts. Trees bordering on acceptance were alternately accepted or rejected. An 8.25 ft radius outward from each sampling point denoted the area in which fixed-radius counts were conducted. Unfortunately, diameters of trees included in the variable-radius counts were not recorded, complicating the subsequent calculation of actual egg mass densities.

Five BT treatments and two chemical treatments were compared for performance (applications and related equipment are summarized in Tables 1 and 2). All applications were accomplished in May, 1979. The chemicals were applied as 25% wettable powders and Thuricide formulations were applied as aqueous concentrates. The Dimilin was prepared by agitation in a mixing tank equipped with a pump and by-pass system. The other materials were agitated within the spray plane hopper during flight to the spray plots.

Dimilin treatment was conducted in early May (Table 1) when hatch was estimated at 50%, coinciding with foliage budbreak. The SIR 8514 applications and initial applications of BT were conducted in mid-May (Table 1) when larvae were predominantly in the 2nd and 3rd instar. The second BT applications (and applications to those plots receiving one late Thuricide 16B treatment) were conducted in late-May (Table 1) when larvae were predominantly in the 3rd and 4th instar. The intended interval between successive BT applications was 7-10 days. However, the spray schedule was disrupted by rainfall which resulted in an interval of 11-17 days between successive BT applications (Table 1).

A Cessna Ag-Truck equipped with a conventional spray system was used to apply the chemicals and the first BT applications (Table 2). The spray plane yielded a 60 foot swath at 125 mph, from 20 feet above tree top level. Unfortunately rainfall delays and scheduling of this spray plane for use in other projects resulted in the use of a different aircraft for the second BT applications. A Bell 47G helicopter equipped with a conventional spray system was used for the second BT applications (Table 2). The helicopter yielded a 60 foot swath at 50 mph, from 20 feet above tree top level.

Radio communication was maintained between pilot, airport team and field crewmen to coordinate spray operations. During aerial applications, plot corners were marked by suspending helium-inflated weather balloons (1.5 ft. diam.) 10-20 feet above the forest canopy. Mixing and spraying equipment was thoroughly cleaned between applications.

Treatment performance was assesed from the standpoint of population reduction by conducting larval and pupal counts in the vicinity of each sampling point in each plot. These counts were accomplished by means of burlap bands stapled to live, overstory trees in the vicinity of each sampling point. Sampling points within chemical-treated plots and their corresponding control plots were surrounded with 10 burlap-banded trees. BT-treated plots only had 5 trees per point. All living larvae and (later) pupae on and within each burlap band were counted once as the season progressed.

Treatment effectiveness was also determined from the standpoint of foliage protection at termination of larval feeding. Five positions (including the outer corners and center of each sampling grid) were used to visually estimate defoliation (20% intervals) of the forest canopy in each plot.

Results and Discussion

As mentioned earlier, positioning of the various plots was based on preliminary egg mass density estimates. Three control plots were intended to represent the various BT plots and three control plots were intended to represent the various chemical plots on the basis of similar egg mass density estimates. However, the pre-spray egg mass counts conducted in the various control plots indicated considerable variation in population levels among them. Hence, the division of the six control plots into two groups was deemed artificial and they were pooled into one group for purposes of comparison with the various BT and chemical treatments (Table 3).

Since the diameters of trees included in the egg mass, larval and pupal counts were not recorded, conventional formulae used to compute densities of various life stages on a per acre basis were not applicable. The egg mass (fixed and variable-radius samples pooled), larval and pupal population densities (Table 3) were calculated using an estimate of 207.25 trees per acre as a conversion factor. This estimate was based on 40 samples of 10 trees measured in the vicinity of various plots, and included only trees measuring 6+ inches dbh. The calculated densities of the various life stages (Table 3) are probably conservative since many trees considered in the conversion factor were smaller in diameter than those employed for population sampling.

Pre-spray egg mass densities in plots receiving chemical treatments ranged 156-270 EM per acre (=205). Plots receiving BT treatments supported pre-spray densities of 22-429 EM per acre (=138). Untreated control plots pre-spray EM densities ranged between 58-411 EM per acre, averaging 247. In chemical plots, the pre-spray densities were above the expected minimum of 150 EM per acre, in agreement with the initial density estimates. However, the expected range of 50-100 EM per acre in BT plots was much narrower than was actually the case. Judging from this and the disparity among EM densities in the various control plots, we question the reliability of the five minute count technique at low densities.

The rainfall experienced in close association with several applications (Table 4) may have adversely affected the performance of some materials. Laboratory studies (Table 5) suggest that the effectiveness of Thuricide 16B may be severely reduced when large amounts of rainfall occur within 24 hours of application.

Larval counts were conducted when larvae were predominantly in the 4th and 5th instars. In general, larval density within individual plots did not reflect pre-spray EM density. Beyond treatment effects, a number of factors may have contributed to this. Large numbers of larvae dead from parasitism, predation and virus infection were found under several burlap bands.

Pupal densities within plots treated with chemicals were greater than the earlier larval densities! In contrast, most plots treated with BT showed a reduction in pupal densities from the earlier larval densities. We have no explanation for this but suspect it is related to intrinsic error in burlap sampling. These reverse trends in population density change are illustrated by the "Corrected Population Reduction" column of Table 3. These values represent larval mortality (occurring between the larval and pupal sampling periods) attributed to treatment with natural mortality (estimated from the control plots) removed.

Among all BT treatments, only the late single BT application failed to reduce the pupal population density below the earlier larval density. Thus, two successive BT applications are recommended over one late application of BT.

The defoliation estimates for the various treatments (Table 3) ranged between 11-30 percent defoliation; greatest defoliation occurred in the control plots. Unfortunately, the defoliation estimates of the various treatments do not reflect differences in treatment performance expressed as corrected population reduction. It is possible that the defoliation estimates actually represent differences in stand composition among the various plots, more so than differences in treatment performance.

Evidently the gypsy moth population was increasing in the general region of this study. In some cases, egg mass densities increased more in treated areas than in untreated areas (Table 3). In only three treatments were the postseason EM densities reduced below the pre-spray densities. Egg mass density was reduced 78 percent following treatment with SIR 8514. Eighty-two percent reduction in EM density resulted from two applications of Thuricide 16B (8 BIU AI/acre) at the rate of 0.5 gallons per acre. The control achieved by all other treatments was negligable.

The poor control afforded by early Dimilin application was disappointing, since similar treatments in previous field trials showed more favorable results. In order to obtain adequate coverage of surfaces likely to be consumed by larvae, we suggest that Dimilin treatments be applied according to conventional timing schedules.

The poor control effected by most Thuricide treatments was partly attributed to rainfall. Apart from disrupting the spray schedule, the rainfall experienced following several BT applications (Table 4) may have washed some deposited material from foliage. Laboratory studies (Table 5) suggest that the efficacy of Thuricide 16B may be seriously reduced by heavy rainfall. Perhaps this is also true of Thuricide 24B, although this remains to be determined under laboratory conditions.

Table 1. Summary of formulations, dosages, and dates of application.

Treatment Material	Formulation	Dose/Acre	Application Date(s)
Dimilin	25W	0.03 lb AI/0.5 gal	May 3
SIR 8514	25W[1]	0.03 lb AI/0.5 gal	May 20
Thuricide	24B[2]	8 BIU AI/0.75 gal	May 18 & 30
Thuricide	24B	8 BIU AI/0.75 gal	May 17 & 31
Thuricide	16B	8 BIU AI/0.75 gal	May 15 & 31
Thuricide	16B	8 BIU/AI/0.5 gal	May 15 & 31
Thuricide	16B	8 BIU AI/0.75 gal	May 27

1 Non-centrifuged formulation

2 Formulation super-centrifuged prior to shipment

Table 2. Specifications of the spray nozzles employed for the various treatment applications.

Application	Orifice	No.	P.S.I.	Screens
Dimilin	8004 flat fan	18	40	50-mesh slotted
SIR 8514	8004 flat fan	18	40	50-mesh slotted
1st application BT	8006 flat fan	18	40	50-mesh slotted
single applic. BT (by spray plane)	8006 flat fan	18	40	50-mesh slotted
2nd application BT (by helicopter)	8004 flat fan	8	40	50-mesh slotted

Table 3. Population density observations in treatment plots.[1]

Treatment	Pre-spray[2] Egg Masses per Acre	Post-season Egg Masses per Acre	Egg Mass[3] Density Change (%)	No. Larvae per Acre	No. Pupae per Acre	Corrected Population Reduction (%)	Percent Defoliation
Growth Regulators: (0.03 lb. AI/Acre)							
Dimilin	200	328	- 64	2543	6369	- 205	22
SIR 8514	210	46	78	894	1024	- 39	23
Thuricide: (8 BIU AI/Acre)							
24B 0.75 gal/Acre x 2[4]	136	133	2	5002	2728	34	19
24B 0.75 gal/Acre x 2[5]	140	187	- 34	5350	3108	29	25
16B 0.75 gal/Acre x 2	99	156	- 58	4518	2727	27	14
16B 0.5 gal/Acre x 2	255	45	82	2222	1149	37	21
16B 0.75 gal/Acre x 1	59	123	-108	2334	1965	- 2	11
Untreated checks:	247	360	- 46	5968	4904	-	30

1 Each treatment was replicated three times; there were six untreated check plots.

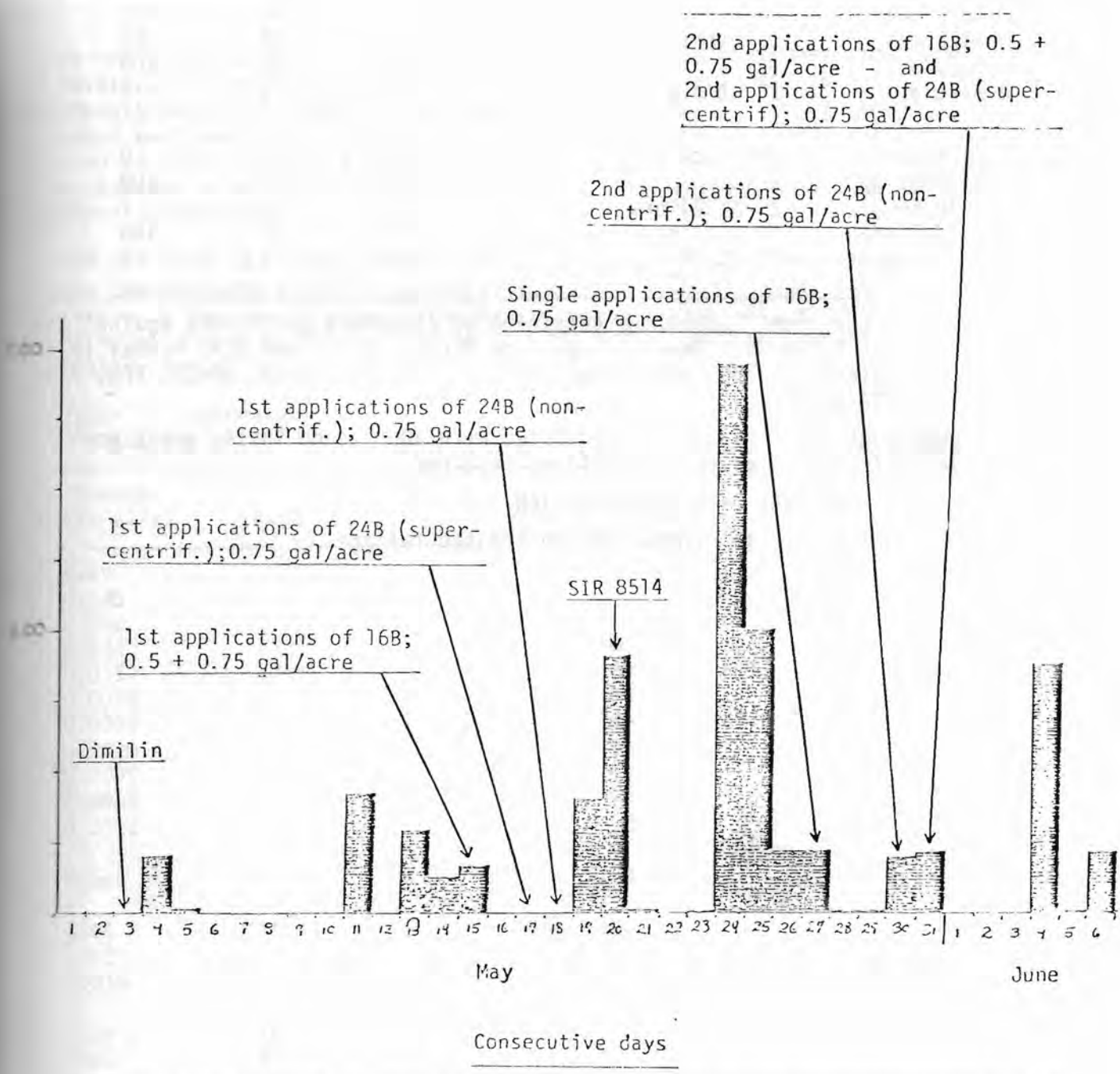
2 Fixed- and Variable-Radius samples incorporated into figures of all Egg Mass Density columns.

3 Abbott's Formula

4 Non-centrifuged formulation.

5 Super-centrifuged prior to shipment.

Table 4. Various application dates and subsequent inches of rainfall [1] encountered during the 1979 spray program.



1 Rain gauge readings from the Canistear Reservoir dam located near the center of the study region. Rainfall recorded at 8:00 a.m. daily.

Table 5. Summary of laboratory tests with Thuricide 16B, demonstrating the influence of rainfall following foliage treatment.[1]

Application[2] Dosage & Rate	Simulated rainfall inches and time administered	Percent Larval Mortality[3-4] 4 Days	8 Days	10 Days
8 BIU AI/2 g/a	-	36	98	100
8 BIU AI/2 g/a	2" within 1 hr	0	18	22
8 BIU AI/g/a	-	35	90	100
8 BIU AI/g/a	2" at 2.5 hrs	0	5	5
8 BIU AI/g/a	2" at 24 hrs	0	0	0
8 BIU AI/0.66 g/a	-	37	92	100
8 BIU AI/0.66 g/a	2" at 24 hrs	0	1	1
8 BIU AI/0.5 g/a	-	15	87	100
8 BIU AI/0.5 g/a	2" at 24 hrs	0	3	4

1 Data extracted from "Laboratory screening of candidate pesticides against the gypsy moth"; Table 25. Interim report by McLane, W.H., and J.A. Finney in the April - September 1979, Semi-Annual Laboratory Report: USDA, APHIS, PPQ, Otis Air Base, MA 02542.

2 Oak seedlings were treated, and infested with 100 second instar gypsy moth larvae following simulated rainfall as required.

3 Hours post-treatment with Thuricide 16B.

4 Days following larval introduction to treated foliage.

Project Number: GM 0.1.2
Project Title: Field Studies with SIR-8514, *Bacillus thuringiensis* and Gypchek
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: W. H. McLane

As new compounds and improved formulations of registered pesticides are developed in the laboratory, it is important that efficacy be established in the field under natural conditions. Typically, these tests are conducted on small plots (40-50A) and are followed by pilot studies that ultimately support registration. Similar studies with registered materials such as BT and Gypchek are conducted to optimize their effectiveness.

BAY SIR-8514, an insect growth regulator from Mobay Chemical Company, has demonstrated excellent activity against 2nd instar gypsy moth larvae in the laboratory. Even when exposed to rainfall, enough material stayed on the foliage to give good kill of test insects (Table 1).

Table 1. Laboratory studies with SIR 8514, an insect growth regulator.

Dosage (lb AI/A)	Rain	Percent Mortality
0.25		100
0.125		98
0.062		97
0.031		100
0.015		100
0.0078		100
0.0039		100
0.0019		98
0.0009		95
0.0004		52
0.0002		32
Check		0
0.06	1"	100
0.03	1"	93
0.015	1"	100
0.06	3"	98
0.03	3"	100
0.015	3"	95
Check		0

Bay SIR-8514 was first field tested in northern New Jersey in 1979. Three 50 acre plots were treated by aircraft with 0.03 pounds of active material per acre. The formulation was applied in a single application at the rate of 0.5 gallons per acre. On the average, egg mass reduction in the sprayed plots was 78 percent. Based on laboratory results, we expected a much greater population reduction than what was realized in the New Jersey field tests.

Because of the excellent laboratory results with this material, Mobay Chemical Company submitted for, and was granted, an experimental use permit to test the material at 0.06, 0.03 and 0.015 pounds per acre. This set of tests was conducted during 1980, on Rhode Island state lands, by the Otis Methods Development Center.

Bacillus thuringiensis (BT) has been registered and used for gypsy moth control for a number of years. The main producers of BT are Abbott Laboratories (Dipel) and Sandoz, Inc. (Thuricide). The material has been in limited use because of a number of factors, namely high cost, two applications are required and inconsistant results are often obtained. Inconsistant results may be due to application techniques, poor weathering characteristics and ultraviolet degradation.

Laboratory tests of 3 formulations of BT showed that each formulation is subject to washoff (Table 2).

Table 2. Weathering tests with Thuricide 16B, Thuricide 32B and Dipel 4L.

				Percent Mortality					
Material	Dosage	Drying Time	Rain	24H	48H	72H	96H	120H	144H
	8 BIU			2	19	55	83	93	96
	8 BIU	1.5 H	3"	0	1	1	3	12	22
4L	8 BIU	72.0 H	3"	4	5	10	13	30	71
	4 BIU	72.0 H	3"	0	2	7	18	33	49
	8 BIU			4	29	51	79	96	99
16B	8 BIU	1.5 H	3"	0	14	19	29	51	65
	8 BIU			2	27	57	80	94	99
	8 BIU	1.5 H	3"	0	0	0	19	26	34
32B	8 BIU	72.0 H	3"	0	3	19	43	65	86
	4 BIU	72.0 H	3"	0	5	12	41	66	86
Check				0	0	0	0	37	62

However, when stickers such as acrylocoat and RA-1645 were added to the BT formulations at 3.0%, the tank mixes weathered much better (Table 3).

Table 3. Influence of Acrylocoat and RA-1645 on weathering properties of 2 formulations of BT.

Material[1/]	Sticker	Drying Time	Rain	Percent Mortality[2/] 24H	48H	72H	96H	120H	144H
	Acrylocoat 3%	0.5 H	3"				67		
Dipel	Acrylocoat 3%	0.5 H	1"	10	24	43	78	89	97
4L	RA-1645 3%	0.5 H	3"				52		
	RA-1645 3%	0.5 H	1"	4	16	33	60	84	90
Thuricide	Acrylocoat 3%	0.5 H	3"				83		
32B	RA-1645 3%	0.5 H	1"	1	11	32	67	85	97
Check				0	0	0	0	0	0

1/ All applications made at 8.0 BIU per acre.
2/ Hours after treatment that mortality readings were made.

Field tests of 8 formulations of BT were conducted on state lands in western Massachusetts during 1980.

Gypchek, the registered formulation of the nucleopolyhedrosis virus (NPV) has seen only limited use since its registration by the Environmental Protection Agency. This could be due to a number of reasons. The material is presently costly to produce, hard to handle, not available except for research, and adversely affected by ultraviolet light and rainfall.

The present label requires 2 applications applied at 25.0 to 125.0 million gypsy moth potency units (GMPU) per acre. Data to back this dosage recommendation came mainly from laboratory experiments. We therefore felt that dosage response information should be obtained from field tests. It was planned that this work would be done in New Jersey by the U. S. Forest Service and in Massachusetts by APHIS. Forest Service involvement was subsequently terminated for various reasons. Plots were established in western Massachusetts on state lands along with BT spray plots. We planned to test Gypchek in the "Pro Tec" formulation at 4 dosages (Table 4).

Table 4. 1980 field test materials, rates and dosages.

Material	Dosage/Acre	No. Gal/Acre	No. App	Plots
SIR-8514	0.015 lbs	0.5	1	3
	0.03 lbs	0.5	1	3
	0.06 lbs	0.5	1	3
Gypchek	25×10^6	2.0	2	4
	6.25×10^7	2.0	2	4
	12.5×10^7	2.0	2	4
	25×10^7	2.0	2	4
Abbott 4L	8 BIU	1	2	4
Abbott 4L + 3% RA-1645	8 BIU	1	2	4
Thuricide 32B	8 BIU	1	2	4
Thuricide 32B + 3% RA-1645	8 BIU	1	2	4
Thuricide 16B	8 BIU	1	2	4
Thuricide 32B	8 BIU	0.25	2	4
Thuricide 32B + 3% Acrylocoat	8 BIU	1	2	2
Abbott 4L + 3% Acrylocoat	4 BIU	1	2	1
Checks				12

Rhode Island Tests with SIR-8514

After obtaining approval from the Rhode Island Department of Environmental Management, 12 50-acre spray plots were established in Pulagki Memorial Forest, Burrillville, RI. This location was selected because population conditions were favorable for our test objectives.

Boundary lines and plot corners were established with a compass and rope, and marked with various colors of surveyor tape. Twenty prism points were established within the center 10 acres of each plot. This was accomplished by having 5 points on each of 4 lines.

At each point, prism trees were identified and labeled with an aluminum tag; 5 trees (generally white oak) were also burlaped at each point. At every other point, 1 egg mass was collected and returned to the laboratory for testing. Pre-spray egg mass counts were made on all prism trees and on the fixed radius plots. The number of egg masses per acre was then computed using Wilson's technique. (Figure #2). Results of this survey are given in Table 5.

Table 5. Pre-spray egg mass data, Rhode Island spray plots 1980.

Plot No.	Dosage	Egg Masses Per Acre	Percent Hatch	Larvae per 50 acre Spray Plot x 10^6 1/
1	0.06 lbs	131	80	2.1
2	0.06 lbs	101	78	2.0
3	0.06 lbs	119	77	2.2
4	0.03 lbs	122	79	2.0
5	0.03 lbs	184	80	3.1
9	0.03 lbs	151	81	3.0
6	0.01 lbs	812	73	12.2
7	0.01 lbs	1,124	61	7.7
8	0.01 lbs	269	91	5.9
C1		448	90	10.0
C2		297	76	6.2
C3		109	81	2.0

1/ Based on the average number of eggs/mass in the 10 samples from each plot.

During April, 1980, the Environmental Protection Agency granted Mobay Chemical Corporation an experimental use permit (3125-EUP-164) for application of SIR-8514 to experimental spray plots in Rhode Island.

On May 27-28 all plots in Rhode Island were treated with the USDA Cessna-Ag-Truck. The aircraft was equipped with 18 flat fan nozzles, number 8004. Spray boom was 90° to slip stream and a pressure of 40 PSI was used. The material was applied at 120 mph in 60 foot swaths, 15 feet above the foliage. Using this technique, a droplet VMD of 100 u was obtained. Plot corners were marked with yellow balloons and radios were used to aid navigation. All treatments were made during the early morning hours under clear weather conditions. Temperature at treatment time ranged from 50-70° F. All mixing and aircraft operations took place out of Danielson Airport, Danielson, Connecticut. Clearance was obtained from the Connecticut pesticide board.

Because SIR-8514 was applied under an experimental use permit, Rhode Island pesticide regulatory personnel visited the mixing and spray areas. They took photograpghs of the experimental label and checked to assure all paper work was in order. They observed the mixing and application of the material. Work was done under the applicator certification of Winfred H. McLane.

Burlaps were checked on a weekly basis starting 16 days following treatment and continued for 6 weeks. Results are summarized in Table 6.

Table 6. Live larval (L) and pupal (P) counts under burlap bands in Rhode Island plots.

		NUMBER OF LIVE LARVAE AND/OR PUPAE											
	Dosage	6/12		6/18		6/26		7/2		7/10		7/20	
Plot	SIR 8514	L	P	L	P	L	P	L	P	L	P	L	P
1	0.06 lbs	144	-	36	-	4	-	2	-	2	-	-	1
2	0.06 lbs	286	-	48	-	17	-	7	2	10	13	-	8
3	0.06 lbs	114	-	8	-	2	-	5	-	3	3	-	1
4	0.03 lbs	401	-	35	-	22	-	51	3	15	9	2	3
5	0.03 lbs	421	-	92	-	10	-	7	-	-	2	-	-
9	0.03 lbs	652	-	283	-	82	2	91	3	121	74	41	142
6	0.01 lbs	611	-	322	-	59	-	30	2	25	9	2	4
7	0.01 lbs	165	-	61	-	24	-	10	-	8	2	1	1
8	0.01 lbs	178	-	89	-	29	-	36	-	54	31	8	58
C1		229	-	-	-	114	4	284	83	388	311	31	338
C2		131	-	203	-	148	-	345	53	348	306	43	397
C3		367	-	310	-	127	-	141	32	209	180	97	191

Defoliation estimates were made at each prism point at the time of peak defoliation. Results are in Table 7.

Table 7. Percent defoliation in 1980 Rhode Island plots treated with SIR-8514.

Dosage	Plot No.	Percent Defoliation [1/]
0.06 lbs/ac	1	12
0.06 lbs/ac	2	12
0.06 lbs/ac	3	19
0.03 lbs/ac	4	13
0.03 lbs/ac	5	17
0.03 lbs/ac	9	19
0.01 lbs/ac	6	7
0.01 lbs/ac	7	12
0.01 lbs/ac	8	17
	CHECK 1	54
	CHECK 2	44
	CHECK 3	32

[1/] Average defoliation of all trees at 20 prism points.

Massachusetts Tests with BT and Gypchek

In cooperation with the Massachusetts Department of Environmental Management, 53 square 50 acre plots were established on state lands in Erving, Northfield, Warwick and Wendall State Forests. These areas were selected because they harbored healthy egg mass populations and little defoliation was noted the previous year.

Plot layout was similar to that described for the Rhode Island study. In Gypchek plots, 2 lines, parallel to the prism point lines, were established on which 10 minute counts were made.

When plots were set up during March and April, 1 egg mass was collected at every other prism point. Five were taken from below the snow line and 5 from above. Egg masses were returned to the laboratory where hatch was determined. Pre-spray egg mass counts were made on all prism point trees and fixed radius plots. The number of egg masses per acre was then computed using Wilson's technique. Results are summarized in Table 8.

Table 8. Pre-spray egg mass data, Western Massachusetts spray plots, 1980.

Material	Dosage	Plot No.	Egg Masses Per Acre	Percent Hatch	Larvae per 50 Acre spray plot x 10^6 [1/]
Abbott 4L	8 BIU/a	17	2,304	84	38.4
		26	436	86	8.9
		39	582	96	13.5
		43	2,047	91	40.1
Abbott 4L + 3% RA-1645	8 BIU/a	28	1,727	83	17.9
		41	1,063	82	19.4
		44	250	92	4.6
		50	3,412	82	44.8
Abbott 4L + 3% Acrylo-coat	4 BIU/a	3	34	92	.6
Thuricide 32B	8 BIU/a	13	3,670	93	57.5
32B		16	2,066	85	30.2
		21	221	87	3.7
		32	925	83	16.8
Thuricide 32B + 3% RA-1645	8 BIU/a	7	908	87	16.7
		18	1,302	83	20.0
		23	215	80	3.4
		47	3.254	95	47.3
Thuricide 32B + 3% Acrylo-coat	8 BIU/a	8	894	94	20.7
		10	493	92	9.8
Thuricide 32B	8 BIU/a	20	2,989	83	57.1
32B		30	760	89	14.4
		40	1,314	90	23.0
		45	107	92	1.0
Thuricide 32B	8/BIU/a	34	334	84	6.8
		38	376	88	6.9
Controls		14	43	93	.9
		22	145	85	3.1
		35	386	88	7.7
		42	895	82	19.5
		49	2,770	83	43.1

Table 8. (Cont'd).

Material	Dosage	Plot No.	Egg Masses Per Acre	Percent Hatch	Larvae per 50 Acre spray plot x 10^6
Gypchek	12.4×10^7	4	97	86	1.9
		9	1,785	92	29.2
		25	266	86	5.0
		27	938	84	16.9
Gypchek	25×10^7	5	194	89	4.2
		19	1,282	87	20.6
		31	1,610	87	37.9
		37	281	86	5.0
Control Gypchek	-	2	245	95	5.1
		24	128	90	2.5
		29	829	87	13.4
		36	1,051	89	17.4

1/ Based on the average number of eggs from the 10 egg masses of each plot.

Because all formulations of BT are registered for gypsy moth control, no experimental use permit was needed for our treatments in Massachusetts. We did have to acquire one for the high dosage of Gypchek and were issued 27586-EUP-27. The state also issued permit number 1-80 for the Gypchek work.

We filed an "Environmental Notification Form" with the Executive Office of Environmental Affairs and notified the public by radio and newspaper. Just a few days prior to spraying, all residences within 0.5 miles of any spray area were notified in person about the spray program. The aircraft pilot took the examination for aerial application certification just before treatments started. The program was conducted under the certification of W. McLane.

We attempted to start our first application of Gypchek on May 20. The carrier for the Gypchek was Pro-Tec (United States Trading International Inc.). We mixed 1 part Pro Tec with 5 parts water. Active material (Gypchek) was mixed with water in 4 15-second bursts of the blender. This mix was added to the Pro Tec-water mix, agitated for about 15 minutes and then loaded into the aircraft.

The spray system was equipped with 8008 flat fan nozzles; all screens were removed from the system. After spraying about 2 swaths the system plugged. Upon examination it was learned that the material had turned hard in the system, thus completely blocking flow.

United States Trading International reported that we received a "bad batch" of Pro Tec. Because all in stock was "bad" they had to prepare another batch. By the time the new material was received most larvae were in the 4th instar. At this time all Gypchek work probably should have been suspended. However, we felt that we might salvage something by trying one application at the 2 highest dosages, namely 25×10^7 and 12.5×10^7 GMPU/A.

On June 6th, 4 plots were sprayed with Gypchek at 25×10^7 and on the following day 2 plots received 12.5×10^7. Nozzles were 80015 with no screens. All applications were made after 3:00 pm.

After spraying each plot the main screen (50 mesh) of the aircraft would have to be cleared of up to 0.5 inches of virus material.

Starting on May 23 plots were sprayed with the first application of BT. Larvae were mainly 2nd instar at this time and foliage was expanded to 75 percent. The aircraft was equipped with conventional 8008 flat fan spray nozzles. Boom pressure was 40 PSI and the aircraft speed was approximately 120 mph.

The second application was applied on all BT plots approximately 7 days later.

Table 9. Schedule of spray applications in western Massachusetts.

Material	Dosage	Plot	1st app.	2nd app.
	8 BIU/gal	17	5/24/80	5/30/80
Abbott 4L	8 BIU/gal	26	5/24/80	6/6/80
	8 BIU/gal	39	5/23/80	5/29/80
	8 BIU/gal	43	5/23/80	5/29/80
	8 BIU/gal	28	5/23/80	5/30/80
Abbott 4L +	8 BIU/gal	41	5/23/80	5/30/80
3% RA-1645	8 BIU/gal	44	5/24/80	6/6/80
	8 BIU/gal	50	5/24/80	5/31/80
Abbott 4L + 3% Acrylocoat	4 BIU/gal	31	5/25/80	6/6/80
	8 BIU/gal	13	5/24/80	5/31/80
	8 BIU/gal	16	5/23/80	5/30/80
Thuricide 32B	8 BIU/gal	21	5/24/80	6/6/80
	8 BIU/gal	32	5/23/80	5/29/80
	8 BIU/gal	7	5/23/80	5/29/80
Thuricide 32B +	8 BIU/gal	18	5/23/80	5/30/80
3% RA-1645	8 BIU/gal	23	5/24/80	6/1/80
	8 BIU/gal	47	5/24/80	6/6/80
Thuricide 32B +	8 BIU/gal	8	5/25/80	6/6/80
3% Acrylocoat	8 BIU/gal	10	5/25/80	6/6/80
	8 BIU/gal	20	5/24/80	6/1/80
Thuricidde 16B	8 BIU/gal	30	5/23/80	5/29/80
	8 BIU/gal	40	5/24/80	5/30/80
	8 BIU/gal	45	5/24/80	6/6/80
Thuricide 32B	8 BIU/gal	34	5/28/80	6/6/80
		38	5/28/780	6/6/80
	$25X10^7$	5	6/6/80	
Gypchek	$25X10^7$	19	6/6/80	
	$25X10^7$	31	6/6/80	
	$25X10^7$	37	6/6/80	
Gypchek	$12.5X10^7$	9	6/7/80	
	$12.5X10^7$	27	6/7/80	

Following treatment of all spray plots, larval counts were made under burlap in the BT and check plots. Live and dead larvae were observed and recorded on a weekly basis. Those data are summarized in Table 10.

Table 10. Larval counts under burlaps in Western Massachusetts BT plots.

Material	6/3		6/11		6/18		6/25		7/9			7/17		
	L	D	L	D	L	D	L	D	L	D	P	L	D	P
Abbott 4L 1/	454	368	693	901	269	381	118	104	613	60	26	543	113	180
Abbott 4L 1/ + RA-1645	303	528	777	1113	165	308	90	79	534	154	24	1003	253	218
Check 2/	154	45	923	200	709	178	507	173	2110	546	654	2294	1313	999
Thuricide 32B 1/	376	226	499	877	242	197	219	121	1502	234	506	1113	1253	449
Thuricide 32B 1/ + RA-1645	521	149	421	747	232	706	187	121	713	104	80	779	462	390
Thuricide 16B 1/	364	437	585	1151	111	171	72	45	595	58	29	318	273	343
Thuricide 32B 3/ 1 Qt/a	55	75	413	406	390	316	138	52	1248	126	226	2388	316	197
Check 4/	266	66	1016	327	646	142	440	119	1906	438	569	1567	1374	1042
Thuricide 32B 3/ + Acrylocoat	214	120	544	794	185	460	91	30	963	146	195	997	693	187
Abbott 4L 5/ + Acrylocoat	-	-	447	248	162	177	91	43	524	46	32	642	93	247
Check 6/	-	-	437	115	379	63	297	67	1037	143	353	1950	398	340

1/ Average live, dead larvae and pupae in 4 plots
2/ Check plots that go with all treatments having 4 replications. Four check plots
3/ Average live, dead larvae and pupae in 2 plots
4/ Average of 2 check plots that go with treatment Thuricide 32B, 8 BIU/qt/acre
5/ Average live, dead larvae and pupae in 1 plot
6/ Check plot for Thuricide 32B + Acrylocoat plots and Abbott 4L + Acrylocoat plot.

Table 11. Results of 10 minute larvae counts in Gypcheck spray plots and checks.

Dosage	June 9-10	June 16-18	June 23-24	June 30	July 7-8	July 14-15
25 X 10^7	335 1/	240	189	282	104	133
12.5 X 10^7	159 2/	235	122	192	67	122
Check	129 2/	113	74	71	68	77

1/ Average live larvae counted in 2 plots
2/ Average live larvae counted in 4 plots

At peak defoliation time, a defoliation estimate was made at each prism point in all plots. The 20 points were averaged and are presented below in Table 12.

Table 12. Defoliation in Massachusetts spray plots and checks.

Treatment	Average Percent Defoliation
Abbott 4L	10
Abbott 4L + 1645	11.5
Abbott 4L + acrylocoat	9
Thuricide 32B	22
Thuricide 32B + 1645	16
Thuricide 32B + acrylocoat	10
Thuricide 16B	9
Thuricide 32B/Qt.	17
Check (BT)	25
Gypchek 25 x 10^7	57
Gypchek 12.5 x 10^7	72
Check (Gypchek)	41

Rainfall was recorded in the general area of the spray plots. No rainfall was recorded for one week following the first application. Approximately 1.24 inches of rainfall was recorded during the week following the second application. The generally low rainfall over the spray period may complicate distinguishing between effectiveness of treatments with and without sticker.

Table 13. Rainfall following BT and Gypchek treatments in Massachusetts.

Date	Measured Rainfall	Date	Measured Rainfall
5/23	0	6/2	.01
5/24	0	6/3	.46
5/25	0	6/4	.20
5/26	0	6/5	.06
5/27	0	6/6	0
5/28	0	6/7	.37
5/29	0	6/8	.14
5/30	0	6/9	0
5/31	0	6/10	.25
6/1	0	6/11	0

Upon the return of the spray aircraft to the airport, from BT spraying, 100 tender northern red oak seedlings were aerially treated with 2 applications of the mix remaining in the hopper. Treated plants were returned to the laboratory and planted outdoors in a shaded area. Plants were watered on a regular basis, however, care was taken to avoid wetting the treated foliage. Natural rainfall was recorded. One-hundred plants were also placed outside as check plants.

Each Monday, Wednesday and Friday 10 plants from each treatment and a check were taken to the laboratory and infested with laboratory reared, 2nd instar gypsy moth larvae, 20 per plant. Mortality readings were made on a daily basis for 6 days. Results are summarized in Table 14.

Data collected at this time indicate that foliage protection was achieved with all BT treatments. However, large numbers of new egg masses have been observed in all plots. This is typical of BT treatments: foliage protection with only limited population reduction.

No foliage protection was obtained in the Gypchek plots and no population reduction is anticipated.

Post season egg mass counts will be made in late October and a final report will be submitted in the next laboratory report.

Table 14. Results of tests conducted on oak seedlings treated by aircraft.

Material	BIOASSAY I			BIOASSAY II			BIOASSAY III			BIOASSAY IV		
	D	R	M	D	R	M	D	R	M	D	R	M
Thuricide 16B	4	0	100	6	.25	98	8	.85	27	11	0.9	57
Thuricide 32B	4	0	48	6	.25	47	8	.85	10	11	0.9	14
Thuricide 32B + RA-1645	4	0	100	6	.25	94	8	.85	73	11	0.9	93
Thuricide 32B + Acrylocoat	5	1.0	97	7	1.0	90	10	2.15	75	12	2.15	70
Dipel 4L	4	0	92	6	.25	82	8	.85	25	11	0.9	36
Dipel 4L + RA-1645	4	0	100	6	.25	83	8	.85	51	11	0.9	51
Dipel 4L + Acrylocoat	5	1.0	92	7	1.0	76	10	2.15	80	12	2.15	40
Check	4	0	0	6	.25	0	8	.85	2	11	.9	3

Material	BIOASSAY V			BIOASSAY VI			BIOASSAY VII		
	D	R	M	D	R	M	D	R	M
Thuricide 16B	13	1.4	71	15	1.4	41	18	2.55	59
Thuricide 32B	13	1.4	16	15	1.4	8	18	2.55	2
Thuricide 32B + RA-1645	13	1.4	86	15	1.4	52	18	2.55	69
Thuricide 32B + Acrylocoat	14	2.15	50	17	2.15	12	19	2.15	63
Dipel 4L	13	1.4	26	15	1.4	15	18	2.55	16
Dipel 4L + RA-1645	13	1.4	43	15	1.4	10	18	2.55	15
Dipel 4L + Acrylocoat	14	2.15	51	17	2.15	17	19	2.15	59
Check	13	1.4	18	15	1.4	2	18	2.55	5

D - Number of days following treatment that bioassay was conducted.
R - Accumulative amount of natural rainfall on treated plants.
M - Percent of mortality after 6 days.

Project Number: GM 7.2.4A
Project Title: Development of Laboratory Evaluation Techniques for Gypsy Moth Male Comparisons - The Effect of Temperature on the Pheromone-mediated Behavior of Laboratory-reared Male Gypsy Moths
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: D. Lance

Introduction

In some Lepidoptera, the temperature regime in which teneral adults are held during maturation has been shown to affect the periodicity of flight (Truman 1973) and the periodicity and magnitude of male responsiveness to pheromone (Bollinger et al., 1977). Gypsy moth males are sexually mature (or nearly so) upon eclosion and commonly live only a few days as adults. Accordingly, the temperature regime in which pupae are maintained was suspected as an agent which could affect the nature and periodicity of the sexual behavior of gypsy moths.

At the Otis laboratory, a series of experiments have been initiated to determine the holding temperature regime for pupae which will result in optimal sterile male sexual behavior. In actograph tests (see GM 0.2.5 in this report), males which were held at relatively cool temperatures (e.g. 20° C) as pupae had a periodicity of activity similar to that of wild-type males. In this study, a sustained-flight wind tunnel is used to define the relationships between pupal holding temperatures and the pheromone-mediated behavior of male gypsy moths.

Methods and Materials

All tests were run in the sustained flight wind tunnel described by Waldvogel et al (in manuscript). The flight chamber of the tunnel is a 2.4 x 1.2 x 0.9 m plexiglass box with screening at either end. For these tests, a wind speed of ca. 0.50 m/sec was maintained within the tunnel.

During the tests, a pheromone source was placed on a 25 cm h plexiglass stand which was 20 cm from the upwind end of the flight chamber. The pheromone source was as 1 cm diameter filter paper disc which was mounted on a stainless steel insect pin and loaded with 0, 2, 10 or 50 ng (+) disparlure in 10 ul hexane. Due to a relatively laminar air flow within the tunnel, the plume of pheromone which was given off by the wick reached a diameter of only 20-25 cm at the opposite end of the tunnel (where moths were placed for testing).

Males moths which were used in the tests were from the New Jersey Standard strain (NJS) and were reared at the Otis facility on the B4 production diet. Larvae were maintained at 25° C until the prepupal (spin) stage. Prepupae were harvested, placed into 480 ml untreated paper cups (50 males/cup) and held at 1 of 4 temperature regimes: a constant 20°, 25°, or 30° C, or a fluctuating temperature regime (FT) which reached a low of 12o C. The pupae were maintained under these conditions until adult eclosion. Adults were harvested on the day of eclosion (ca. 1600 hrs) and held overnight at 20 per 480 ml paper cup within the flight tunnel and under the temperature conditions in which they would be tested the following day. A photophase of 0500 to 2100 hrs was maintained throughout rearing and testing.

In order to examine the periodicity of pheromone response, tests were conducted on an hourly basis from 0830 to 1600 hrs (in field tests, a large majority of male recaptures occur within this time period). At the start of each hour, moths were chilled (13° C) and placed into 7 cm diameter x 12 cm long cylindrical plastic mesh cages (3 moths/cage). The moths were then maintained at flight tunnel temperatures until they were used.

For testing, the cage containing moths was placed on a plexiglass stand (22 cm high) within the flight tunnel, but not within the pheromone plume. After a 2-5 minute acclimation period, the upwind end of the cage was removed, and the plexiglass stand was pushed gently into the pheromone plume.

Once exposed to the pheromone, moths were observed for 5 minutes, and the following was noted: the number of moths initiating wing-fanning, the number initiating anemotactic flight, the occurrence of sustained flight within the plume (only if moths entered the plume within 15 seconds of initiating flight), and the times at which wing-fanning and flight were started.

Table 1 shows the number of moths flown at each of four pheromone concentrations, under each of four temperature regimes, at each hourly period; the July simulation (JS) regime is a fluctuating temperature regime with a high of 30° C from 1200 to 1400 hours and a low of 20° C from 0400 to 0600 hours.

Results

Effect of Pheromone Concentration

The percent of males which initiated wing-fanning within 5 minuts increased from 9-23% at 0 ng (+) disparlure up to nearly 100% in the 50 ng tests (Fig. 1). In general, the proportion of males which responded did not vary much among treatment groups; however, a significantly higher ($p<0.05$) proportion of males from the FT pupal regime initiated wing-fanning in the 0 ng tests. In 1979 flight-tunnel tests, males which were eclosed in an outdoor insectary similarly responded at 0 and 2 ng more frequently than males which were maintained at constant temperatures (unreported data). Fluctuating pupal temperature regimes may result in males which are more prone to appetitive flight than males which were maintained at constant temperatures.

At all pheromone concentrations, males from the different pupal treatment groups showed little difference in the time to initiate wing-fanning (or response latency period=RLP; Fig. 2). The variation in the 0 ng tests was primarily a result of small sample size (13-29 males). In general, RLP's decreased with increased pheromone concentration. The duration of pre-flight wing-fanning (DPF) also varied little among the 20°, 25°, and FT males (Fig. 3); however, the 30° males had longer DPF's at all 4 pheromone concentrations, indicating that holding pupae at 30° may result in reduced adult flight potential.

Effect of Temperature During the Tests

In general, the percent of males which responded to 2 ng wicks increased gradually with increased temperature (Fig. 4). RLP's varied little with flight temperature; no trends in RLP vs. temperataure were evident among moths from any of the pupal treatment groups (Fig. 5). In general, DPF decreased with increased temperature (Fig. 6). Among the 20°, 25° and FT males, no differences in DPF were evident except at 23°, where moths which were held at cooler pupal temperatures had shorter DPF's. Males from the 30° pupal treatment had longer DPF's at all 3 flight temperatures.

Periodicity of Response

For constant flight temperatures, the proportion of moths which initiated wing-fanning are shown by hour of the day in Fig. 7. The responsiveness of the 25° males was relatively constant from the 0900 tests through the 1430 tests, but rose in the 1530 tests; this is in close agreement with the results of Waldvogel et al (in manuscript). In general, the responsiveness of males from the different pupal treatments was similar in the afternoon tests, but, in the morning tests, males from the cooler pupal holding regimes tended to be more responsive than moths from the warmer pupal treatments.

When exposed to a simulated July day temperature regime during the tests, males from the 20°, 25°, and FT treatments showed a definite periodicity in response, with very few moths responding in the 0900 hours tests, and maximum responsiveness reached at 1100 or 1200 hrs (Fig. 8). In the morning, the 20° and FT males were more responsive than the 25° males. The responsiveness of the 30° males showed less periodicity and was generally lower than that of the males from the other pupal treatments.

Hourly RLP's for the JS tests were quite variable, and no definite trends were evident among pupal treatment groups or among hours of the day (Fig. 9). Hourly DPF's were similar for the 20°, 25°, and FT males (Fig. 10); however, 30° males had consistently longer mean DPF's than males from the other pupal treatments.

Initiation of Wing Fanning vs. Flight

Males which wing-fanned did not always take flight. Table 2 shows the proportions of wing-fanning males which took flight within the 5 minute test period. Chi^2 analysis shows that the proportion of wing-fanning 30° males which took flight was significantly ($p<0.01$) lower than the proportions for 20°, 25°, or FT males. The 30° males' longer DPF's may be partially responsible for this, i.e., since 30° males wing-fanned longer than the other males, the time at which they initiated flight would have occurred more frequently after the end of the 5 minutes. However, many of the males (all treatments) which responded but did not fly either stopped wing-fanning without taking flight or appeared to attempt to fly (they faced into the wind and repeatedly jumped up ca. 1-2 cm, but couldn't sustain flight. Some moths which did this would land on their backs and then have trouble righting themselves).

Flight in the Plume

While some moths showed sustained flight within the plume at 2 and 10 ng, only the 50 ng tests provided sufficient numbers (or almost, anyway) for analysis. The proportions of moths which initiated sustained flight within the plume, are shown in Table 3. While the proportion of 20°, 25° and FT males entering the plume were similar, the proportion of 30° males entering the plume was significantly lower.

Discussion

Pupal holding temperatures did not appear to markedly affect the ability of adult male gypsy moths to perceive pheromone. In general, at a given temperature and pheromone concentration, the proportion of males which responded, and the time to response, was similar among moths from all 4 pupal temperature treatments; however, a number of differences in males performance did arise, and these differences may have implications for male competitiveness:

1. In the morning, males which were held at the cooler temperatures as pupae were more responsive than males which were held at warmer temperatures. In field studies, wild-type males tend to be recaptured at pheromone sources earlier in the day than laboratory-reared males (25° C throughout development). In actograph studies, the activity of 20° males, FT males, and wild-type males, peaks earlier in the day (i.e., ca. 1000 hrs) than the activity of 25° males (ca. 1200 hrs). Pending field studies this summer, the periodicity of sexual behavior in sterilized male gypsy moths appears to be optimized by the cooler pupal holding temperatures.

2. Males which are exposed to fluctuating pupal holding temperatures may be more prone to appetitive flight than males which are exposed to a constant temperature as pupae. Appetitive flight may increase a males chances of encountering a pheromone source. Thus, males which are exposed to a fluctuating pupal temperature regime may be more competitive than males which are exposed to constant temperatures as pupae.

3. 30° males required a longer period of pre-flight wing-fanning than males from other pupal treatment groups.

4. 30° males responded to pheromone in proportions similar to those in which the 20°, 25°, and FT males responded, but a significantly lower proportion of the responding 30° males took flight within the 5 minute test period.

5. Compared to males from the other pupal treatment groups, a significantly lower proportion of the 30° males showed sustained flight in the pheromone plume.

6. When exposed to a temperature regime which simulted a "typical" July day, the 30° males did not show a periodicity of responsiveness similar to that of the other males.

Differences 3-6 indicate that 30° males may be weaker fliers than the other males in these tests; this, along with "abnormal" periodicity, indicates that relatively high pupal holding temperatures may reduce male competitiveness.

Literature cited

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Truman, J.W. 1973. Temperature-sensitive programming of the silkmoth flight clock: a mechanism for adaptation to the seasons. Science *182*: 727-729.

Waldvogel, M.G., V.C. Mastro, C.H. Collinson, and E.A. Cameron. An evaluation of the pheromone-mediated responsiveness of laboratory-reared irradiated, laboratory-reared non-irradiated, and wild male gypsy moths. (In manuscript).

Table 1. Number of moths per pupal treatment flown at each hour for each combination of temperature and pheromone concentration.

Pheromone concentration	Temperature during test 23°	28°	33°	JS
0	N.C.[1]	9	9	9-12
2	12	9-30	15-24	24-36
10	N.C.	15-24	21-24	N.C.
50	N.C.	9-24	12	9-12

[1] N.C. = Series of tests not complete

Table 2. Proportions of wing-fanning males gypsy moths which took flight.

Pupal holding temperature	# males wing-fanning	# males flying	# flying/ #wing-fanning
FT	842	817	0.970 a
20°	916	880	0.961 a
25°	856	811	0.947 a
30°	685	606	0.888 b

[1] Proportions not followed by the same letter are significantly different ($p<0.01$) as determined by contingency table analysis.

Table 3. The proportions of male gypsy moths showing sustained flight within a pheromone plume (50 ng wick).

Pupal holding temperature	No. of males flown	No. in plume	No. in plume/ No. flown
FT	144	19	0.131 a
20°	166	18	0.108 a
25°	242	26	0.107 a
30°	128	4	0.031 b

[1] Proportions not followed by the same letter are significantly different ($p<0.05$) as determined by contingency table analysis (so I cheated a bit including the 30° bugs - so what?) !!!

Figure 1. The effect of pheromone dose on the percentage of male gypsy moths which initiated wing-fanning in 5 minute flight tunnel tests.

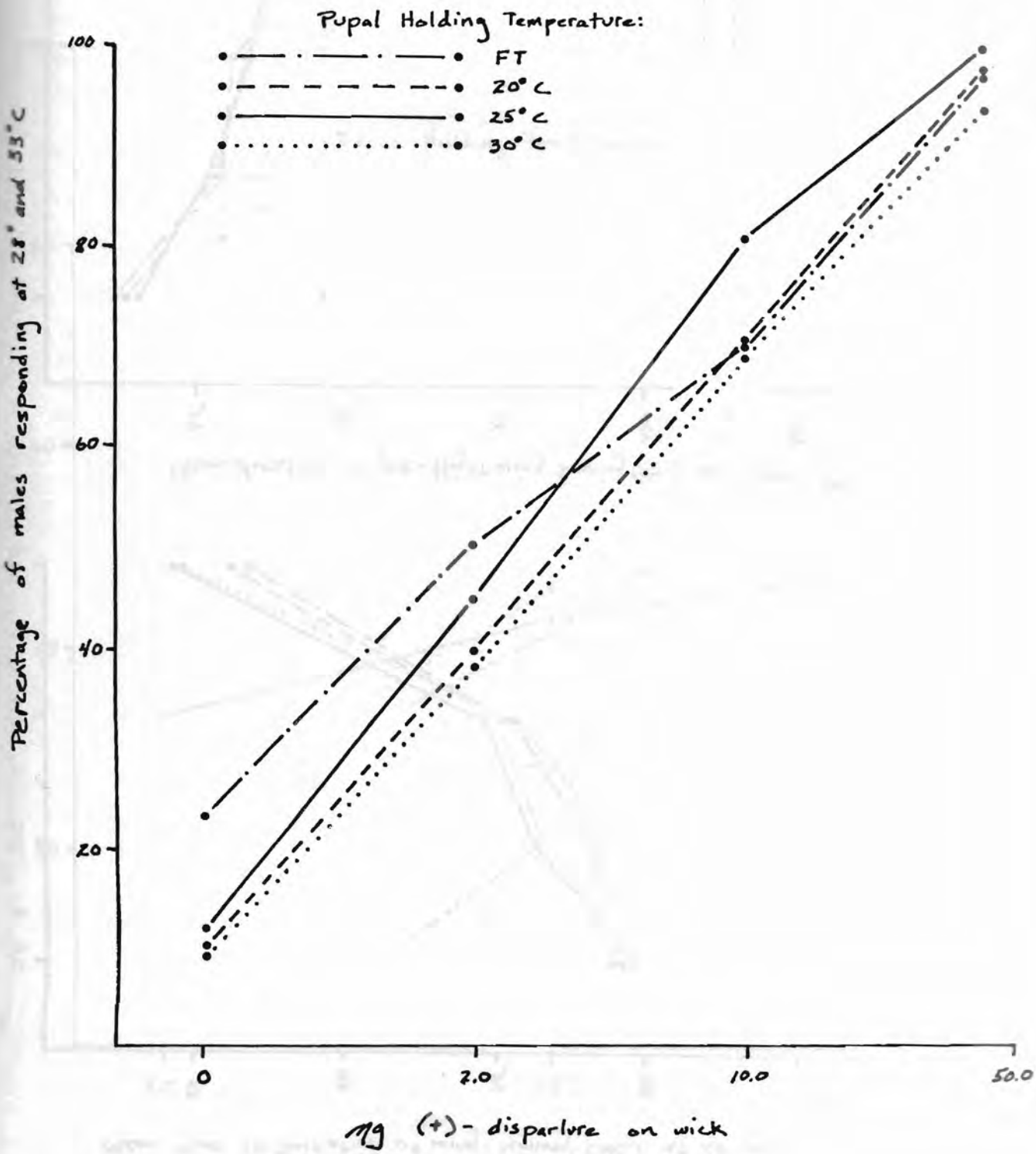
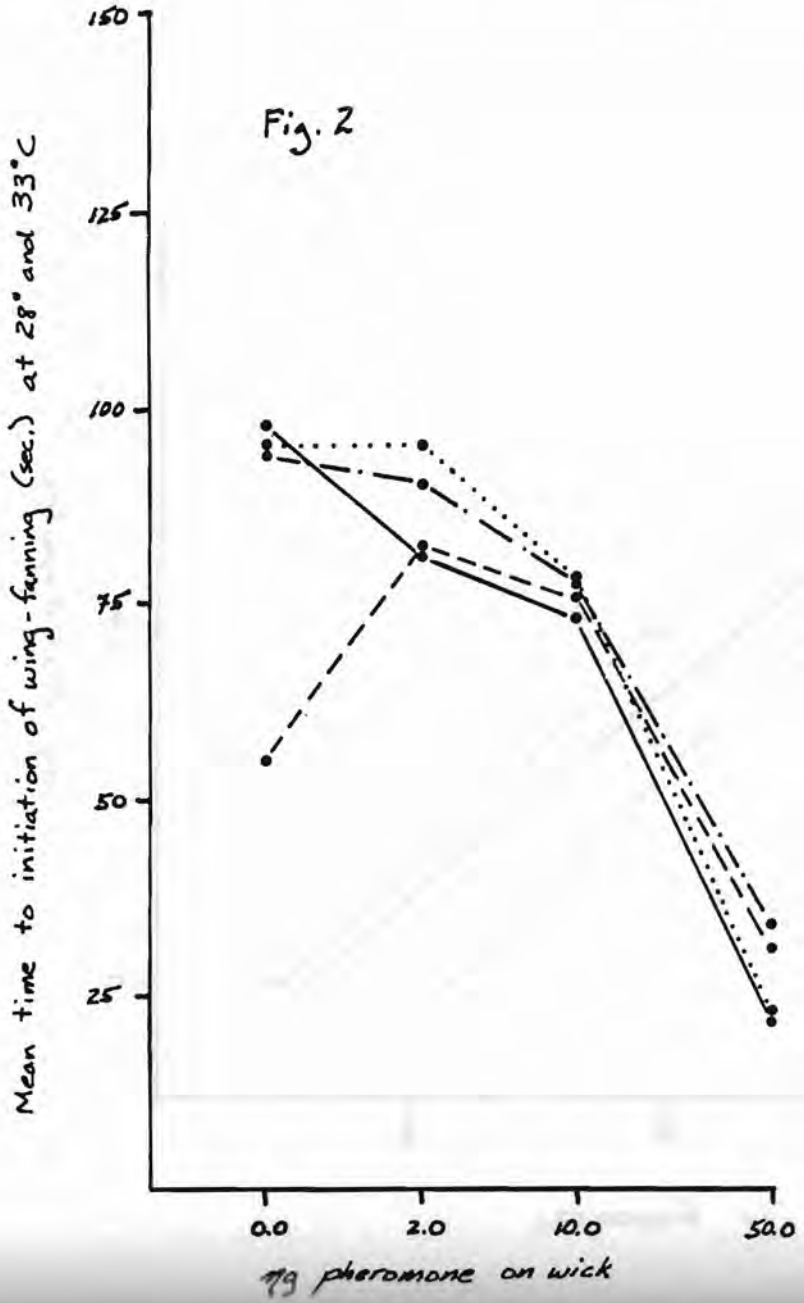


Figure 2 and 3. The effect of pheromone dose on the response latency periods (Figure 2) and durations of pre-flight wing-fanning (Figure 3) of gypsy moth males.



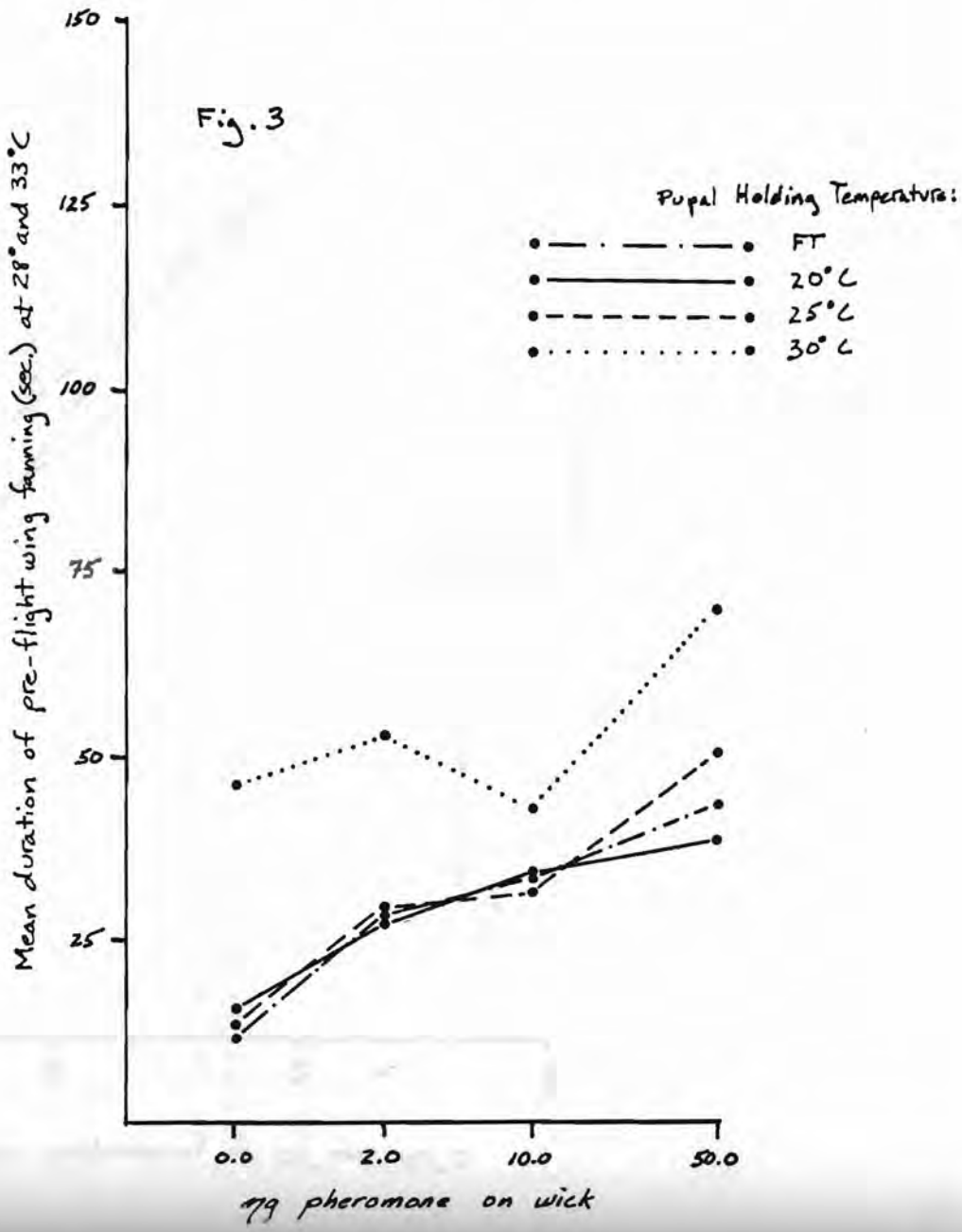


Figure 4. The effect of temperature on the percentage of male gypsy moths responding to wick containing 2 mg (+) disparlure.

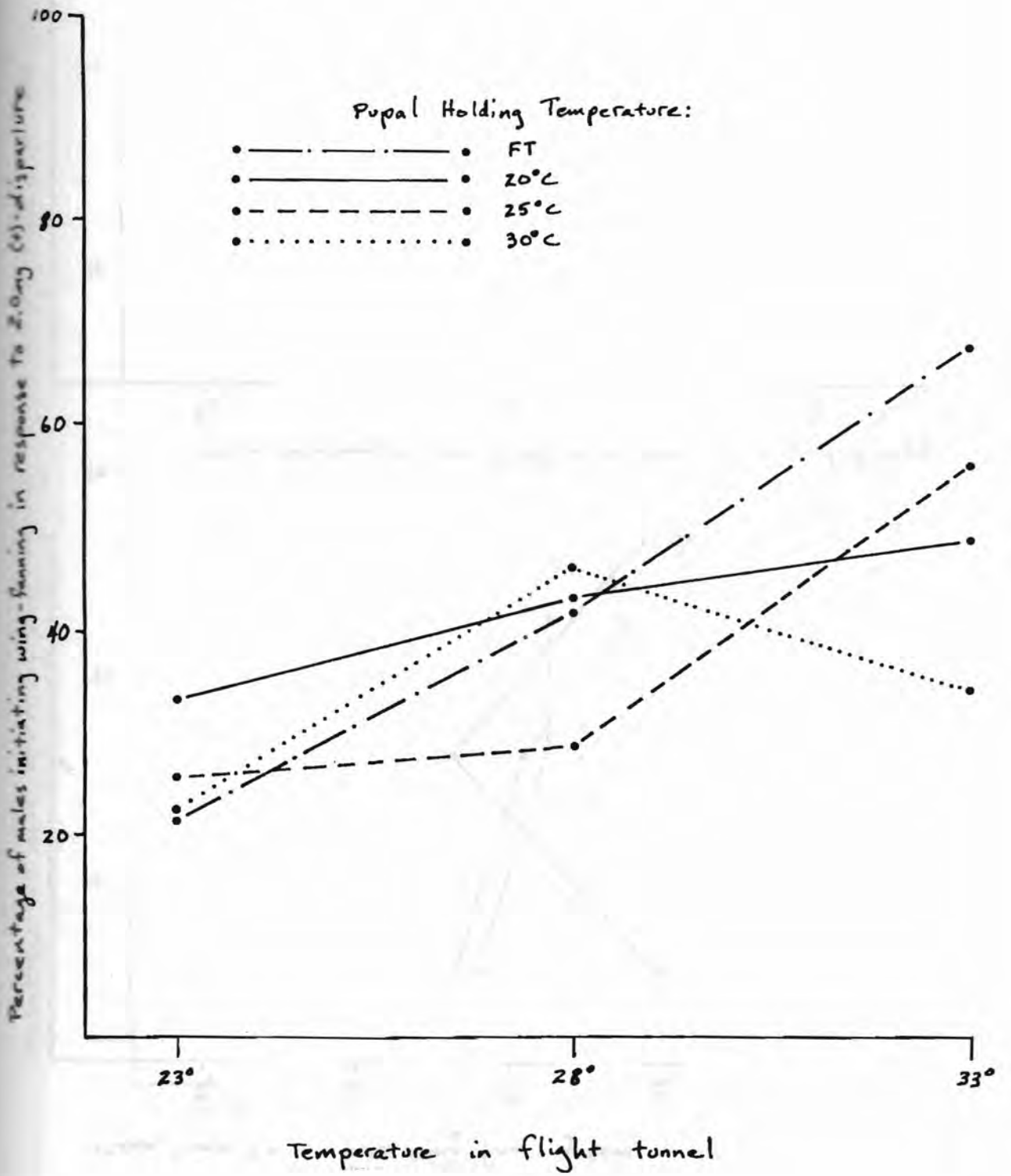
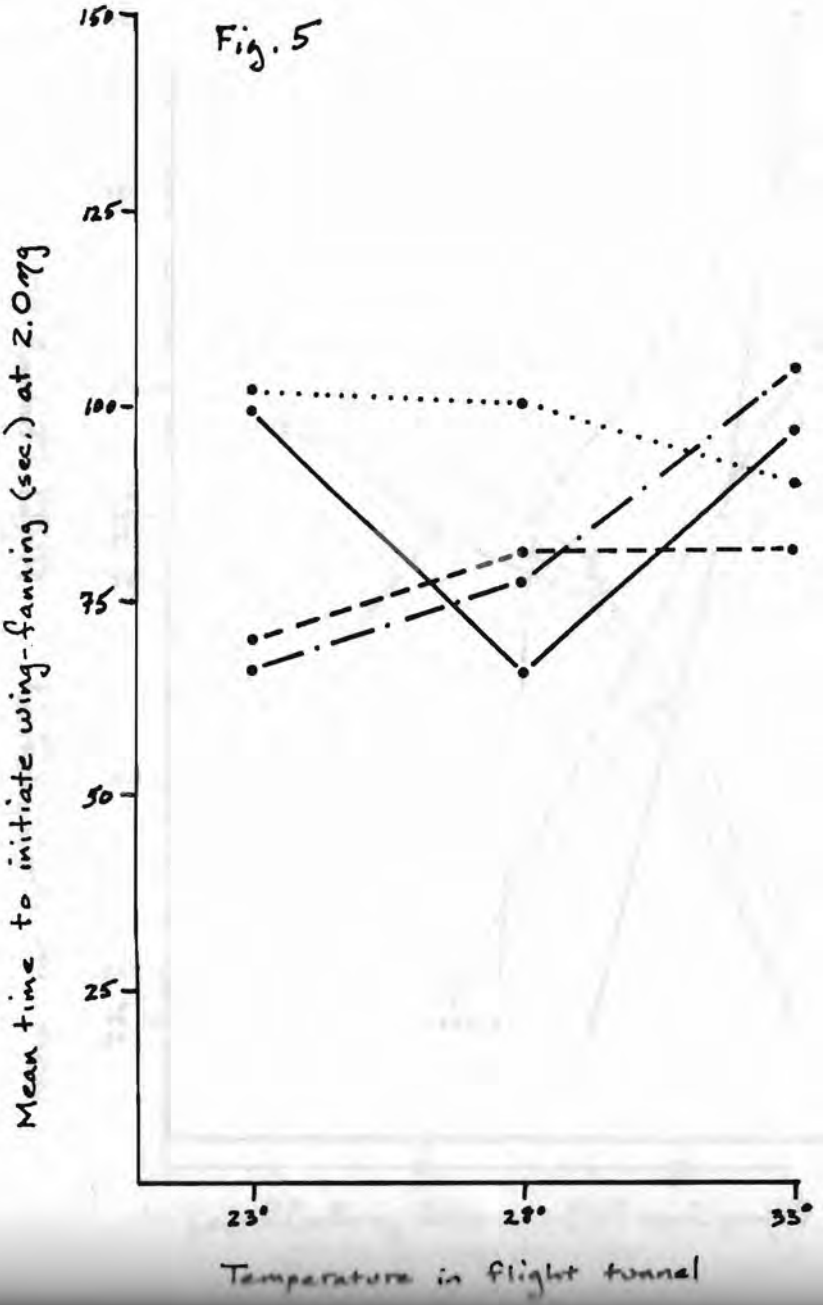


Figure 5 and 6. The effect of temperature on the response latency period (Figure 5) and durations of pre-flight wing-fanning (Figure 6) of male gypsy moths.



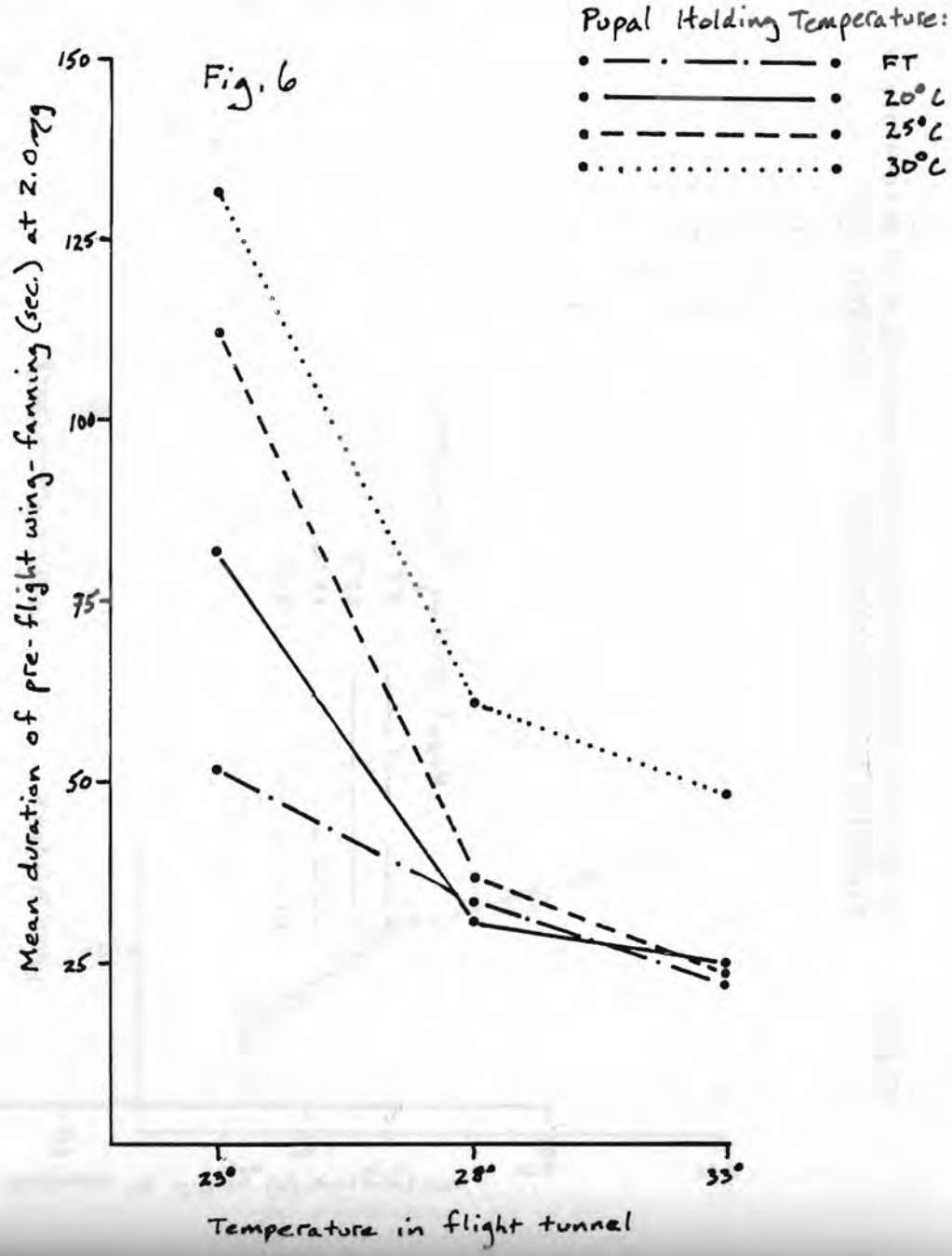


Figure 7. The percentage of male gypsy moths responding, per hour, to (+) disparlure in wind tunnel tests (constant temperatures in flight tunnel).

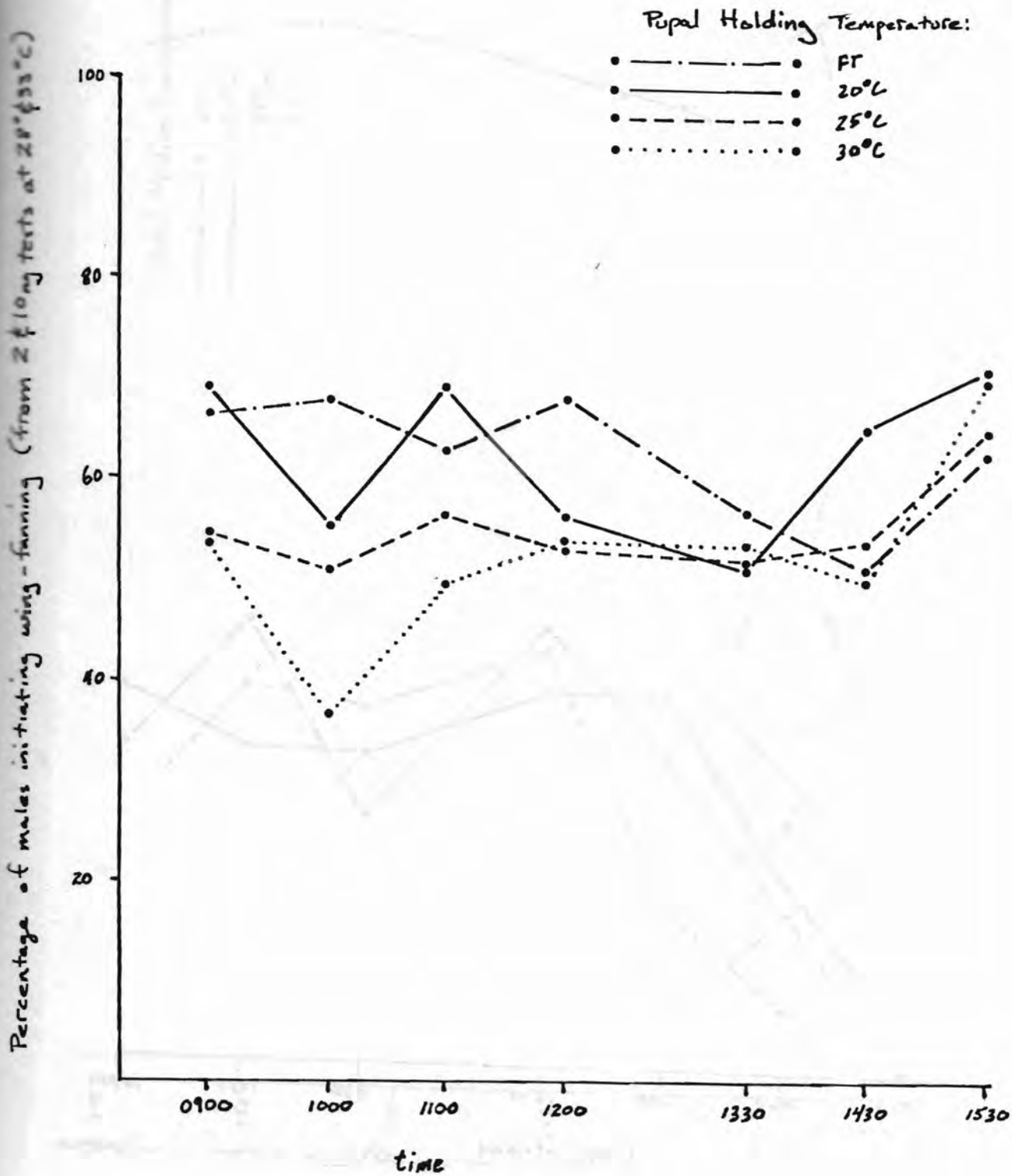


Figure 8. The percentages of gypsy moth males which took flight in response to a 2 mg pheromone while exposed to a simulated July day temperature regime.

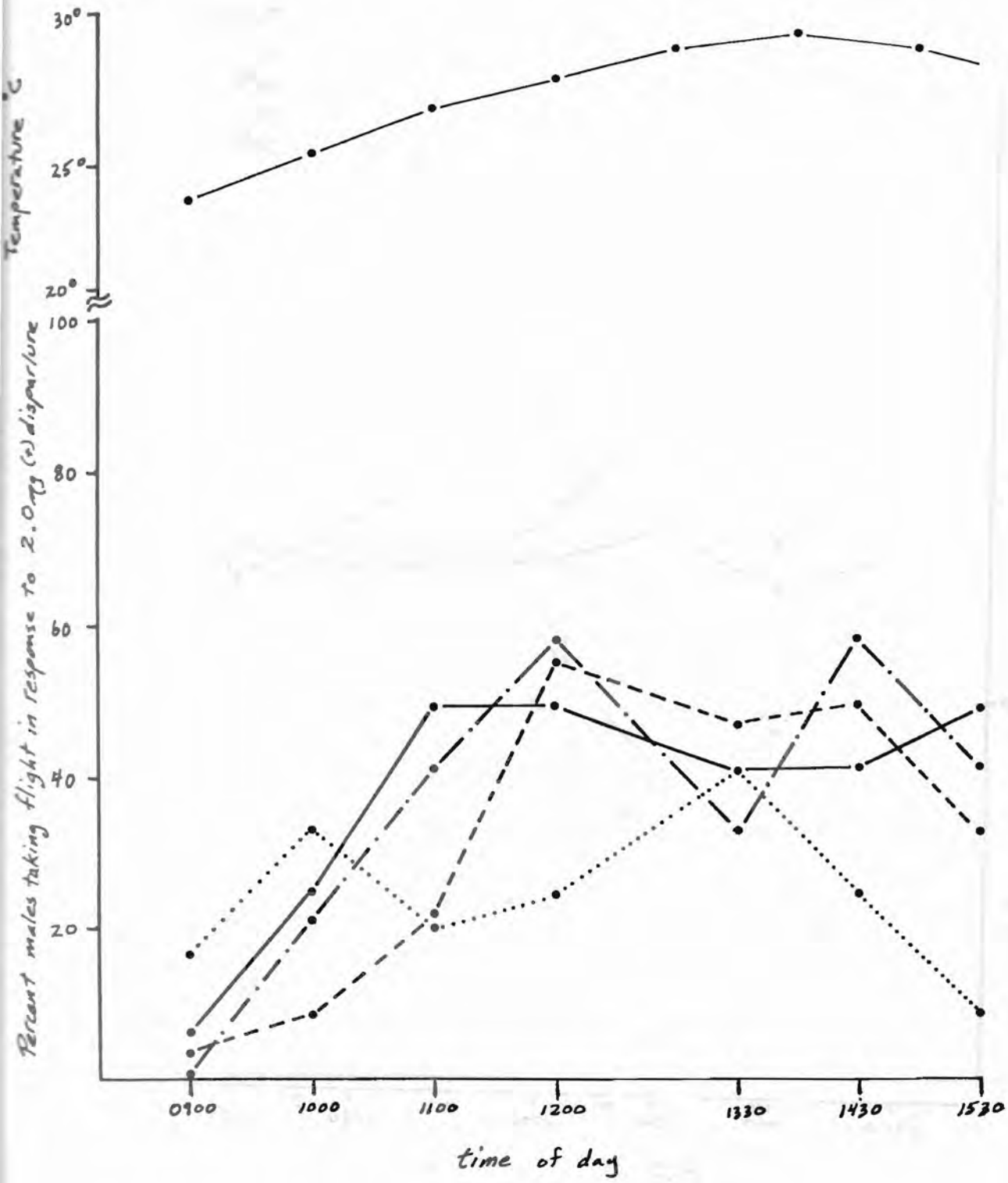


Figure 9. Response latency periods for gypsy moth males which were exposed to a 2 mg wick and a simulated July day temperature regime.

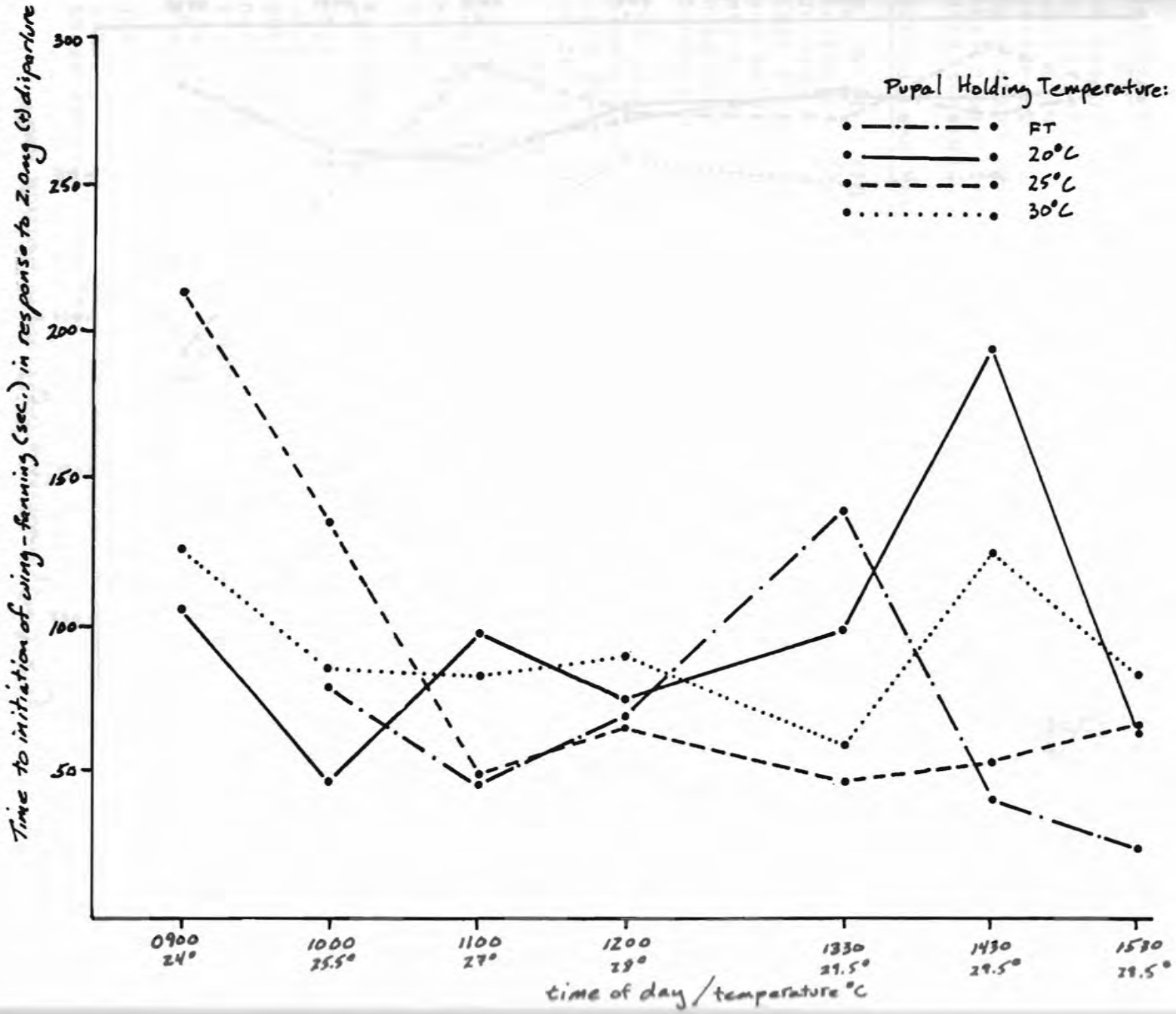
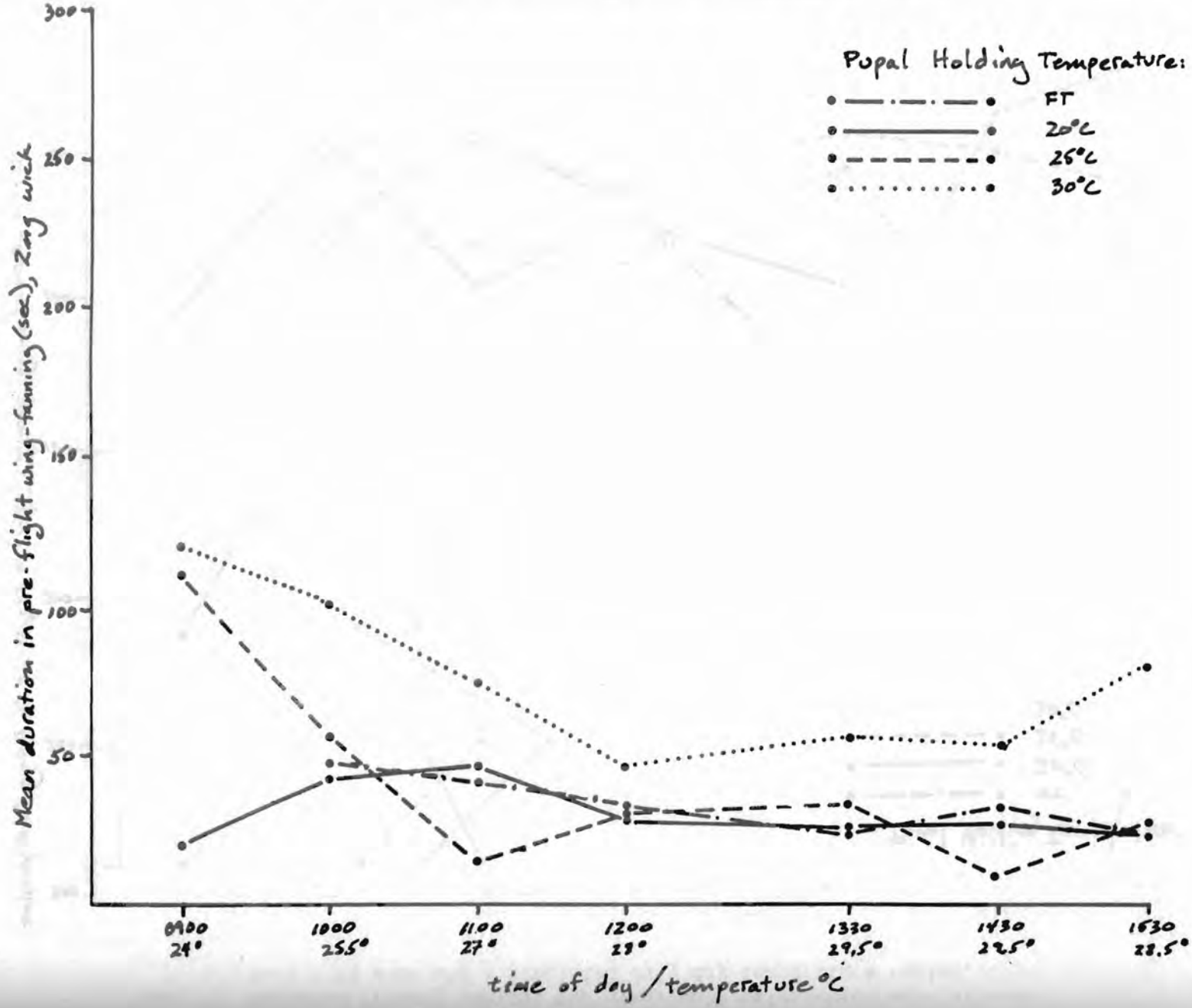


Figure 10. Durations of pre-flight wing-fanning for gypsy moth males which were exposed to a 2 mg wick and a simulated July day temperature regime.



Project Number: GM 7.2.4B
Project Title: Development of Laboratory Evaluation Techniques for Gypsy Moth Male Comparisons - Comparing the Pheromone-mediated Behaviors of Wild vs Laboratory-reared Males
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leader: D. Lance

Introduction

At the Otis laboratory, the sustained flight wind tunnel is being developed as a tool for determining sterile male quality. Before wind tunnel data can provide useful comparisons among males from different laboratory strains or rearing treatments, we must understand the pheromone-mediated behavior of wild males. For this study, flight tunnel data have been taken on males from 4 wild strains and one laboratory-reared strain.

Methods and Materials

To obtain wild males for this study, gypsy moth pupae were collected from beneath burlap bands in (1) New Lisbon, New Jersey, (2) Hamden, Connecticut, (3) Waterboro, Maine, and (4) East Brewster, Massachusetts. The pupae were placed 16-oz. cups and held in an outdoor insectary until eclosion. Laboratory males (NJS-F20) were reared on the B4 production diet and were held under standard rearing conditions (25° C, 50-60% RH, 0500-2100 hrs. Pp) throughout development.

The adult males were exposed to pheromone in a sustained-flight wind tunnel. The test procedures were similar to those which were described in GM 7.2.4A of this report. All tests were run at 25° C. Wicks were loaded with 0, 2, 10 or 50 ng of synthetic (+) disparlure.

For each of the 4 geographic strains of wild males, 84 moths were tested at each concentration of pheromone (only 42 Maine moths were tested at 10 ng); 126 NJS males were exposed to each concentration.

Results and Discussion

Table 1 shows the percentages of moths which initiated wing-fanning in response to the various concentrations of pheromone. Except for the Massachusetts wild males, the percentage of response did not vary significantly among the strains. Differences between the Massachusetts wild strain and the other strains may (or may not) have been caused by an early season application of BT at the East Brewster site.

The Response Latency Periods (RLP's) of the different strains (Table 2) will not be compared until the statistical analysis of these data are complete.

The mean durations of pre-flight wing-fanning (DPF's) are shown in Table 3. The DPF's of the NJS males were consistently longer than those of the wild males. There were few major differences in DPF's among the wild strains, except that the New Jersey wild males tended to have relatively short DPF's.

Compared to the wild males, a significantly lower proportion of NJS males showed sustained flight in the pheromone plume (Table 4). For the wild strains, the proportion of males which flew in the plume increased with mean forewing length. Male size (reflected in forewing length) is affected by diet and larval density, and appears to be a good measure of the degree of environmental stress to which a population is exposed. In this study, the smaller (i.e., more stressed) males were less capable of picking up pheromone plumes.

In conclusion, NJS and wild males appear to be similar in their abilities to detect (+) disparlure. However, compared to the wild males, the NJS males had longer DPF's and were less capable of sustaining flight in a phermone plume. In other tests, holding conditions for pupae have been shown to affect both DPF's and flight in the plume (see GM 7.2.4A). By changing the laboratory rearing conditions (e.g. by holding pupae at cooler temperatures), it may be possible to reduce the behavioral differences between NJS males and wild males.

Table 1. Percentages of adult male gypsy moths which initiated wing-fanning in response to various concentrations of (+) disparlure in a sustained-flight wind tunnel.

Strain of Moths	PERCENT OF MALES RESPONDING[1] ng of (+) disparlure on wick			
	0.0[2]	2.0	10.0	50.0
New Jersey wild	.0	35.7 ab	76.2 ab	100.0
Connecticut wild	4.8	38.1 ab	86.9 a	100.0
Maine wild	10.7	46.4 a	76.2 ab	100.0
Massachusetts wild	14.3	25.0 b	75.0 b	100.0
NJS laboratory-reared	1.6	44.4 a	85.7 ab	100.0

1 Within a column, percentages not followed by the same letter represent proportions which are significantly different ($p<0.05$) as determined by chi^2 analysis.

2 Number of observations per cell was too small to allow chi^2 analysis.

Table 2. Mean Response Latency Periods[1] for adult males gypsy moths which responded to various concentrations of (+) disparlure in a sustained-flight wind tunnel.

Strain of Moths	RESPONSE LATENCY PERIOD (Sec.) ng (+) disparlure on wick 2	10	
New Jersey wild	59.1	59.4	8.9
Connecticut wild	85.7	70.7	10.0
Maine wild	89.7	62.7	9.1
Massachusetts wild	94.2	51.3	18.4
All wild	82.1	61.0	11.6
NJS laboratory-reared	93.6	50.5	10.1

[1] Adjusted for independent variables and covariates. Test to separate means had not been run at the time of this writing.

Table 3. Mean Duration of Preflight Fanning[1] for adult male gypsy moths which responded to various concentrations of (+) disparlure in a sustained-flight wind tunnel.

Strain of Moths	DURATION OF PREFLIGHT FANNING[2] ng (+) disparlure on wick 2	10	50
New Jersey wild	18.2	21.3	31.5
Connecticut wild	21.3	30.6	46.16
Maine wild	16.6	43.1	57.65
Massachusetts wild	21.5	30.2	56.1
All wild	19.0	29.4	47.9
NJS/LR	44.9	49.8	65.8

1 Adjusted for independent variables and covariates. Tests to separate means had not been run at the time of this writing.

Table 4. Percentages of adult male gypsy moths which demonstrated sustained flight in the pheromone plume of a wick which was loaded with 50 ng of (+) disparlure.

Strain of Moths	Forewing Length	S.D.[1]	PERCENT IN PLUME[2] Among all strains	wild vs lab-reared
New Jersey wild	20.6	1.2	26.2 a	
Connecticut wild	17.1	1.3	10.7 b	
Maine wild	17.4	1.4	15.5 ab	17.8 a
Massachusetts wild	19.7	1.6	19.1 ab	
NJS/LR	22.0	1.4	10.3 b	10.3 b

1 Mean length of forewing (mm) for 100 males from each strain

2 Within a column, percentages not followed by the same letter represent proportions which are significantly different ($p<0.05$) as determined by chi^2 analysis.

Project Number: GM 8.2.2
Project Title: Radiological Sterilization of Male Gypsy Moths
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leader: C. P. Schwalbe

This project has been fully reported in previous Laboratory Reports. The results have led to the adoption of treating 8-11 day old male pupae with 15 krads as the standard sterilization procedure. This treatment reduces hatch to ca. 6% and results in essentially no survival in the F_1 generation. Moths released in the 1980 trial in Berrien County, Michigan were sterilized according to this formula.

During the course of investigations it was also discovered that male pupae exposed to substerilizing amounts of radiation and mated with normal females yield F_1 egg masses that hatch. Adults developing from these egg masses are sterile. Consequently, additional studies will be conducted to determine the precise dosage and age formula that will maximize yield of sterile F_1 progeny. It is hypothesized that releasing substerile males will have greater impact on the target population because of the additional effect of "native" sterile F_1 males the following year.

Project Number: GM 8.2.3
Project Title: Gypsy Moth Mating Behavior
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leader: C. P. Schwalbe

This report is being finalized at this time since no additional studies are planned. Previous reports dealt with mating frequency, duration of copulation and sperm transfer in sterile and fertile male moths. Studies on the timing of sperm translocation within the female reproductive system was also reported. Future studies concentrating on sperm transfer by sterile F_1 males will be reported in GM 8.2.2.

Project Number: GM 8.2.6
Project Title: Large Capacity Milk Carton Traps - Design Improvements
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: E. C. Paszek, C. P. Schwalbe

Introduction

In the summer of 1978 a large capacity 1/2 gallon milk carton (MC) trap was tested for trapping large numbers of moths in dense infestations.

Several modifications of that trap were bioassayed in the field and laboratory in 1978-79. The modification that appeared to be the most promising was designed with 12 slotted (1x5/16") entrance ports, 3 per side, 1/2" apart and 1" below the top of each panel. The hood was constructed from a 3.75 x 3.75 piece of heavy polyethylene coated paper slipped over the MC and taped in place. The hood's 4 sides tapered away from the MC with its skirts lowest point projecting 1.5" away from the sides of the trap. This overhang protected the entrance ports, and directed the moths into a partially confined space where they readily entered the trap. The trap that was finally recommended for field use in the summer of 1979 was a green colored modified version of the above design with 12 slotted (1x5/16") entrance ports. The hood was dye cut out of polyethylene coated paper with a 1.125" tab projecting above each of the 4 panels. It was easily assembled into shape by folding and creasing on the perforated lines and was positioned over the top of the MC where it was held in place by inserting the tabs into pre-cut 1.125" slits. The tabs were then stapled to the body of the trap. The lure dispenser and a 3 g DDVP flea tag were attached to a 6" piece of plastic twist-em plant tie which was suspended inside the trap. The bottom of the trap was closed and sealed with tape. The trap could be opened and inspected through the spout or through the bottom. This trap was made commercially at a cost of 42 cents/trap by assembling parts from the following companies:

International Paper - 1/2 gal MC	-	.06
Sandia Die & Cargridge - Hood	-	.07
H. F. Loeb - Die cutting MC	-	.03
Zoecon Corp. - 3 g vapona flea tag	-	.26
Total cost	-	.42

The price of the Hercon disparlure dispenser was not included in the above cost.

Several modifications of the 1/2 gal MC were field tested in 1979. However, definitive results for optimizing efficiency were not obtained. Consequently, several tests were designed to determine the optimum trap size, hood overhang size, entry port size and number of entry ports. All traps were baited with 500 ug of (+) disparlure (Hercon dispenser) and a 3-1/2" piece (3.6 g) Zodiac dog flea collar was used as the killing agent. The studies were conducted in moderately infested oak and pine forest in the Freetown-Fall River State Forest.

Effects of trap size and number of entrance ports were tested in this study. Four MC trap sizes and varying numbers of entry ports/trap were compared (10 replicates each) in a 16 x 10 grid (ITD = 100 m). Traps were placed 7/25 and checked 3 times 7/27, 7/29 and 8/11. Results are summarized below.

Trap size and entry port study - Total moths captured in 10 replicates

	Entry ports/trap			
Trap size	4	8	12	16
Pint	4,551	4,246	2,686	1,770
Quart	10,168	8,127	5,789	5,592
1/2 Gallon	9,283	9,403	5,861	5,866
Gallon	12,155	15,418	13,863	11,085

Generally the larger trap sizes captured greater number of males. It also appears that trap effectiveness was greatest with 8 entry ports. Catch in traps with 12 and 16 entry ports was substantially reduced.

In a study to determine the influence of the size of the trap hood on the efficiency of the 1/2 gal trap, 3 hood overhang sizes were compared with a hoodless trap. Ten replicates were tested in a 6 x 7 grid with spacings 100 m apart. Results are summarized below.

Hood size study - Average number of moths captured in 10 replicates

Date checked	No hood	1/3 size hood	2/3 size hood	Full hood
7/27	170	208	274	278
7/29	84	109	168	195
8/1	61	70	110	127
8/4	40	92	118	107
8/6	23	54	85	80
Total	378	533	755	787

The 1/2 gal traps with the full hood and the 2/3 size hood captured more moths than the traps with 1/3 size hood. Eliminating the hood resulted in further reduction in catch. Results were consistent among the 5 sample intervals.

A comparison of 4 entry port sizes (listed in the following table) was conducted to evaluate the effect of this variable on trap efficiency. This study was done on the 1/2 gal MC trap with 8 entry ports in a 7 x 6 grid (ITD=100 m). Results are summarized below.

Entry port size study - Average number of moths captured in 10 replicates.

	3/16 x 1"	1/4 x 1"	5/16 x 1"	3/8 x 1"
7/27	314	309	359	370
7/29	292	276	326	299
8/1	113	123	184	158
8/4	58	56	89	74
8/6	20	26	45	38
Total	797	790	1,003	939

The 1/2 gal traps with 5/16 x 1" size entry ports generally captured more moths than other treatments. However, 3/8 x 1" ports gave approximately the same results. Smaller entry ports dramatically reduced catch.

The gallon size MC with eight 5/16 x 1" size entrance ports and a full size hood (2-7/8" overhang) was selected as the most efficient trap from the above 3 studies. It was compared to the Delta survey trap on 8/8/80 in an oak-pine forest east of the housing area on Otis AFB. Twenty replicates of the Delta and 20 gallon size MC traps (10 with eight 5/16 x 1" entrance ports/trap and 10 with twelve 5/16" x 1" entrance ports/trap) were randomly placed 100' apart along 2 wood trails parallel to each other in a light infestation. The traps were set out 11:00 am and were checked 2-1/2 hours later at 1:30 pm, moths were removed and they were checked again 2 hours later at 3:30 pm.

The gallon size MC with 12 entrance ports had an average catch of 13 moths, that with 8 ports captured 10 and Delta traps averaged 7 moths/trap.

Herculite Products, Inc. marketed a trap nearly identical to the USDA 1/2 gal MC trap on a limited scale in 1980. The pheromone dispenser was the Hercon lure tape with 13% racemic disparlure. Hercon Insect Tape (10% Baygon) was used as the killing agent. Six of the self adhering 1 x 4" Baygon strips (6.3 g) were placed on the inside trap wall below the entrance ports. The 1980 USDA MC traps contained 500 ug (+) disparlure in the Hercon dispenser and a 3-1/2" piece (3.6 g) of the Zodiac dog flea collar (FC) (9% DDVP) as the killing agent. Five replicates of eight treatments (listed in the following table) were field tested 8/4 - 8/18. The traps were spaced 100 m apart in a 8 x 5 premarked grid at Freetown-Fall River State Forest.

When the Hercon traps were assembled, the hood tabs were stapled to the trap body to prevent the hood from slipping down and blocking the entrance ports. This slippage occurred in a preliminary field test in Sandwich in July.

Most moth flight occurred before 2 heavy rainstorms. In the second week of the study the rain soaked Hercon traps collapsed from water absorption. Results (average number of moths/trap) are summarized below.

Hercon Baygon Racemic	USDA Baygon Racemic	Hercon DDVP-FC Racemic	USDA DDVP-FC Racemic	Hercon Baygon (+)	USDA Baygon (+)	Hercon DDVP-FC (+)	USDA DDVP-FC (+)
3	3	7	3 (n=4)	21	26	35	33 (n=4)

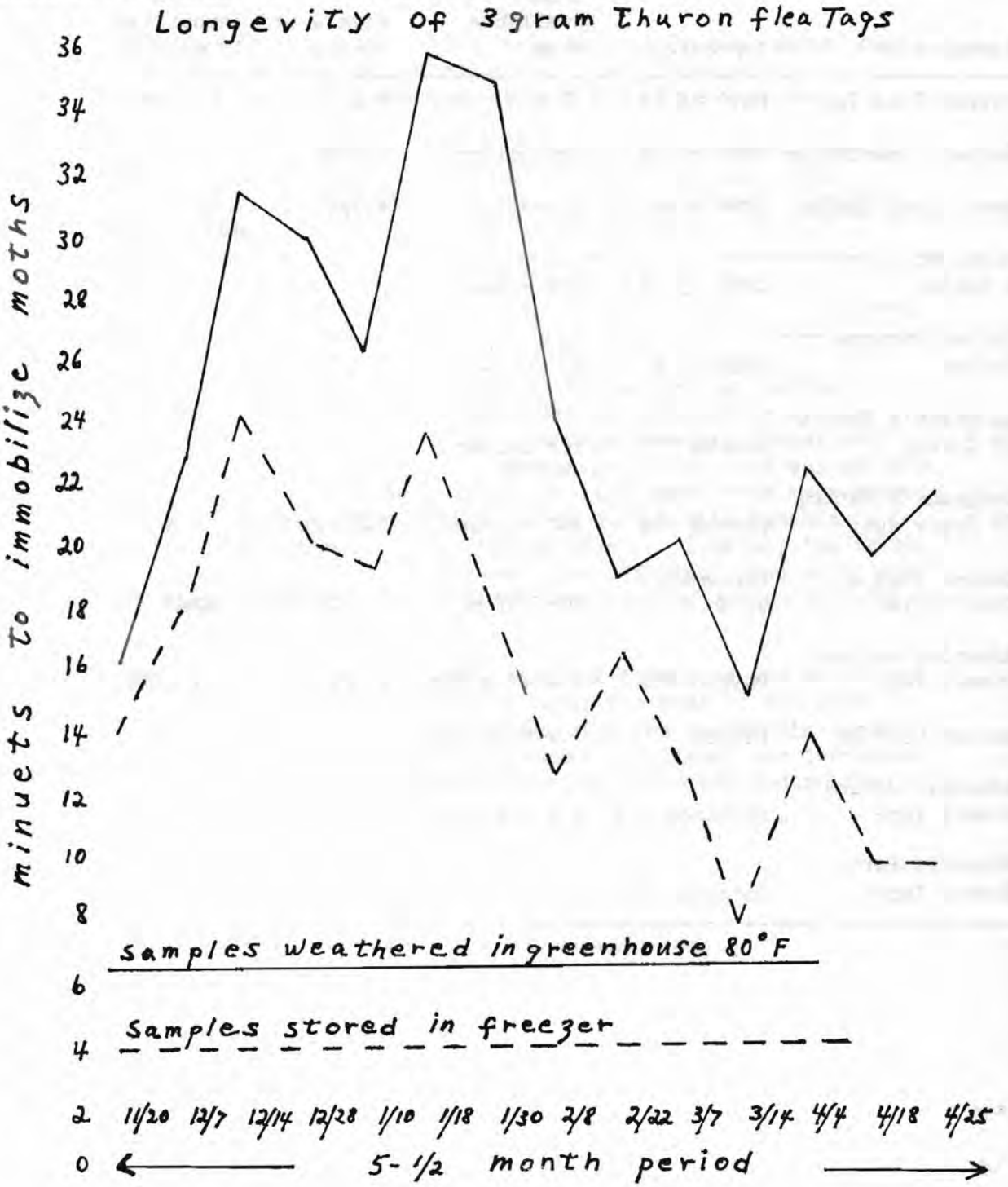
Both the Hercon and USDA traps baited the (+) disparlure and Zodiac (DDVP-FC) were much more efficient in capturing moths than the similar Hercon and USDA traps baited with Hercon lure tape (racemic disparlure) and the Zodiac (DDVP-FC) killing agent. Hercon and USDA traps baited with (+) disparlure and the Baygon insect strips killing agent were much more efficient in capturing moths than similar traps baited with racemic lure-tape and the Baygon Insect strip killing agent. However, the traps with the Baygon killing agent were somewhat less efficient than both sets of traps using the combination of (+) disparlure and the Zodiac DDVP-FC killing agent.

The Hercon trap should be made of water resistant cardboard and the hood should stay in place when fitted in the slots of the body of the trap. With the remedying of these two structual deficiencies the Hercon trap compares favorably to the USDA trap. The capture efficiency was increased in both sets of traps when (+) disparlure was used with the Zodiac flea collar killing agent.

From September 1979 through February 1980 twelve killing agents were screened in the greenhouse and compared in terms of time required to immobilize male moth placed in the trap. Formulation descriptions and results are summarized below.

Formulation	Active Ingredient	Commercially Available Form	size + wt Tested	Minutes to Immobilize 10 male moths
Thuron Flea Tag	DDVP 18.6%	1" dia. disc	3 g	5 - 14
Zodiac Flea Collar	DDVP 8.37%	33" collar	3-1/4"(3g)	5 - 13
Shell Flea Collar	DDVP 9.6%	26 collar	*(3g)	4 - 11
Hartz Mt 2 in 1 + Collar	DDVP 13.7%	29" collar	2-1/2"(3g)	90+
UniPet, Upjohn Collar	DDVP 9.5%	22 collar	3-3/5" (3g)	37 - 135
Sergeant's Sentry IV Collar	Dibrom 15%	16" collar	3-1/2" (3g)	14 - 22
Sergeant's Sentry IV Heavy duty	Dibrom 15%	16" collar	2-17/20 (3g)	15 - 26
Zodiac Flea + Tick Collar	Carbamate 9.4%	30" collar	2-1/2" (3g)	40+
Adhesive Hercon Insect Tape	Baygon 10%	1 x 4" strip	6.3 g	24 - 34
Hercon L227-36	Dibrom	1 x 6 strip	1.5 g	25 - 38
Adhesive Hercon Insect Tape	Diazinon 10%	1 x 4 strip	6.3 g	70+
Adhesive Hercon Insect Tape	Dursban 10%	1 x 4	6.3 g	70+

A greenhouse aging study was done with the Thuron 3 g flea tags. From 11/16/79 - 4/25/80 ten flea tags were weathered in the greenhouse in individual 1/2 gal MC traps at temperatures of approximately 80° F. They were compared with 10 flea tags that were stored in a freezer. This study established a curve on the longevity of the killing agent. After 5.5 months of aging 22 minutes was required to immobilize moths with the weathered flea tags and 10 minutes with controls. Traps using this killing agent should maintain peak efficiency up to 5.5 months as shown on following longevity curve. Fluctuations are most likely due to variations in bioassay temperature conditions.



In order to select an inexpensive replacement for the Thuron flea tag now in use, an aging study of 7 controlled release formulations selected from the 12 killing agents screened in the greenhouse bioassay was set up. Ten replicates of each were weathered outdoors in MC traps at 3 time intervals (12 weeks, 8 weeks and 4 weeks). The aged formulations and unaged (0-day) controls were bioassayed in a moderate infestation 7/15-21/80. Results are summarized below.

Formulation	Total No. Moths Captured in 10 replicates 12 week	8 week	4 week	0-day
Zodiac DDVP 8.4% flea collar 3.6g	5410	6827	6929	4260
Shell DDVP 9.6% flea collar 3.2g	4537	6364	5581	5175
Thuron DDVP 18.6% " " 3.0g	4522	3726	4152	4142
Hercon Baygon 10% Insect tape 6.3g	3593	4098	3346	2510
Sergeants Dibrom 15% flea collar 3.4g	3266	3726	2818	2443
Hercon Dibrom Resin strip 3.0g	2454	1980	2478	1950
Hercon Dibrom Resin strip 1.5g	1726	1941	2423	1885

The Zodiac DDVP flea collar ranked first in capturing moths at all age intervals. Shell's DDVP flea collar ranked second and the presently used (1979-1980 survey program) Thuron flea tag ranked third. The Zodiac and Shell flea collars containing DDVP are the best replacements for the Thuron flea tag. Since the Shell flea collar is made of extruded plastic, it is difficult to cut into uniform sized pieces. It is sold only through veterinary clinics at $3.50/collar retail and one collar can be cut into 8 pieces. The Thuron flea tag in 1980 costed $1.98 retail and sold at a wholesale bulk rate of $0.42/tag. It was not a good seller and has since been taken off the market. The most economical replacement for the flea tag is the 33" Zodiac flea collar for large dogs (Net wt. 1.0 oz). It retails at $1.85 and wholesales at $1.27/collar. The collar is divided into 9 equal 3.5" pieces with a cost of $0.14/trap.

Additional testing was conducted to determine the optimum weight of Zodiac DDVP flea collar in the 1/2 gal and gal MC traps. Eight treatments, 10 replicates per treatment were field tested 8/4-18 in a 8 x 10 grid with trap spacings of 100 m. The following table summarizes the test.

1/2 gallon traps				1 gallon traps			
3 g	6 g	9 g	12 g	3 g	6 g	9 g	12 g
560	512	581	617	632	635	824	819

These results show that optimum DDVP concentrations vary with trap size. More moths were captured in 1 gal traps containing 9 and 12 g DDVP than in similar traps with 3 and 6 g. This is expected because the larger volume requires more pesticide to immobilize moths before they can escape. This same phenomenon was not observed in the smaller 1/2 gal traps. Similar results may have been observed if less than 3g had been tested in 1/2 gal traps.

Combining results of all studies reported here (and discounting possible interactions among variables) the trap design expected to capture the most male moths would have the following specifications:

Body - 1 gallon MC
Hood - Full or 2/3 size
Number Entry Ports - 8 or 12 per trap
Size of Entry Ports - 5/16 x 1"
Killing Agent - 9 g Zodiac Flea Collar (8.4% DDVP)
Pheromone - 500 ug (+) disparlure (Hercon dispenser)

Project Number: GM 9.2.2
Project Title: Efficacy of Different Overflooding Ratios of Sterile Males in Simulated Populations
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: V. C. Mastro

Introduction

Mating success (i.e. competitiveness) of laboratory-reared (LR) male gypsy moths, will determine the overflooding ratio required to effectively suppress a feral population. Fully competitive sterile males would allow the lowest overflooding ratio to effect control of a wild population. Lower degrees of competitiveness, it follows, would require larger sterile:fertile ratios to effect the same degree of control. Competitiveness is a term often used to describe a set of parameters -- behavioral, physiological and possibly morphological -- which are used to compare laboratory reared insects with their wild counterparts. In sterile insect release programs, these parameters are chosen with the hope that they will reflect the mating success or "overall competitiveness", of released insects under field conditions. Often, the relative importance of these parameters is not known and male strain comparisons can not be tied directly to field mating success.

In field trials (1977-1978), LR male gypsy moths (both irradiated and untreated) and wild-type (WT) males were similar in most parameters measured. These parameters included longevity (as measured by age of capture), ability to detect and respond to a pheromone source (as measured by captures at placed females and synthetic pheromone sources), propensity to disperse (distance from release site to capture site), and eclosion and dispersal periodicities (observations). Also, close-range orientation and precopulatory behavior appeared similar. However, periodicity of trap capture (or mating) was asynchronous between WT and LR males in some 1977 trials. On cooler days, peaks of capture at pheromone sources were nearly synchronous for WT vs. LR males, but on warmer days, peaks of capture occurred nearly 3 hours apart. Although 1978 trials did not demonstrate a difference in periodicity, 1980 field and laboratory tests did. Holding and release techniques were altered from year to year, and may account for the variation among test results. Although they are potential indicators of male competitiveness, the parameters which we have measured have not been correlated with the overall competitiveness of sterile males released in a native population. For example, we do not know the degree to which a difference in sexual periodicity will or will not affect overall competitiveness. The tests which are described here were to determine how different sterile male:feral male ratios affect mating success and subsequent egg mass viability under a controlled set of field conditions. However, constraints placed on the tests also limit the interpretation of the data.

Methods and Materials

Ratio Plots

In the first series of tests, 3 plots were provisioned with LR insects (untreated) to simulate a sparse, native, low-density population. Sterile males were released into these plots at different rates to provide 50S:1F and 10S:1F overflooding ratios. The third plot received no sterile males and served a control. In each plot, 67 fertile males and 67 fertile females were placed daily using a replacement-randomization scheme. Placement points (270 available per plot) were randomized daily. The same release scheme was used for all 3 plots on any given day.

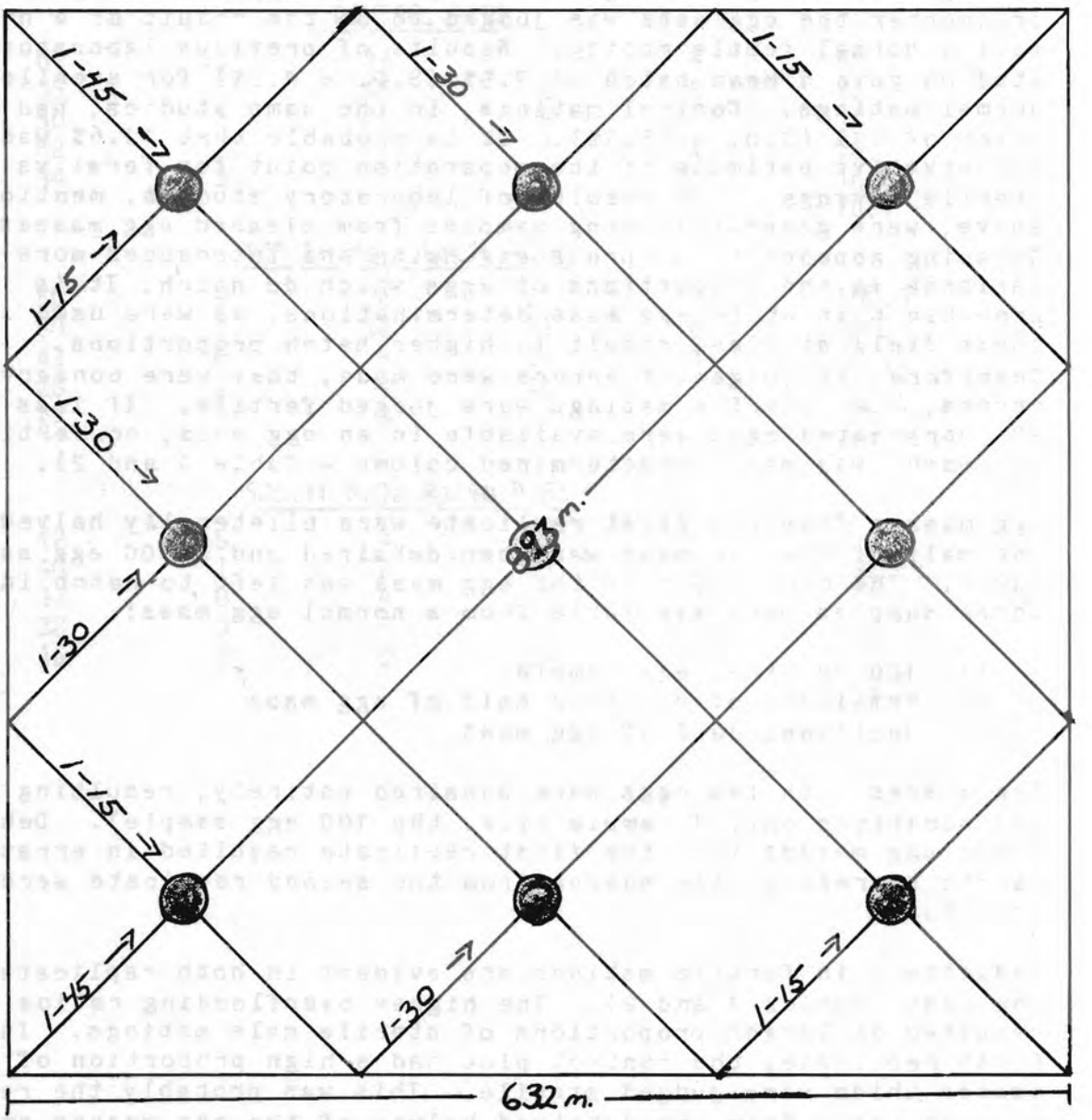
Fertile LR males were held as pupae in an insectary until eclosion. Because of the harvesting technique used, male pupae were of an unknown age when placed in the insectary. On the morning following the day of eclosion, males were collected and taken to field sites for release.

Sterile males (irradiated as adults) were held and allowed to eclose in the laboratory. Eclosion trays were provisioned with 1,000-1,500 male pupae. Daily, newly eclosed males were removed from trays and allowed to expand and harden their wings (minimum 1 hr). On the same day, males were chilled (50° F), irradiated (15 krads), and held at 50° F until their release on the following morning. Sixhundred and seventy sterile males were released daily in the 10S:1F ratio plot and 3350 sterile males were released daily in the 50S: 1F ratio plot. Males were not available for a planned 100S:1F release plot.

To monitor male mating success, females (one-day old virgins which had been held in an insectary until eclosion) were placed in small wooden A-frames at selected sites in the field plots. On the day following placement, females (and egg masses, if oviposition had begun), were collected and returned to the laboratory. Egg masses were embryonated for 28 days (78° F, 50-60% RH) and then chilled for 120 days (4° C). After chilling was complete, individual egg masses were placed in small petri dishes and allowed to hatch. For hatch, all egg masses were held in a high humidity chamber (80% RH - 78° F). All hatching larvae, unembryonated eggs, embryonated unhatched eggs, and parasites were counted.

Two replicates were run. The first replicate was complete before native flight had begun in the test area (June 26-30). The second replicate was run during the period of native male flight (August 1-4). Five large capacity milk carton traps were placed in each plot and were checked daily to monitor native moth density. Because all sterile males were marked with fluorescent Day-glo powder, it was possible to distinguish them from fertile males (laboratory-reared released and wild) and compute overflooding ratios.

RATIO PLOT DESIGN



RELEASE SITES LOCATED 20 m. APART

● - STERILE MALE RELEASE SITES

Results and Discussion

Mating success of sterile and fertile males was monitored using placed native females. Fertility judgements were made on individual egg masses on the basis of the proportion of embryonated eggs which hatched. If the percentage of embryonated eggs which hatched was less than or equal to 11.5%, the egg mass was judged to be the result of a sterile male x normal female mating. If the percentage of embryonated eggs hatching was 11.6% or greater the egg mass was judged to be the result of a normal male x normal female mating. Results of previous laboratory studies gave a mean hatch of 3.5% (S.D. = 4.2%) for sterile x normal matings. Control matings, in the same studies, had a mean hatch of 59% (S.D. = 35.3%). It is probable that 11.6% was a conservative estimate of the separation point for feral vs. sterile matings. The results of laboratory studies, mentioned above, were generated using samples from cleaned egg masses. Cleaning appears to suppress egg hatch and introduces more variance in the proportions of eggs which do hatch. It is probable that whole egg mass determinations, as were used in these field studies, result in higher hatch proportions. Therefore, if judgement errors were made, they were conservative errors, i.e. sterile matings were judged fertile. If less than 20 embryonated eggs were available in an egg mass, no fertily judgement was made (undetermined column - Table 1 and 2).

Egg masses from the first replicate were bilaterally halved. One half of the egg mass was then dehaired and a 100 egg sample taken. The other half of the egg mass was left to hatch intact. Three samples were available from a normal egg mass:

1) 100 dehaired egg sample
2) Remainder of dehaired half of egg mass
3) Uncleaned half of egg mass

Egg masses with few eggs were dehaired entirely, resulting in 2 and sometimes only 1 sample (i.e. the 100 egg sample). Dehairing these egg masses from the first replicate resulted in erratic hatch; therefore, egg masses from the second replicate were left intact.

Reductions in fertile matings are evident in both replicates of the test (Tables 1 and 2). The higher overflooding ratios resulted in larger proportions of sterile male matings. In the first replicate, the control plot had a high proportion of egg masses which were judged sterile. This was probably the result of poor hatch from the dehaired halves of the egg masses and may be partially due to misjudgment of the number of unembryonated eggs. On the first few days of release, only a small number of males were available to find females (e.g. only 67 males were released/plot), and temperatures were cool and near the flight temperature threshold. Also some sterile males did migrate to control plots (Table 3). Evaluation of egg masses from treated plots resulted in a much higher incidence of sterile

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Table 1. Results of releasing sterile males in 40 ha plots with 67 fertile male and female (NJF_{19}) gypsy moths released daily. Replicate 1.

Release Date	No. EM recovered	No. EM undetermined	No. EM unembryonated	No. EM sterile (11.5% hatch)	No. EM fertile (11.6% hatch)	% of mated sterile
			CONTROL PLOT			
6/26	61	16	33	7	5	58
6/27	55	1	46	2	6	25
6/28	50	3	21	5	21	19.2
6/29	52	2	12	4	34	10.5
6/30	34	3	6	4	21	16.0
						$\bar{x}$=20.1
			10S:1F Male Ratio Plot			
6/26	50	9	14	27	0	100.0
6/27	50	1	38	11	0	100.0
6/28	43	6	5	28	4	87.5
6/29	28	2	5	14	7	66.7
6/30	27	0	8	13	6	68.4
						$\bar{x}$=84.5
			50S:1F Male Ratio Plot			
6/26	42	5	4	32	1	97.0
6/27	40	1	12	25	2	92.6
6/28	31	0	4	24	3	88.9
6/29	38	0	6	30	2	93.8
6/30	46	2	9	34	1	97.1
						$\bar{x}$=94.2

Table 2. Results of releasing sterile males in 40 ha plots with 67 fertile male and female (NJF_{19}) gypsy moths released daily. Replicate 2.

Release Date	No. EM Recovered	No. EM undetermined	No. EM unembryonated	No. EM sterile (11.5% hatch)	No. EM fertile (11.6% hatch)	% of mated sterile
			CONTROL PLOT			
7/31	60	3	1	1	55	1.8
8/1	66	5	3	6	52	10.3
8/2	63	1	8	2	52	3.7
8/3	55	4	4	5	42	10.6
						$\bar{x}$= 6.5
			10S:1F Male Ratio Plot			
7/31	49	0	1	19	29	39.6
8/1	61	19	4	7	31	18.4
8/2	55	1	4	26	24	52.0
8/3	54	2	13	20	19	51.3
						$\bar{x}$=41.1
			50S:1F Male Ratio Plot			
7/31	48	0	0	20	27	42.6
8/1	42	1	2	20	19	51.3
8/2	47	1	3	33	10	76.7
8/3	52	3	10	24	13	64.0
						$\bar{x}$=58.43

Table 3. Male trap captures on simulated ratio plots. Replicate 2.

Date	Total Captures	Sterile Males	Fertile Males	Ratio S:F
		CONTROL		
8/1/79	67	2	65	
8/2/79	28	1	27	
8/3/79	29	0	29	
8/4/79	22	13	9	
		Σ=16	Σ=130	1:8.1
		10 Sterile:1 Fertile		
8/1/79	40	40	0	
8/2/79	131	128	3	
8/3/79	49	43	6	
8/4/79	72	65	7	
		276	16	16.7:1
		50 Sterile:1 Fertile		
8/1/79	118	118	0	
8/2/79	222	207	15	
8/3/79	211	202	9	
8/4/79	234	234	0	
		761	24	31.7:1

egg masses. Eighty four percent of the egg masses from the 10S:1F plot were judged to be from sterile matings. Sterility increased to 97 percent in the 50S: 1F ratio plot.

Replicate 2 resulted in lower proportions of sterile egg masses. However, sterile:fertile male ratios were complicated by the presence of native males. Estimates of sterile:fertile male ratios were calculated from male captures in pheromone-baited traps placed in each plot (see Table 3). In these plots, since the calculated ratios are lower than intended ratios, a higher proportion of viable egg masses would be expected.

If sterile males were as competitive as their fertile counterparts, one would expect 90.9% and 98.0% of the matings to be sterile in the 10S:1F and 50F:1S plots, respectively. Proportions of egg masses judged sterile in the first replicate approach these degrees of sterility. However, even using adjusted overflooding ratios (Table 3) to compute the expected proportions of egg masses which should be sterile does not explain the decrease in the observed proportions found sterile in the second replicate. Several explanations are possible: e.g. native males were much more competitive, changing weather conditions (warmer temperatures) favored competitiveness of fertile males, etc. However, none of these has been demonstrated. Additionally, more precise determinations of sterility through egg mass hatching may provide a better estimate of the actual mating success.

Project Number: GM 9.2.3
Project Title: Trial Sterile Male Field Test in a Simulated Sparse Population
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leader: V. C. Mastro

Introduction

The objective of the following tests was to evaluate the sterile male technique when applied to a gypsy moth population which was the approximate density and covered the approximate geographic area as an envisioned target population. Detection traps, when placed on an appropriate grid, normally detect new infestations before they cover 640 acres. However, it is not possible to predict the density and geographic distribution for the following year and therefore, this trial was completed using a simulated moth population. The simulated population was manipulated to approximate the increase in adult density that one would expect in a natural population. Numbers of sterile males released per day remained constant, as in an operational program, resulting in lower sterile:fertile ratios as the test progressed. Test area size, release numbers and overflooding ratios differ from those described in the work plan since moth availability was limited.

Methods and Materials

A population was simulated using laboratory reared NJ-F_{17} male and females. Laboratory-reared NJ males have been shown to be as competitive as feral males in field trials. Also NJ females have demonstrated attractancy similar to feral females in field tests. Release rates of fertile insects were incremented weekly to simulate a natural eclosion pattern. Placement of fertile insects was randomized each release date. Obviously, a simulated population differs from a native population. Native populations normally do not occur randomly distributed but rather in aggregate clumped distributions; and densities of native populations do not increase in 7 day time frames. The simulation we used for this test, however, did provide the information critical for evaluation of mating success of sterile males.

Fertile males and females used for simulation were held and handled in same manner as insects used in ratio plots (GM 9.2.2). Fertile insects were released over a square 156 ha area (1250 X 1250 m). Nine parallel lines (125 m between lines) with 42 points (25 m between points) were established for release of fertile insects, or a total of 369 possible release sites. Sterile males were released uniformly over a larger area (ca. 306 ha) on a uniform 250 m square grid. The 250 m wide release zone bordering the simulated population was intended to act as a buffer treatment area, as would likely be necessary in a real population where insect distribution is not precisely known.

Sterile males were irradiated and handled in the same manner as sterile males which were used in ratio plots (GM 9.2.2). A total of 2857 sterile males were evenly distributed daily on the 49 release sites. Fertile males were released at increasing rates; 29 males for the first 7 days, 57 males for the next 7 days and 286 males for the last seven days of the test. This release schedule resulted in approximate sterile to fertile overflooding ratio of 100:1, 50:1 and 10:1.

Fertile females and egg masses were also handled in the same manner described in report GM 9.2.2. Hatch determinations were done using whole egg masses which had not been dehaired.

Results and Discussions

Larger overflooding ratios resulted in a greater proportion of females mated with sterile males (Table 1). An average of 87% of the females recovered during the first week of the test deposited egg masses which were determined sterile. This average dropped to 73% during the second week of the trial (50S:1F ratio) and to 26% during the third week (10S: 1F male ratio). Theoretically if sterile males were equally competitive with fertile males and release strategy did not effect mating success, the proportion of females which mated with sterile males should have been greater. At a 10S:1F overflooding ratio, 90.9% of the matings should have been with sterile males. Greater overflooding ratios should have also resulted in higher proportions of sterile matings (i.e. 98.04% for a 50:1 ratio and 98.99% for the higher 100S:1F ratio). However, overflooding ratios were computed using the number of sterile males released in the entire plot including the buffer zone. If ratios are computed based on only the number of males released in the treated area (1465/day) overflooding ratios are halved (Table 2). Past field experience demonstrated that the majority of males do not disperse beyond 200 m. Therefore, sterile males released outside of the simulation area would not be expected to impact appreciably on the fertile released population. However, our limited ability to define a native population distribution and our poor understanding of early instar dispersal would necessitate the release of sterile males in a "buffer" area in dealing with real target population.

Table 3 presents mean percent of sterile matings for each week of the study and expected percent sterility computed using both total male release and males released only in the simulation area. Proportion sterility for the last week of release (7/23 - 7/29) was much lower than expected. Native male flight had begun during this period and undoubtly lowered the proportion of sterile mating. Unfortunately no measure of the native density inside the release area is available for this period. Generally, it appears that sterile males were not as competitive as their fertile counterparts, e.g. in the first week of the study only 37.5% of the matings were sterile, a degree of sterility which one would theoretically expect from a 7S:1F overflooding ratio. Several explanations of why these poor results occurred are evident. Avian predation was observed in the test site and appeared to increase as the test progressed.

Birds soon "learned" that workers would be releasing sterile male "box lunches" at sites and aggregate there. Unlike fertile males, sterile males were in a chilled condition at the time of release and not capable of flight for several minutes. Second and perhaps more important, irradiated, chilled adult males tested in competition plots, during the same period this test was being conducted, displayed abnormal activity periodicity. On the day of release irradiated, chilled males were most active in the evening hours, well after normal male activity tapered off. Little activity of these chilled males occurred at normal peak flight.

It is unfortunate that during the 1979 field season we chose to irradiate and release adult insects. However, as noncompetitive as released sterile males were, they did impact measurably on the fertile population. With development of good pupal aging and irradiation methods and the ability to manipulate male activity, results of such a release can only improve. Avian predation will also continue to be a problem, even with pupal release, unless more emphasis is placed on developing methods to retard feeding at release sites.

Table 1. Fertility of females released in a 156 ha area into which 2857 sterile males had been released daily.

Release Date	Total No. females or egg masses recovered	No. of egg masses undetermined	No. of egg masses unembryonated	No. of 1/ egg masses sterile	No. egg 1/ masses fertile	Percent females sterile
	(First 7 days of test - 29 fertile males & 29 fertile females released/day)					
7/9	26	2	6	16	2	88.9
7/10	27	0	2	22	3	88.0
7/11	23	1	1	20	1	95.2
7/12	26	3	3	18	2	90.0
7/13	22	0	2	19	1	95.0
7/14	29	0	7	24	3	88.9
7/15	21	1	0	13	7	65.0
	(Second 7 days of test - 57 fertile males & 57 fertile females released/day)					
7/16	41	0	10	28	3	90.3
7/17	42	6	3	23	10	69.7
7/18	51	0	5	41	5	89.1
7/19	48	1	0	33	14	70.2
7/20	45	0	2	22	21	51.2
7/21	44	1	4	30	9	76.9
7/22	47	3	2	28	14	66.7
	(Third 7 days of test - 286 fertile males & 286 fertile females released/day)					
7/23	224	10	9	92	113	44.9
7/24	246	10	10	87	139	38.5
7/25	233	3	11	66	153	30.1
7/26	239	0	13	66	153	30.1
7/27	246	9	11	30	196	13.3
7/28	224	15	9	33	167	16.5
7/29	236	7	10	21	198	9.6

1/ Matings were considered sterile when 11.5% or less of the total embryonated eggs in a mass hatched.

Table 2. Sterile:fertile male ratios computed using the total number of sterile males released in entire project area (306 ha); and using sterile males released only in the simulation area (156 ha).

Release dates	Total SM released/ day	No. SM released inside simulated area/day	No. fertile males released/ day	Overall S:F ratio	S:F ratio inside simulation area
7/9-7/15	2857	1465	29	98.5:1	50.5:1
7/16-7/22	2857	1465	57	50.1:1	25.7:1
7/23-7/29	2857	1465	286	10.0:1	5.1:1

Table 3. Actual mating success of sterile males compared to expected values computed using the total sterile males released and only sterile males released inside the simulation area.

Release dates	Observed percent sterile matings by week	Expected percent sterile matings (SM released inside simulated area)	Expected percent sterile matings (total SM released)
7/9-7/15	87.5	99.0 (98.5:1)	98.1 (50.5:1)
7/16-7/22	73.4	98.0 (50.1:1)	96.4 (26.7:1)
7/23-7/29	26.1	91.0 (10.1:1)	83.7 (5.1:1)

Project Number: GM 9.2.5
Project Title: 1979 Field Evaluation of Micro dispersable Formulations of Disparlure for Mating Disruption
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: C. P. Schwalbe and E. C. Paszek

This study, detailed in the April - September, 1979 Laboratory Report, was conducted to evaluate the relative effectiveness of Conrel fiber, Hercon flake and NCR microcapsule formulations of disparlure in disrupting mating. Application rate was 2 and 20 g/ha. Tests were performed in Freetown State Forest where gypsy moth populations were sparse. The Hercon and Conrel formulations reduced mating and trap catch greater than the microcapsule treatment at 20 g/ha; no differences were observed at 2 g/ha. These tests led to continuing studies of the Hercon and Conrel formulations at different population densities. GM 0.2.5. reports the results of that work.

Project Number: GM 9.2.6
Project Title: Monitoring Populations of Gypsy Moth in Campgrounds with Disparlure-baited traps
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leader: V. C. Mastro

During the 1979 field season data to assess regulatory risk were collected from 104 campgrounds in 6 northeastern states. Each campground was inspected and assigned a risk rating. These ratings (1 = low risk, 3 = high risk) are a summarization of objective and subjective observations. To provide objective estimates of adult male density, pheromone baited large capacity milk carton traps were placed in each campground. Traps were serviced at the end of the flight season and the total male capture recorded. Another estimate of insect density was provided by postseason egg mass counts. It was hoped that these two estimates of adult density would be helpful in developing a technique for assessing regulatory risk of a campground.

Regression analysis of the data was accomplished by computing correlation coeffficients for the pairs of variables.

Unfortunately only 65 of the 104 campgrounds were numerically risk rated so the size of the data set for the two of the analysis was limited.

The following table provides a summary of the analysis.

Variables	No. of Cases	R^2
Males trapped vs egg mass density	104	.00045
Risk rating vs egg mass density	65	.07186
Risk rating vs males trapped	65	.02498

Results of this test indicate that there is not a strong relationship between egg mass density in compgrounds and male capture in pheromone baited traps. Furthermore, it does not appear that male trapping alone is a good measure of regulatory risk. The poor correlation between male capture and risk rating is not entirely unexpected. The second estimate of density (egg mass counts) was not related to risk rating. Relationships between egg mass density and male trap captures have been reported previously, however.

Many variables, other than insect density, impact on how the regulatory risk associated with a campground is determined. Risk ratings provide a method for assessing regulatory hazard. Objective measurements of insect density, out of state visitor use, incidence of contact between visitors and pests, and survival and establishment of various life stages when transported, will provide the information for a risk rating system which more accurately reflects regulatory hazard.

Project Number: GM 0.2.1
Project Title: Whether Wicks Weather
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: D.H. Simser, C.P. Schwalbe, J.E. Richardson, H.H. Knight

Various design features, such as surface area and lure content affect emission rate of laminated plastic lure dispensers. The standard (+) dispenser used in the 1980 detection and delimitation program was produced by Hercon and contained disparlure synthesized by Dr. D. Farnum of Michigan State University. The dispenser was a 1/8 x 1" laminated plastic strip containing 500 ug (+) disparlure. Because of their small size, these dispensers are difficult to install into traps. Modifications of the standard design (I) have been developed for testing. Type (II) dispensers are similar in lure content to (I), but have a greater surface area, measuring 3/16 x 4". Type III dispensers are standard size, but are backed by an adhesive coating which facilitates installation in traps. In the present study, the three dispenser designs were field aged for 2 week intervals up to 10 weeks, after which they were bioassayed for attractiveness to male moths.

Methods and Materials

Ten wicks of each design (30 total) were aged at 2 week intervals for 10 weeks. At the onset of an interval, wicks were removed from freezer storage and stapled, (I and II), or pressed (III) within a milk carton trap in a standardized position. Traps were sealed and suspended ca. 1 m above ground level in a wooded area near the laboratory.

A 10 x 20 grid with an inter-trap distance of 33.4 m was established in Freetown State Forest, MA. All traps containing wicks were collected from the aging area and a 2 inch strip of Zodiac Pet Products Flea Collar with DDVP was stapled in standardized position in each. Zero-week aged wicks were removed from freezer storage and installed in traps. Ten traps baited with 100 ug (+) disparlure in 100 ul n-hexane applied to a cotton dental wick, and ten blank traps containing only the flea collar were prepared as comparative standards. In total, 20 treatments with 10 traps per treatment were assayed. Treatments were randomized every other line, and were suspended at ca. breast height from host trees. Traps were checked for moth capture 24 h following trap placement, and on three consecutive 24 h intervals thereafter.

Results

Results of this study are summarized in Table 1 and Figure 1; several interesting conclusions can be drawn. Dispenser designs I, II or III lost little, if any, activity when field aged to as long as 10 weeks. Average numbers of moths caught per aging interval were consistent within all treatments.

Secondly, wick III captured significantly fewer moths when compared to I or II. Apparently the adhesive backing of wick III retards release rate and reduces trap catch. Fresh wicks (0 weeks old) consistently captured fewer moths than other age intervals for all wick designs tested indicating an activation period is required for wicks to maximize lure emission. Since fresh wicks were in the field for ca. 120 h during this study without revealing any increase in moth capture, this activation period may be rather long.

Results of this study form the basis for recommending that type II dispensers be used in 1981 survey. Their large size, 3/16 x 4", and efficient use of lure (500 ug/unit) make them improved replacements for the 1/8 x 1" dispenser containing 500 ug (+) disparlure.

Table 1. Average number of feral moths captured in traps baited with wicks aged for varying intervals.

	Average number of males per trap [1] [2]					
Dispenser	0 Wk	2 Wk	4 Wk	6 Wk	8 Wk	10 Wk
Design						
I	4.5 de	10.9 b	10.7 b	10.0 bc	10.5 b	14.2 a
II	3.8 e	12.2 ab	9.8 bc	12.4 ab	11.9 ab	11.8 a
III	3.3 e	7.1 cd	4.4 de	7.3 cd	5.7 de	5.7 de
100 ug std	10.3 b					
blank	0.2					

1 Based upon 10 traps per treatment per replication, with 4 replications.

2 Values followed by the same letter are not significantly different (p .01) with Duncan's Multiple Range Test.

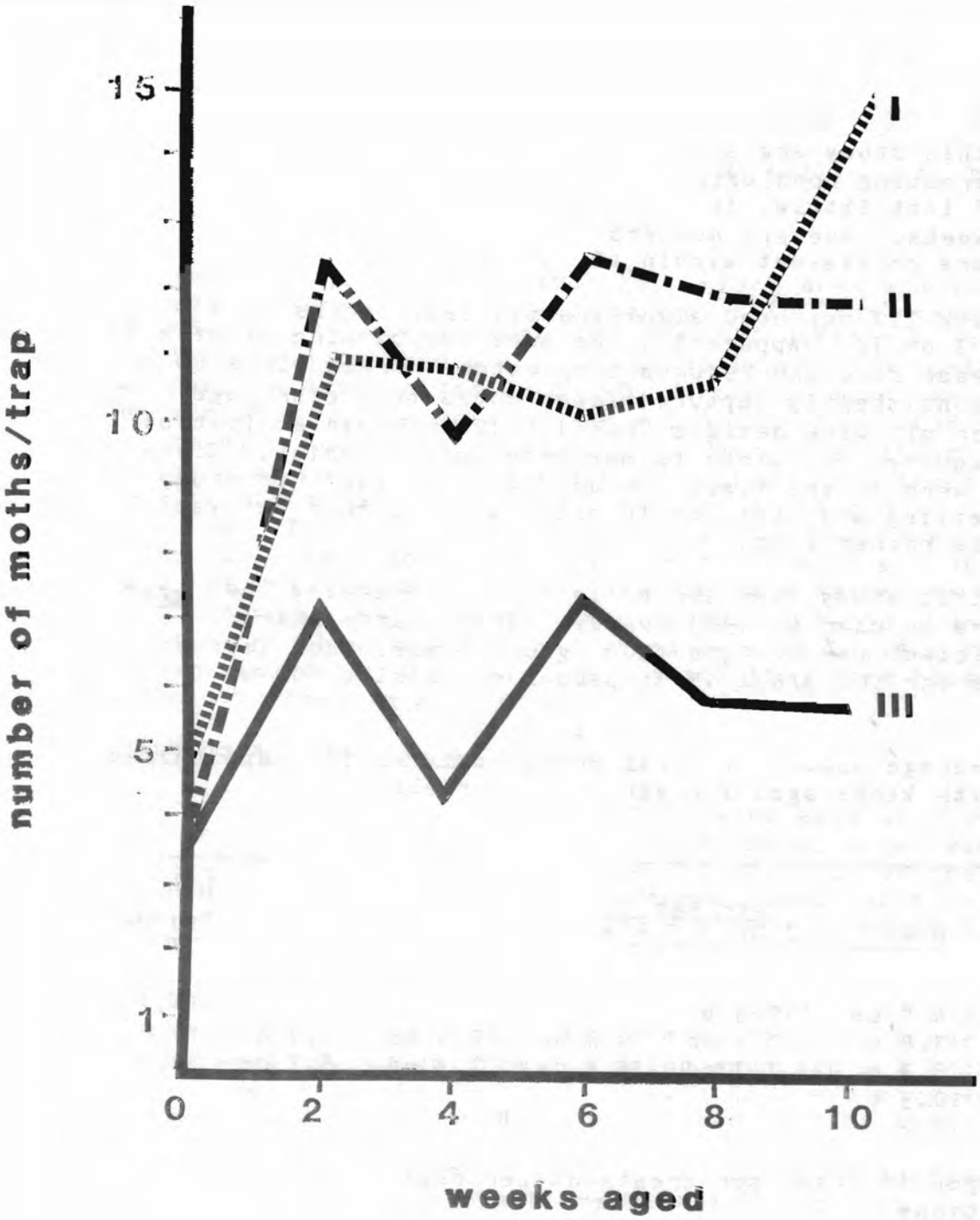

Fig. 1. Comparison of aged wick designs based upon number of feral males captured.

Project Number: GM 0.2.2
Project Title: Preseason Bioassay of Recently Synthesized (+) Disparlure
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: D. R. Lance, V. C. Mastro and C. P. Schwalbe

Introduction

To satisfy contractual agreements with producers of (+) disparlure (Dr. Donald Farnum, Chemistry Department, MSU and Andrulis Research Corporation, Bethesda, MD), samples of newly synthesized (+) disparlure were bioassayed in field trials at Brownsville, Texas in March, 1980. Also, during the course of these tests, possible effects of internal dye markers on male response to pheromone, were evaluated.

Materials and Methods

Pheromone

The disparlure for these trials came from 5 sources:

1) (+) disparlure produced for the 1978 field season by Farnum at Michigan State University. As the biological activity of this pheromone had been determined in previous tests, it was used as a standard for the present set of trials.

2) (+) disparlure which was reclaimed (by hexane extraction) from Hercon wicks, which had been used in the field for the 1979 detection and delimitation programs.

3) (+) disparlure produced by Farnum for the 1980 field season (a pooled sample of 10 ul from each of 5 lots).

4) (+) disparlure produced by Andrulis, from a 300 mg sample received for testing.

5) Unused Hercon wicks, containing 500 ug (+) disparlure each, from the 1979 field season.

With the exception of treatment number 5, all pheromone samples were tested using cotton dental wicks (1 cm dia. x 1 cm h). Wicks were loaded by applying 0.1 ml hexane solutions containing 0.1 and 1.0 mg disparlure/ml. Solutions for sources 1 and 3 were prepared directly from neat disparlure. The concentration of the disparlure solution from source 2 was determined by GLC analysis and then diluted to 0.1 and 1.0 mg/ml. Andrulis disparlure was sent to us in ampules containing solutions of appropriate concentrations. However, the ampules were improperly sealed and the concentration also was determined by GLC analysis. The 0.1 mg/ml solution was accurate within the limitations of analysis; the 1.0 mg/ml solution was measured at ca. 0.85 mg/ml.

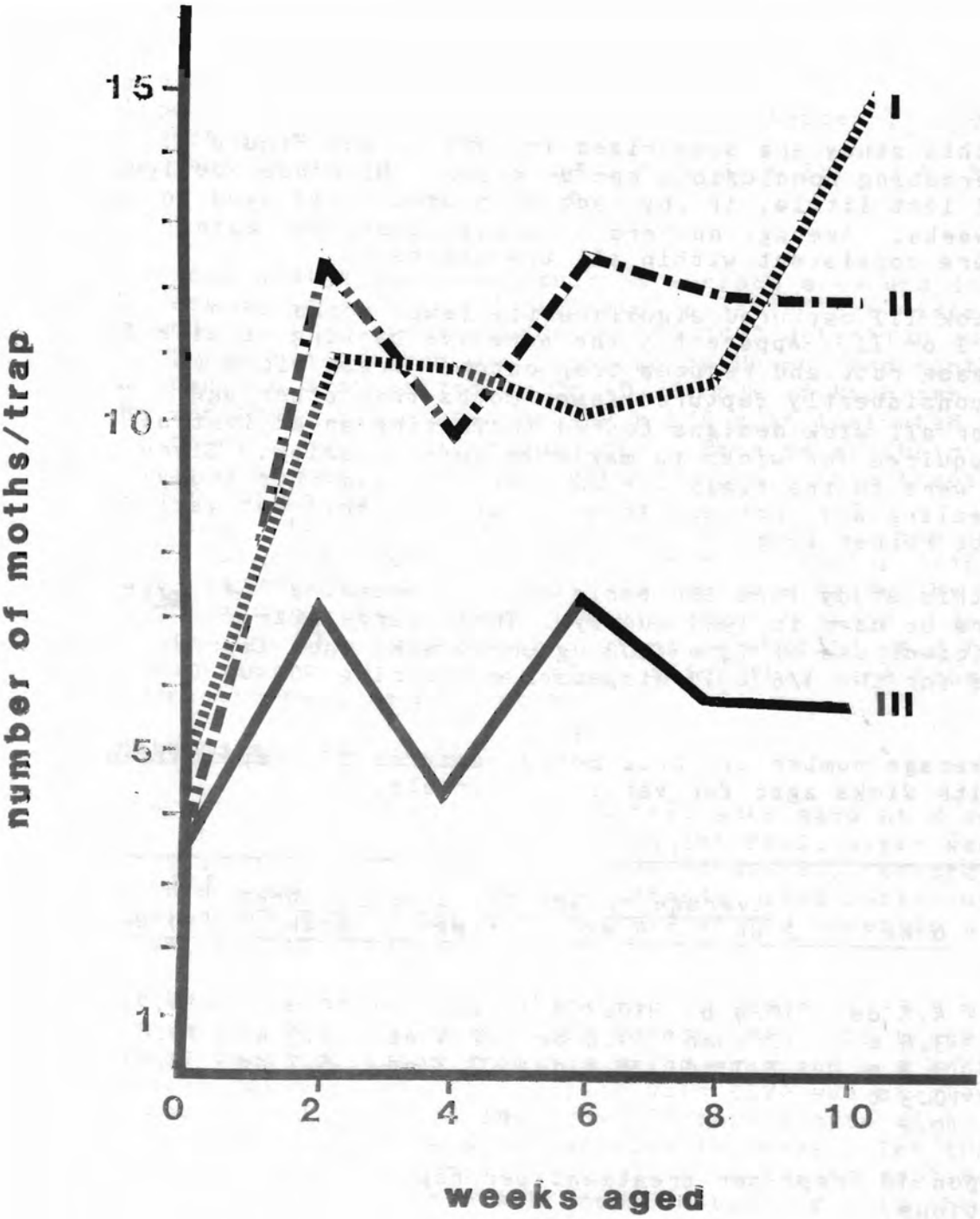

Fig. 1. Comparison of aged wick designs based upon number of feral males captured.

Project Number: GM 0.2.2
Project Title: Preseason Bioassay of Recently Synthesized (+) Disparlure
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: D. R. Lance, V. C. Mastro and C. P. Schwalbe

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Insects

Male gypsy moths used in the test were reared at the Otis Methods Development Center on the B4 production diet. A portion of the males were marked internally by adding 0.3 g/l of either red Calco dye or blue No. 35 dye (both in 3 ml linseed oil carrier) to the B4 diet. Male pupae were transported, in biomailers, as airline luggage, and were eclosed in the Brownsville Methods Development Center (at night) and in the field (during the day). Before release, undyed males were marked by dusting their antennae with Day-glo powder.

Plot and trial design

In a grapefruit orchard in Brownsville, two concentric rings of Delta traps baited with (+) disparlure, were placed at 70 m and 140 m from the central release point. The 70 m ring was divided into 3 120 degree arcs, or sectors; the 140 m ring was divided into 6 60 degree sectors. Traps were evenly spaced along the rings, and, within a sector, each combination of pheromone source (e.g. Farnum 1978) and concentration (e.g. 10 or 100 ug) was randomly assigned to a trap point. In Trials 1 and 2, 7 pheromone treatments (Farnum 78, Farnum 80, and reclaimed, each at 10 and 100 ug, and Hercon wicks) were tested. For Trials 3-5, Andrulis disparlure was included as 2 treatments (10 and 100 ug) and Hercon wicks were dropped. Within sectors, the positions of pheromone treatments were rerandomized before each trial, except before Trial 5, when only the 70 m line was rerandomized.

In Trial 5, males marked with internal dyes were tested against undyed males. On the first day of Trial 5, undyed moths were dusted with blue Day-glo pigment (previously released males had been dusted yellow), and dyed males were not dusted. On the second release day, all males, dyed and undyed, were dusted with pink Day-glo powder.

Results

In total, 2788 males were released and 853 (30.6%) were recaptured. No significant differences in moth catches were observed among pheromone sources (Table 1 - 3). In Trials 1 and 2, collectively, (but not in Trials 3-5), traps containing 100 ug wicks caught significantly ($p < 0.05$) more males (89.5 males/Trial) than traps baited with 10 ug wicks (44.0 males/Trial).

In general, the Farnum 1980 (+) disparlure appears to be of high quality. Traps baited with Andrulis disparlure did not catch significantly fewer moths than traps baited with disparlure from other sources (Table 3); however, Andrulis captures were the lowest, and as Andrulis is a new producer, further testing of their product is warranted.

For males released on the first day (but not the second day) of Trial 5, blue-dyed males were recaptured in a significantly lower proportion than either red-dyed or undyed males (Table 4). The low proportion of recapture of the dyed males which were released on day 1, and the extremely high proportion of dyed day-2 males which were recaptured, leads us to suspect that some of the (undusted) dyed day-1 males may have been "accidentally dusted" while in the traps (they would have been subsequently counted as day 2 males). A high proportion of the recaptured males which were released on day 2 were heavily dusted. In the traps, some of their Day-glo powder may have been transferred to undusted, dyed males, effectively marking them. Further studies (investigating both dye type and concentration) are required before any conclusions can be made concerning the competitiveness of internally dyed males.

Table 1. Total numbers of laboratory-reared male gypsy moths captured by (+) disparlure-baited Delta traps in a circular plot in Brownsville,TX (March 1980).

		TRIAL 1		TRIAL 2		TOTAL 3/	
Pheromone Source	Pheromone on wick (ug)	70 m 1/	140 m 2/	70 m	140 m	Treatment	Source
Farnum 1978 (Standard)	10	13	2	5	2	22	81
	100	33	6	17	3	59	
Farnum 1980	10	16	2	7	4	29	93
	100	25	6	29	4	64	
Reclaimed 1979	10	25	2	6	4	37	93
	100	31	5	18	2	56	

1/ 3 traps per treatment
2/ 6 traps per treatment
3/ No significant differences, as determined by ANOVA, were found among pheromone sources, however, 10 ug traps caught significantly (p 0.05) fewer moths than 100 ug traps.

Table 2. Total numbers of laboratory-reared male gypsy moths captured by (+) disparlure-baited Delta traps in a circular plot in Brownsville, TX (March 1980).

Pheromone Source	Pheromone on wick (ug)	TRIAL 70 m 1/	TRIAL 140 m 2/	TRIAL 2 70 m	TRIAL 2 140 m	TOTAL 3/
Farnum 1978	100	33	6	17	3	59
Farnum 1980	100	25	6	29	4	64
Reclaimed 1979	100	31	5	18	2	56
Hercon 1979	500	32	16	18	5	71

1/ 3 traps per treatment
2/ 6 traps per treatment
3/ ANOVA detected no significant differences in trap captures among pheromone sources.

Table 3. Total numbers of laboratory-reared male gypsy moths captured by (+) disparlure-baited Delta traps in a circular plot in Brownsville, TX (March 1980).

Pheromone Source	TRIAL 3 70 m 1/	TRIAL 3 140 m 2/	TRIAL 4 70 m	TRIAL 4 140 m	TRIAL 5 70 m	TRIAL 5 140 m	TOTAL 3/ Treatment	TOTAL 3/ Source
Farnum 1978	15	12	3	1	9	3	43	119
	19	7	6	4	27	13	76	
Farnum 1980	18	7	1	2	31	5	64	153
	28	4	3	5	40	9	89	
Reclaimed 1979	16	3	3	2	47	7	78	140
	23	6	2	3	27	4	62	
Andrulis	7	8	0	1	7	4	27	104
	23	8	5	4	26	10	77	

1/ 3 traps per treatment
2/ 6 traps per treatment
3/ ANOVA detected no significant differences in trap captures among pheromone sources.

Table 4. Numbers of internally dyed and undyed male gypsy moths which were released, and the proportion which were recaptured in (+) disparlure-baited Delta traps, in a circular plot in Brownsville, Texas (March 1980).

Type of dye	Moths released on day 1				Moths released on day 2			
	# released	# recaptured	% recapture[1]		# released	# recaptured	% recapture[1]	
Calco red	118	31	26.3	a	51	31	60.8	a
Blue No. 35	85	12	14.1	b	44	24	54.5	a
Undyed	134	47	35.1	a	237	122	51.5	a

1 Within a column, percentages not followed by the same letter represent proportions which are significantly different at the $p<0.05$ level (for analysis, actual numbers, not proportions, were used in multiple uncorrected 2 x 2 contingency table comparisons).

Project Number: GM 0.2.3
Project Title: Evaluation of (+) Disparlure used in Detection and Delimitation Survey Programs
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leader: D.H. Simser and C.P. Schwalbe

Enantiomerically pure (believed to be >99%)(+) disparlure has been obtained from several sources. Material used in 1979 and 1980 survey programs was prepared under cooperative agreement by Dr. D. Farnum, Michigan State University. However, in the interest of developing commercial availability of this compound, a contract has been negotiated with Andrulis Research Corporation, Bethesda, Maryland to synthesize 200 g (+) disparlure for program use.

In the present study, (+) disparlure synthesized by Andrulis Research was compared with that prepared by Dr. D. Farnum, and several other (+) or (±) preparations. Activity was based upon capture of laboratory-reared male moths in traps baited with the various formulations.

Methods and Materials

The source of each material tested, and code designation of each treatment is:

Treatment Code	Source
(F)	Sample from 20.046 grams (+) disparlure received from D. Farnum of Michigan State University. This material was used in all 1980 line trapping programs (where (+)-disparlure was used).
(AI)	Preproduction test sample of 0.3 g provided under contract SB3-4-7-8 (a) 9-C-1100 with Andrulis Research Corp., Bethesda, MD, received 3-13-80.
(AII)	Sample of 19.2 grams received 4-17-80 under under above contract. This material was not used in 1980 trapping program and is presently in storage for future use.
(RC)	Sample extracted from Hercon dispensers used in 1979 trapping program. The original source was Michigan State University.
(RA)	Sample of racemic disparlure, GM-IX-57-2 received from B. Leonhardt, SEA-AR, Beltsville, MD.
(B)	Blank control - cotton dental wick

Laboratory preparation

Neat (F),F(AI), and (AII) were diluted to 100 ug disparlure/100 ul n-hexane; identical solutions were prepared from (RC) and (RA). All treatments were serially diluted to 10 ug/100 ul and 1 ug/100 ul. To insure identical concentrations, all materials were analyzed on a Tracor 550 gas chromatograph (oven temp. 188° C, inlet temp. 225° C, detector temp. 250° C; column: 3% ov-1 on chromosorb W/HP 80/100, 6' x 1/4" od, glass)

Each treatment and concentration was applied to a cotton dental wick of standard size with a Drummond Dialamatic Microdispenser. Following solvent evaporation, individual wicks were threaded on copper wire and the wire stapled within a high capacity milk carton trap. Wick placement within traps was standardized. In addition, a flea tag (Zodiac Pet Products) containing DDVP, was stapled within each trap in a standardized position. Blank traps containing cotton dental wicks and flea tags were prepared and isolated from treated traps to prevent contamination. Treatments were repeated in 10 traps; thus, a total of 160 traps were prepared.

Field preparation

A 10 x 16 grid with an inter-trap distance of 50 m was established in a wooded area (primarily scrub oak with dense understory) on Otis Air Base, Massachusetts during June 1980. Traps were suspended from host trees at ca. breast height, away from the tree bole. Each of the 16 treatments was represented within a grid line, and within line trap order was randomized prior to placement.

Within the grid, six fixed equidistant male release sites were designated. Initially, four consecutive 24 h releases of male moths were planned; however, unseasonally cold weather conditions within this 96 h period necessitated an additional release. Newly eclosed moths (NJ F_{21}) were held under constant conditions for 24 h. Thus, all released moths were 24-48 h old. A total of 28,500 moths were released (day 1, 4200; day 2, 4500; day 3, 4200; day 4, 4200; day 5, 8400) in equivalent numbers per site per release day.

Traps were checked for moth capture 24 h following release 1, 2, 4 and 5. During each checking period, all treatments within lines were re-randomized and replaced on an adjacent line. A total of 4 replicates of 10 traps/treatment/replicate were recorded for evaluation.

Results

In comparison with currently used (+) disparlure, (F), traps baited with the commercial formulations, (AI) and (AII) captured similar numbers of released moths. Disparlure reclaimed from used wicks (RC) also captured large numbers. (Table 1) Dose dependent responses were noted in (F), (AI) and (AII) but not, interestingly, in (RC). Statistical analysis yielded poor separation of results; however, comparative activity of standard (F) and candidate (AI) and (AII) is evident.

Pre-season field studies such as this are hampered by shortcomings in the experimental design and procedure. The use of fixed male release sites did not provide for uniform distribution of males throughout the study area. Males tended to cluster at the release sites and were captured by traps in the near proximity. Variance in trap catch, even within treatments, was extremely high. For example, trap catch of (F) ranged between 2-231 moths during a single replicate. Pre-season weather conditions varied greatly during the test period, which resulted in inconsistent numbers of moths trapped (Table 2). Such factors necessitate additional in-season field studies before choice decisions can be made.

Table 1. Average number of male moths captured in traps baited with disparlure from various sources.

Source	Concentration (ug)	Average Number of Moths [1] [2]
(F)	100 (+)	13.15 a
	10 (+)	3.75 bc
	1 (+)	2.00 bc
(AI)	100 (+)	4.98 bc
	10 (+)	3.88 bc
	1 (+)	0.98 c
(AII)	100 (+)	8.75 ab
	10 (+)	6.40 bc
	1 (+)	2.65 bc
(RC)	100 (+)	5.00 bc
	10 (+)	4.98 bc
	1 (+)	6.55 bc
(RA)	100 (±)	1.13 c
	10 (±)	4.10 bc
	1 (±)	0.60 c
(B)	-	0.20 c

[1] Based on the average capture of ten traps per treatment per replicate, with four replications.

[2] Values followed by the same letter are not significantly different at 10% level using Duncan's Multiple Range Test.

Table 2. Recovery of released moths into a grid of traps baited with disparlure from various sources. Each captured in 10 traps.

Source	Concentration (ug)	1	2	3	4	Total
(F)	100	52	20	400	54	526
	10	8	25	103	14	150
	1	18	9	43	9	79
(AI)	100	27	15	140	17	199
	10	24	16	89	23	152
	1	4	8	21	5	38
(AII)	100	40	25	248	37	350
	10	5	12	215	24	256
	1	8	10	70	18	106
(RC)	100	34	26	101	39	200
	10	36	22	100	41	199
	1	32	4	217	8	261
(RA)	100	5	1	38	1	45
	10	3	4	149	8	164
	1	6	3	8	10	27
(B)	-	4	0	3	1	8

Project Number: GM 0.2.4
Project Title: Comparative Evaluation of (+) and (±) Disparlure Formulations in Eliciting Male Trap Catch
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: D. Simser, C. P. Schwalbe

The present study is a continuation of project GM 0.2.3. However, that preseason evaluation of (+) disparlure formulations was based upon recapture of released laboratory-reared male moths. Also, in this study two racemic (±) formulations were assayed for field activity.

Laboratory Preparation. Experimental laboratory procedures were as described previously unless otherwise indicated. Source of the additional (±) was:

(Bie) Sample from 205 Kg shipment received from Chemsampco, Columbus, Ohio, under 1980 contract by SEA-AR, Beltsville, MD.

(Sch) Sample from 195 Kg received by APHIS, Otis Air Base, Massachusetts, under above contract.

All materials were tested at 10 ug/100 ul n-hexane. Racemic disparlure was diluted to concentration from neat material. Concentration was verified by gas chromatographic analysis before applying to cotton dental wicks. The blank (cotton wick only) was treated with solvent only. All wicks were fastened within traps as previously described; however, a 2 inch strip of Zodiac Pet Products Flea Collar replaced the flea tag.

Field Preparation and Procedure

A 9 x 9 grid with an inter-trap distance of 50 m was established in a forested area of Sandwich, Massachusetts during June, 1980. Visual observations and monitor trap captures of moths indicated that flight activity throughout the test grid had commenced.

Milk carton traps baited with disparlure formulations were suspended from host trees in a completely randomized design. Traps were checked for moth capture after three consecutive 24 h periods following trap placement.

Results

The number of male moths captured (Table 1) indicates similarities in (+) formulations. All (+) enantiomer-baited traps captured large numbers of moths. However, (AI)-baited traps captured significantly more moths than the others. Preseason data are compared with results of this study in Table 2.

Racemic disparlure captured significantly fewer moths than any (+) formulation (Table 1). Similar numbers of moths were captured, although only (Sch) captured significantly more moths than the blank.

Conclusions

1) The (AI) and (AII) material is comparable in activity to previously used (RC) and currently used (F) (+) disparlure, which warrants purchase of this material for future use.

2) The (±) formulations, (Sch) and (Bie) are active in the field, but predictably, are less effective in capturing male moths than any of the tested (+) formulations.

Table 1. Average number of male moths captured in traps baited with 10 ug (+) or (±) disparlure from various sources.

Source	Enantiomeric Composition	Number of Feral Moths Trapped [1] [2]
(AI)	(+)	136.3 a
(RC)	(+)	129.9 ab
(AII)	(+)	115.3 b
(F)	(+)	102.6 b
(Sch)	(±)	46.3 c
(Bie)	(±)	37.7 cd
(RA)	(±)	30.9 cd
(B)		17.6 d

[1] Base on an average of three replicates, with ten traps per treatment per replicate.

[2] Values followed by the same letter are not significantly different at 1% level with Duncan's Multiple Range Test.

Table 2. Comparison of male moth capture in traps baited with 10 ug (+) disparlure.

Source	Preseason test [1]	Seasonal Test [2]
(F)	3.75	102.6
(AI)	3.88	136.3
(AII)	6.40	115.3
(RC)	4.98	129.9

[1] Average of 4 replicates, with ten traps per replicate.

[2] Average of 3 replicates, with ten traps per replicate.

Project Number: GM 0.2.5
Project Title: 1980 Field Evaluation of Hercon and Conrel Formulations of Disparlure for Disrupting Mating at Varying Population Densities
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: C.P. Schwalbe and Don Albright

The objective of these tests was to determine the degree of mating disruption effected by disparlure applied to plots of varying population densities. Application rate was constant throughout the tests at 50 g/ha; Hercon flakes and Conrel fibers were the formulations used.

Sixteen ha plots were established in south central Pennsylvania. Prior to treatment, 16 subplots (.0625 ha each) were established in the center 4 ha of each plot. Egg mass counts performed in these subplots were used to establish preseason population densities. Plots were selected so that 12 plots could be identified in each of 3 densities. Low, medium and high densities averaged (n=12) 3.1, 39.5 and 213.6 egg masses per ha, respectively. A total of 36 plots were ultimately established.

After hatch, 4 burlap bands were placed in each subplot (64 bands/plot). These were examined periodically to give further measure of populations within the plot. Treatment of plots commenced shortly after first observed pupation. Conrel fibers were applied July 2-4 and Hercon flakes were applied July 4.

Treatment effectiveness was monitored by male moth captures in 1/2 gal traps baited with (+) disparlure and incidence of mating of female moths (laboratory-reared) placed in the plots. There were 8 traps and 8 female moths in each plot. Female moths were replaced every other day and any eggs deposited were collected.

Eggs were examined for embryonation ca. 6 weeks after they were collected. No dissection for determining insemination was performed. Final assessment of efficacy will be based upon post-season egg mass densities which are scheduled to be done Oct. 20 - Nov. 7, 1980.

A summarization of data collected to date appears in Table 1. Reduction of trap catch appears to be greatest at low preseason population densities. Similarly, the incidence of successful mating was lower at sparce densities and higher at heavy densities. Final conclusions regarding the efficacy of these treatments, however, will not be drawn until fall egg mass counts have been completed and analyzed.

These results indicate that disrupting gypsy moth mating with broadcast applications of disparlure is density dependent. The rate of increase of the target population is a major factor in determining if control would be effected by the degree of mating disruption observed in these tests. A more detailed report concerning this will be included in the next laboratory report when all data have been collected and analyzed.

Table 1. Number of male moths caught in traps and female moths mated in plots treated with Hercon and Conrel formulations of disparlure. Four replicates of each treatment were sprayed with 50 g/ha disparlure.

	Low Density			Medium Density			Heavy Density		
	Hercon	Conrel	Check	Hercon	Conrel	Check	Hercon	Conrel	Check
Preseason EM/ha [1]	2.0	3.6	3.6	45.6	29.3	43.7	204.0	200.0	236.8
Total ♂♂ Captured [2]	515	181	7,944	3,540	1,287	25,483	8,253	6,348	27,704
% ♂♂ Reduction	93.5	97.7	0	86.1	95.0	0	70.2	77.1	0
# ♀♀ Determined [3]	183	153	184	205	196	245	216	243	252
% ♀♀ Mated	16.4	10.2	57.1	28.3	35.7	82.0	35.2	45.3	73.4
% Mating Reduction	71.3	82.1	0	65.5	56.5	0	52.0	38.3	0

1 Average of 64 subplots, .0625 ha each
2 Season-long total of males caught in 4 plots conbined (8 traps/plot)
3 Season-long total of females determined from 4 plots combined

Project Number: GM 0.2.6
Project Title: Development of Mass Marking Techniques for Gypsy Moth Male
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: M. Shorb and V.C. Mastro

Introduction

One method of marking male gypsy moths for field studies is to dust the insects with Day-glo powder. In previous field tests, the powder has been brushed onto the antennae of the newly eclosed males. Obviously, this method of individual marking would not be practical in a large scale sterile male program. This report describes two alternative marking techniques.

Objective

The purpose of this study was to determine whether male gypsy moths could be marked with Day-glo powder in large numbers with a minimum of worker attention.

Methods and Materials

In a 16 oz. squat cup, fifty male pupae, 8-11 day old, were liberally dusted with Day-glo "Aurora Pink" fluorescent pigment. Insects from this first treatment were placed in a 53 cm x 61 cm x 173 cm h plexiglass chamber. The adults were allowed to eclose at ca. 24° C, 30% RH and high humidity conditions ca. 24° C, 75% RH. The adult males were allowed to fly about the chamber until all had died. The absence or presence of marking was determined under an ultraviolet light. This test was repeated using Day-glo "Horizon Blue" fluorescent pigment. The pupae were placed in the same eclosion chamber as the pupae which had been treated with "Aurora Pink".

For the second treatment, the interior sidewall of a 16 oz. paper squat cup was abraded with medium grain sandpaper. The sidewall was then dusted with Day-glo "Aurora Pink" fluorescent pigment taking care not to dust the floor of the cup. Fifty male pupae were placed in the powder-treated cup exercising caution not to dust them. The cup was then placed in a 53 cm x 61 cm x 173 cm h plexiglass chamber and the insects were allowed to eclose under (normal laboratory conditions) and high humidity conditions. Adult males were left in the chamber until all had died. The dead insects were evaluated under UV light. This procedure was repeated using Day-glo "Horizon Blue" fluorescent pigment.

Results and Discussion

All the insects from both treatments were checked under a UV light and found to be well marked. Day-glo powder could be detected on wings, legs, abdomen and antennae of the moths. The males' eyes were also found to be covered. The role of vision in mating activity (see Brown and Cameron, 1977) has not been clearly defined and the effect of dusting the eyes on male performance is unknown.

Project Number: GM 0.2.7
Project Title: Sterile Male Trial Berrien County, Michigan
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leader: V.C. Mastro

Introduction

A pilot test of the sterile male technique was initiated in 1981 in Berrien Coutny, Michigan. The primary purpose of this study was to determine the degree of interaction between released sterile males and native males in a sparse, isolated population. The Berrien County site was chosen because the population there appeared to be isolated and relatively sparse. A delimitation survey of the area in 1979 resulted in capture of 90 malees in a small area. In addition, the survey did not detect any native males in the surrounding 9 square miles. Our secondary objective was to determine what difficulties and deficiencies would be encountered in an operational program. Implicit in these objectives was an effort to test new procedures which would improve efficiency of all phases of the project including rearing, harvesting, marking, handling, shipping, release and evaluation. Several unexpected problems arose and attempts were made to remedy them. In all, the program was successful, in that the above objectives were met. However, several deficiencies still exist which directly impact on the practical feasibility of using this technique as an operational part of our program. These will be discussed in the results section of this narrative.

The majority of the Berrien County pilot study data has now been summarized and will be reported in the following discussion.

Unfortunately, the degree of sterile male mating success, perhaps the most critical element, must be estimated from egg mass hatching determinations, and these data will not be available until March, 1981.

Methods and Materials

Sterile Male Production - To facilitate a single harvest of pupae from each production (infest) date, a sampling system was devised which checked the developmental rate of each days production. This led to the procedure in which, four days after pupation had reached 10 percent of the total insects infested, all pupae were harvested. Generally, greater than 80 % of the insects had pupated by the harvest date. Using this procedure ca. 87 % of the pupae which were harvested were within a four day age range (i.e. 1-4 day old). The remaining pupae were 5 day old or older.

Once harvested, pupae were held an additional 7 days under standard rearing conditions and then were irradiated. Using this schedule for harvest and irradiation, the majority of pupae were treated 8-11 days after pupation. Fifteen krads was chosen for complete sterilization of males. Ten krads, the dose outlined in the work plan, was not used because of the difficulties in evaluating a partially-sterile male release, and an incomplete understanding of the dosage-age relationship for maximizing carry over sterility into the F_1 generation.

Competitiveness of males treated with 15 krads has been evaluated in 1978 field trials. Males treated as 10-11 day old pupae performed as well as untreated laboratory-reared males. However, males which were treated as 8-9 day old pupae, were slightly less competitive.

After irradiation treatment males were packaged the same day and shipped to Benton Harbor, MI. Generally, unless delayed in shipment, males were placed in the field the following day.

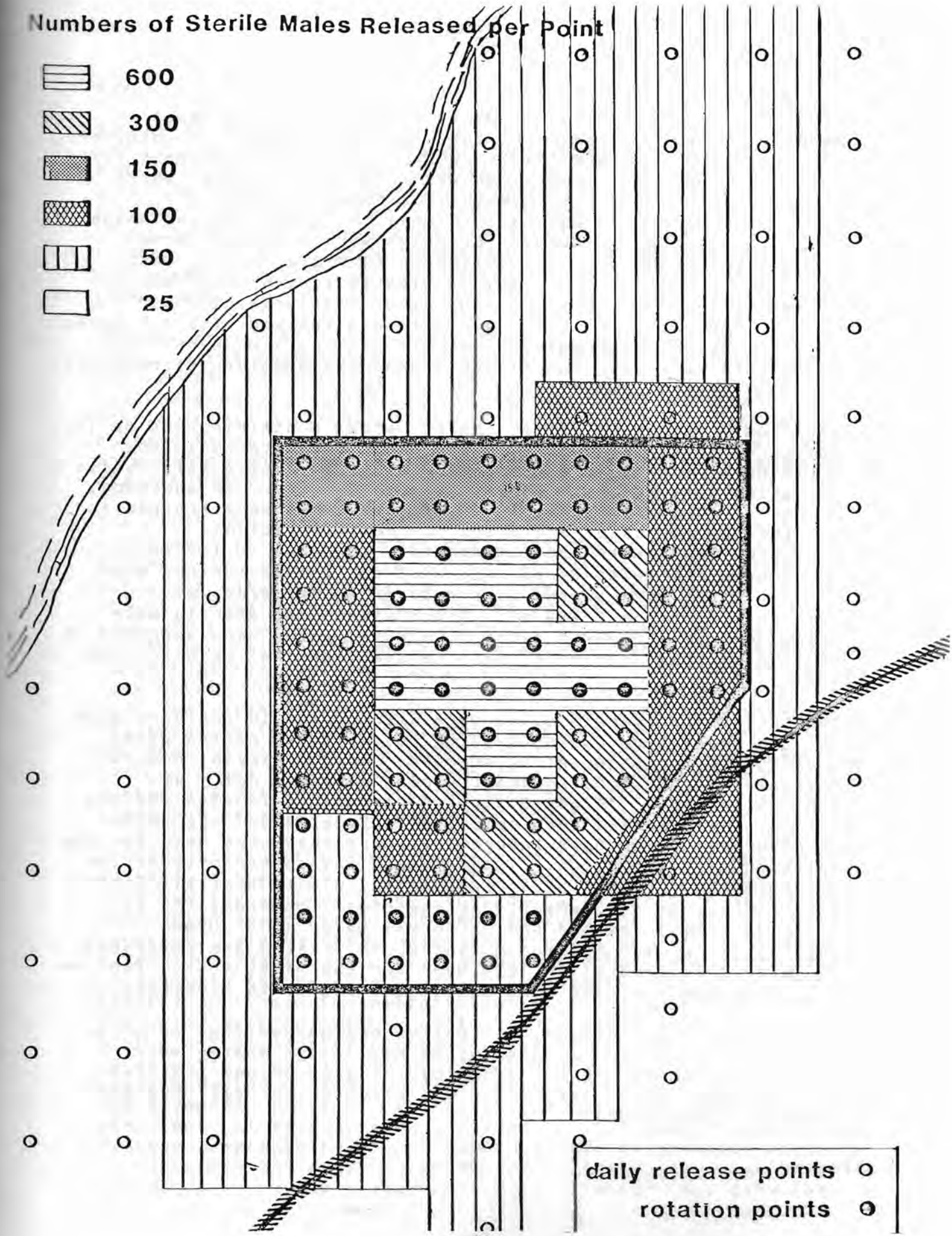
Plot Design - Layout of the test site was completed by the second week in June. A grid system was established which provided sites for: burlap banding (larval survey), sterile male release points and trap sites. A total of 297 banding sites were established on a uniform 47.5 m grid. Bands were checked weekly beginning June 23. During the second week (June 30-July 5) larvae were found at several banding sites. When a site check was positive for larvae, additional host trees within a 20 m radius of that site were also banded. On July 8 the first prepupa was found beneath a band. A summary of the weekly (7/8-13) check is presented in Table 1. A total of 102 larvae (mostly 5th and 6th instar) and pupae were found at this time. These positive sites confirmed infestation in a core area of ca. 4.5 ha. The following weekly check (completed on 7/15-16) revealed only 45 insects under bands. These include; 6 pupae, 25 prepupae and 16 larvae. The loss of insects between checks can be attributed to *Calosoma spp* predation and to the disturbance of insects caused by lifting the burlap when checking the site. To minimize this loss, no further bands were checked until native flight was complete.

Table 1. Positive burlap banding sites - Benton Harbor, Michigan (7/8-13, 1980).

SITE	Number of Larvae	Number of Prepupae	Number of Pupae	TOTAL
J-13	8	1	1	10
J-16	1	0	0	1
K-15	47	3	2	52
K-16	1	0	0	1
K-17	1	0	0	1
L-12	1	0	0	1
L-13	4	0	0	4
L-14	2	1	0	3
L-15	10	0	0	10
L-16	5	0	0	5
L-17	1	0	0	1
M-13	1	0	0	1
M-15	3	1	0	4
M-16	3	1	0	4
M-17	3	0	0	3
N-12	1	0	0	1
	92	7	3	102

Sterile Male Release - Beginning on July 11 approximately 9,000 male pupae a day were placed in the pilot study site. Releases continued until 8/21/80 for a total release of ca. 373,500 male pupae. A weighted release system, using the results of the burlap-banding survey, was used to distribute the sterile male pupae. Within the confirmed infested "core area" ca. 6,000 males were released per day. The number of males that any site received was calculated by multiplying the proportion of the number of larvae and pupae found under bands at that site by the total to be released at all sites. Within the core area and adjacent to it, release sites were spaced 47.5 m apart on a grid. The grid was partitioned into squares each containing 4 release sites. Each square was provisioned with 6 release containers (1 container on 2 sites and 2 containers on 2 sites). This arrangement provided for a possible 6 day rotation of release sites. Depending on eclosion rate of sterile male moths, this rotation could be shortened to 4 days and lengthened to 6 days.

Outside of the core area, release sites were spaced 95 m apart. As the distance from the core area increased, the number of sterile males released per day decreased. On the outside fringe of the treated area only 25 males were released/day/site (Fig. 2).


Numbers of Sterile Males Released per Point
600
300
150
100
50
25
daily release points
rotation points

A modified quart milk carton was used for release of small numbers of males (<50 males). Water Brainage from the container was facilitated by replacing the bottom with plastic screen (1/8" mesh). The top of the container was folded in a manner which would provide a 1.25" x 5" opening for male dispersal. A double-ended wire hook was used to hang the container and shape the exit port. Larger numbers of males were released using circular wire mesh (1" x 1") cylinders (12" dia. x 7 h) with solid plastic tops and bottoms. A plastic screen (1/8" mesh) basket (8" dia. x 2" h) placed inside of the large cylinder served to contain the pupae and provided a resting site for males to expand and dry their wings. At mid season, a wrap of 1/8" mesh plastic screening was placed around the outside cylinder (5" h) to retard bird predation.

Evaluation - Pheromone baited (Hercon wicks containing 500 ug (+) disparlure) half-gallon milk carton traps were placed in the sterile male release area to monitor sterile:fertile male ratios and provide some estimate of native moth density. In addition, areas adjacent to the sterile male release area were trapped to determine the degree of isolation of the infestation. Milk-carton traps were used immediately adjacent to the male release area and Delta traps were used for points further away. Both trap types were placed at a rate of 32 traps/sq. mi. (Inter-trap distance = 262 m). Traps within the sterile male release zone were checked daily. Captured males were examined to determine if they were internally marked (sterile) or wild-type males.

Laboratory-reared female moths were also placed in the core area (i.e. the area where burlap-banding had detected native gypsy moths) to monitor male mating success. Twenty virgin, one-day old females were placed in small wooden A-frames daily at individual sites. The day following placement, females and any portions of egg masses were collected. Females and egg masses were returned to the Otis laboratory where they were held for the completion of oviposition. Twenty eight days after oviposition was completed, egg masses were placed in cold treatment. Control matings were done concurrently at the Otis laboratory (25 IR males x 25 normal females and 25 normal males x 25 normal females). Males for control mating (both treated and untreated) were taken from production lots used for the pilot test. Females were taken from the same lots as females placed in Michigan.

After native flight ceased, all native egg masses that could be found were collected. A total of 48 native egg masses were located under bands and by searching the area around positive banding sites. These egg masses are also now in cold treatment at the Otis laboratory.

Results and Discussion

At the initiation of the sterile male pilot study, little information was available about the population in Benton Harbor. Ninety males had been trapped in 1979; however, an extensive survey by Michigan Department of Agriculture personnel had failed to locate any egg masses or evidence of any life stage. Given that the precise location of the infestation was poorly defined, release of sterile males would have had to be more or less broadcast over the suspected area. This would have been undesirable.

Burlap-banding of trees provided an alternative method for locating the population and distributing sterile males so that the largest proportion would be placed where the native density was the greatest. Although information about spacing of banding points for this purpose does not exist, the grid spacing chosen appears to have achieved the desired goal. That is, it provided information on the population's geographic and numerical distribution.

The first native male was captured on 7/25-17 days after the first male prepupa was observed under burlap. The mean pupal period under standard rearing conditions is 14 days and, thus, it appears banding can be accurately used for prediction of male flight. Peak male flight occurred between 8/1 and 8/9 and was complete by 8/21. This is a 27 day flight period, which is within the range that has been reported in the literature. The largest native capture (8/6) was greater than 13% of the season long total. Overflooding ratios during peak male flight, as would be expected, decreased. Table 2 presents capture data for all traps in the sterile male release area and capture ratios for the whole area. Also included in Table 2 are capture ratios for 6 control traps in the inner trap zone (ITZ) that consistently captured feral males. Sterile:fertile ratios in the ITZ were lower but parallel with those for the release area as a whole.

If male trap captures can be used as indicators of male mating success, either set of calculated sterile:feral ratios (Table 2) were too low at peak flight to provide any great measure of population suppression. Several reasons for these low ratios are apparent. Ninety feral male were trapped in 1979 as compared to a total of 274 in 1980 (272 males inside the release area 2 males adjacent to the release area). This apparent increase in native density depressed the potential overflooding ratios by a factor of 3 e.g. sterile:fertile ratios would have been 3 times larger if the native density remained constant. Perhaps more importantly, only 10,841 sterile males were captured during the release period. This 3 percent recovery rate is much lower than was expected. Earlier field tests, designed to estimate efficiency of trap arrays, resulted in 9.1% male recovery for a similar trap spacing. A 9% recovery rate is probably a conservative estimate of the actual recovery rate when trapping a native, more randomly distributed, population (see Schwalbe - GM 7.2.1 April 1 - September 30, 1978).

Table 2. Sterile and feral male trap captures from traps inside the release area (Traps 4-17).

DATE	7/15-7/23	7/25	7/26	7/28	7/29	7/30	7/31	8/1	8/3	8/4	8/5	8/6
No. Sterile Males Captured	$\bar{x}$=213.3	140	194	309	296	175	342	427	274	226	284	503
No. Feral Males Captured	0	1	0	0	3	1	11	17	20	29	13	36
S:F Ratio (Rounded to whole numbers)		140:1	194:0	309:0	99:1	175:1	31:1	25:1	14:1	8:1	22:1	14:1
S:F Ratio for trap ITZ area only					63:1	113:1	23:1	18:1	9:1	7:1	17:1	13:1

DATE	8/7	8/8	8/9	8/10	8/11	8/12	8/13	8/14	8/15	8/16	8/17	8/18
No. Sterile Males Captured	567	422	455	186	245	179	95	153	141	146	50	179
No. Feral Males Captured	32	18	33	9	16	12	8	4	1	1	1	1
S:F Ratio (Rounded to whole numbers)	18:1	23:4	14:1	21:1	15:1	15:1	12:1	38:1	141:1	146:1	50:1	179:1
S:F Ratio for trap ITZ area only	15:1	20:1	13:1	16:1	14:1	7:1	7:1	29:1	100:1	105:1	31:0	151:1

DATE	8/19	8/20	8/21	8/22-8/25	8/26-9/2
No. Sterile Males Captured	177	450	514	$\bar{x}$-360.25	Σ=141
No. Feral Males Captured	2	2	1	0	0
S:F Ratio (Rounded to whole numbers)	89:1	225:1	514:1		
S:F Ratio for trap ITZ area only	125:1	176:1	306:1		

The low recovery rate in Benton Harbor may be due to many factors, a few of which became very obvious to us during the course of the field season. Bird predation appeared to exert more pressure on released males this summer than in any past field release. Although not quantified, bird predation at release sites, trapping sites, and throughout the sterile male release area was easily and constantly observed. Although the effort which was made to decrease predation at release sites (i.e. addition of fine plastic-screening to the outside of release cylinders) may have decreased predation at those sites, it did not eliminate predation throughout the release area. Interestingly, recovery rates in field trials near the Otis laboratory this past year were dramatically depressed. Again bird predation was extremely obvious.

Yellowjacket (Vespula maculifrons) predation was also observed at release sites. Predation by Vespids was only observed once in the past, during a short period in 1977. Although an attempt was made to quantify this predation, a good estimate is not available on how greatly this depressed the sterile male population. Typically, yellowjackets would enter a release container and prey on males whose wings were not fully dry or hardened. Signs of predation were easily noticed even when yellowjackets were not present. Typically, a yellowjacket would go from male to male excising the head from the thorax. Several decapitated males were usually found at release sites where this was a problem.

Generally, bird predation appears to exert a greater influence on the sterile male density than that by yellowjackets. Pressure from birds appeared to be high throughout the release area, whereas pressure from Vespids appeared to be localised. Of the two, bird predation appears to be a more complex problem and one which should be given increased attention.

Undoubtedly other factors decreased the sterile male population. Several violent storms occurred during the field season resulting in battered and wet pupae in release containers, which reduced percent eclosion. The cylindrical release containers, provisioned with 600 pupae, dryed slower after a rain than the modified milk carton and may have had poor eclosion rates from them. Cool wet weather also affected eclosion rates and a 6 day rotation during these periods may not have been long enough.

Asynchrony of sterile laboratory-reared male and feral male activity was demonstrated in the Massachusetts field trials and actograph work; this pilot study, however, has not provided enough detailed periodicity of flight information to corroborate those findings.

Hatch determinations from egg masses of monitor females will provide an assessment of male mating success. Perhaps the most conclusive data will be collected from determinations of native female egg masses. Egg mass determinations should be completed by April, 1981 and will be included in the next annual report.

Project Number: GM 0.2.8A
Project Title: Factors Affecting Sexual Periodicity in Male Gypsy Moths - Actograph Studies
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: D.R. Lance, V.C. Mastro, T.M. ODell and M.D. Shorb

Introductiton

To effectively compete with feral males, laboratory-reared sterile males must (1) be active during the period of the day when feral females are receptive, and (2) be in synchrony with feral males or, if asynchronous, begin activity slightly before feral males. In field tests, laboratory-reared male gypsy moths (NJSS) are recaptured at pheromone sources in proportions equal to or greater than males which were field-collected as pupae; however, peak recapture of laboratory-reared males occurs later in the day than peak recapture of wild-type males (see Fig. 1). To identify the causes for this difference in periodicity, an actograph (activity monitor) was constructed and is being used to investigate the effects of photoperiod and temperature on the activity rhythms of adult male gypsy moths.

Materials and Methods

In a 2.3 x 3 M sound-dampened room, 10 cages have been set up for continuously monitoring the activity of male moths. Each cage consists of a 7.5 cm radius clear plastic hemispherical dome which is enclosed, at the bottom, by a 4mm mesh circular plastic "dish" (7.5 cm radius by 3 cm deep). The cages are suspended on rubber bands from a frame of 2 cm diameter PVC pipe.

A phonograph needle (ceramic cartridge) rests on the top center of each dome. Signals created by vibrations within the dome (e.g. from wing-fanning or flight) are picked up by the needle and transmitted to an electronic compiler, which accumulates total activity readings for each cage, over a period of preset length (5 min to 1 hr). When a period of activity accumulation is over, the amounts of accumulated activity are printed out by a strip-chart recorder in a series of 10 bars, one each for cages 1-10; the height of each bar is proportional to the amount of activity which occurred during the period. Also at this time, the compiler resets to zero and begins accumulating activity readings for the next period.

When a test is run, 5 newly eclosed males are placed in each actograph cage. The room is then sealed off for at least 64 hours (to monitor activity of moths at 0, 1 and 2 days old). After a test, bar heights for each cage are measured to determine the amount of activity during each time period (normally 1 hour). Each hour's data are then transformed from total hourly activity into the percentage of total daily activity which occurred within that hour. This is done to compensate for possible differences in sensitivity among cages and factors such as variation among treatments in the size of moths (e.g. when comparing wild-type vs. laboratory-reared moths).

All of the laboratory-reared males which were used in these tests came from the NJSS strain and were reared on the B4 production diet. Unless otherwise specified, all laboratory-reared males were maintained throughout development under standard laboratory rearing conditions (or SLRC; at 25° C, 0500 to 2100 hrs Pp). Wild-type males were collected, as pupae, from an infestation in Dighton, MA, and were held, until adult eclosion, in an outdoor insectary adjacent to the Otis facility.

Up to this time, a large number of tests have been run to identify the factors which affect the activity rhythms of adult male gypsy moths. The bulk of these tests fall into one of the following areas:

1) Activity rhythms of wild-type males vs. laboratory-reared males. Over a series of 4 test periods, 13 cages (= replicates) of laboratory-reared moths and 9 cages of wild-type moths were tested. Also during these tests, 13 replicates of laboratory-reared moths which were transferred to the outdoor insectary as "spins" (prepupae) and held in the insectary through adult eclosion, were tested. In previous field tests, when laboratory-reared male pupae were held in the outdoor insectary, the resulting adults showed a periodicity of trap capture similar to that of wild-type males.

2) Activity as related to photoperiod during the test. The activity of laboratory-reared males was tested in continuous light (6 cages) and continuous dark (7 cages). In both tests, the moths were placed in the cages during the photophase (ca. 1600 hrs). For the continuous dark test, lights were shut off at 2100 hrs.

In a second series of tests, laboratory-reared males were tested in continuous darkness, but the scotophase was started at various times, i.e. 1200 (8 cages), 1800 (4 cages) and 2400 hrs (8 cages) on the day of eclosion, and 0600 (5 cages) and 1200 (5 cages) on the following eclosion.

3) The effect of photoperiod during development. Larvae were maintained at SLRC until 21 days post-infest and then transferred to one of the following photoperiods:

1) 0800 to 2400 hrs (5 cages)
2) 0500 to 2400 hrs (4 cages)
3) Continuous light (3 cages)
4) 0500 to 2100 (standard rearing conditions) (4 cages)

The moths were held under these conditions (all at 25° C) through adult eclosion. Adults were placed in the actograph cages, where they were exposed to continuous dark starting at 1800 hrs on the day of eclosion.

4) The effect of pupal holding temperature on adult activity. In some lepidopterans, the temperature at which teneral adults are held during maturation has been shown to affect the subsequent activity rhythms of the mature adults. As gypsy moths are sexually mature (or nearly so) upon eclosion, studies were undertaken to examine the relationship between pupal holding temperature and adult periodicity.

Laboratory males were placed, as pre-pupae, into 1 of the 4 following temperature regimes:

1) 20° C
2) 25° C (standard rearing conditions)
3) 30° C
4) Fluctuating temperature (a high of 25° C in the early afternoon, with a gradual decrease to 12° C in the early morning and a gradual increase back to 25° C near 1200 hrs). Males were held at these temperatures until adult eclosion (Pp: 0500 to 2100 hrs in all cases). All tests were run at 27° C with a 0500 to 2100 hrs Pp.

5) The effects of packaging, shipping and marking procedures on adult male activity (not reported here). In field tests, most recapture of released males occurs on the day following eclosion (= day 1); therefore, it is critical that released male activity by synchronous with feral male activity on that day. Accordingly, the discussion of actograph findings will concentrate on day 1 of adult life.

Results

1) Laboratory-reared vs. wild type-moths

Results of actograph tests comparing laboratory-reared vs. wild-type males are presented in Fig. 2. In these tests, the activity peak for wild-type males occurred ca. 2 hours ahead of the activity peak for males which were reared and eclosed in the laboratory. However, the periodicity of laboratory-reared males which were placed in an outdoor insectary as prepupae, was similar to that of wild-type males. Two important findings, then, came from these tests:

a) Differences in periodicities of the activity rhythms of wild-type males vs. laboratory-reared males can be demonstrated in laboratory tests, and are similar to differences in periodicities of trap captures observed in field tests (compare Fig. 1).

b) Holding conditions during the pupal stage can affect the periodicity of adult activity rhythms.

2) Activity as related to photoperiod during the test

When males were exposed to continuous light, they showed no apparent activity rhythms. Males which were exposed to a "lights off" showed a circadian activity rhythm (Fig. 3) that continued for over 48 hours despite the lack of further extrinsic photoperiod cues. When the time of "lights off" was varied, the time of peak activity varied accordingly (Fig. 4), generally occurring ca. 19-20 hours after the males were exposed to "lights off". The initiation of the scotophase appears to be the stimulus which sets the phase of circadian activity rhythms in male gypsy moths. However, when "lights out" was moved to only several hours away from what would be a "normal" lights out (i.e., 2100 hrs), the resulting shift in the activity peak appeared to be less than the shift in the time of lights out (i.e., the peaks appeared to be somewhat clumped around the moths "normal" activity period - compare Figure 3, solid line). This apparent clumping may be simply the result of experimental error, or it may be caused by photoperiod entrainment which occurred during the larval or pupal stage.

3) Activity as related to the photoperiod during development

No differences in male periodicity were apparent among the 4 photoperiod treatment groups (Fig. 5). In these tests, the photoperiod to which male gypsy moth pupae are exposed had no discernable effects on the periodicity of adult activity.

4) The effects of pupal holding

The effects of pupal holding temperature on the periodicity of adult activity rhythms are shown in Fig. 6. The activity of moths which were held at cooler temperatures (20° C or 12° C 25° C) peaks earlier than the activity of moths maintained at 25° C; the difference is similar to that observed in tests comparing wild-type vs. laboratory-reared males (see Figure 2). Similarly, the activity of moths held, as pupae, at 30° C peaks later than that of moths held at 25° C. Thus, the cooler the temperature in which male pupae are maintained, the earlier the peak of activity. Pending confirmation in field studies, the periodicity of activity rhythms in laboratory-reared males can apparently be adjusted, by manipulating pupal holding temperature, to match that of feral males. Studies are currently underway to determine whether a temperature-mediated shift in periodicity can be achieved by placing pupae (or pre-pupae) into cooler temperatures for only a short length of time (e.g. 2 or 3 days. As cooler temperatures significantly lengthen the pupal stage (which would decrease the capacity of the rearing facility by increasing the space X time required to rear a moth), finding such a window would save time and space in an operational SMT program for the gypsy moth.

Conclusions

1) The actograph can be used to detect differences in periodicities of activity among gypsy moth males from different strains or treatments.

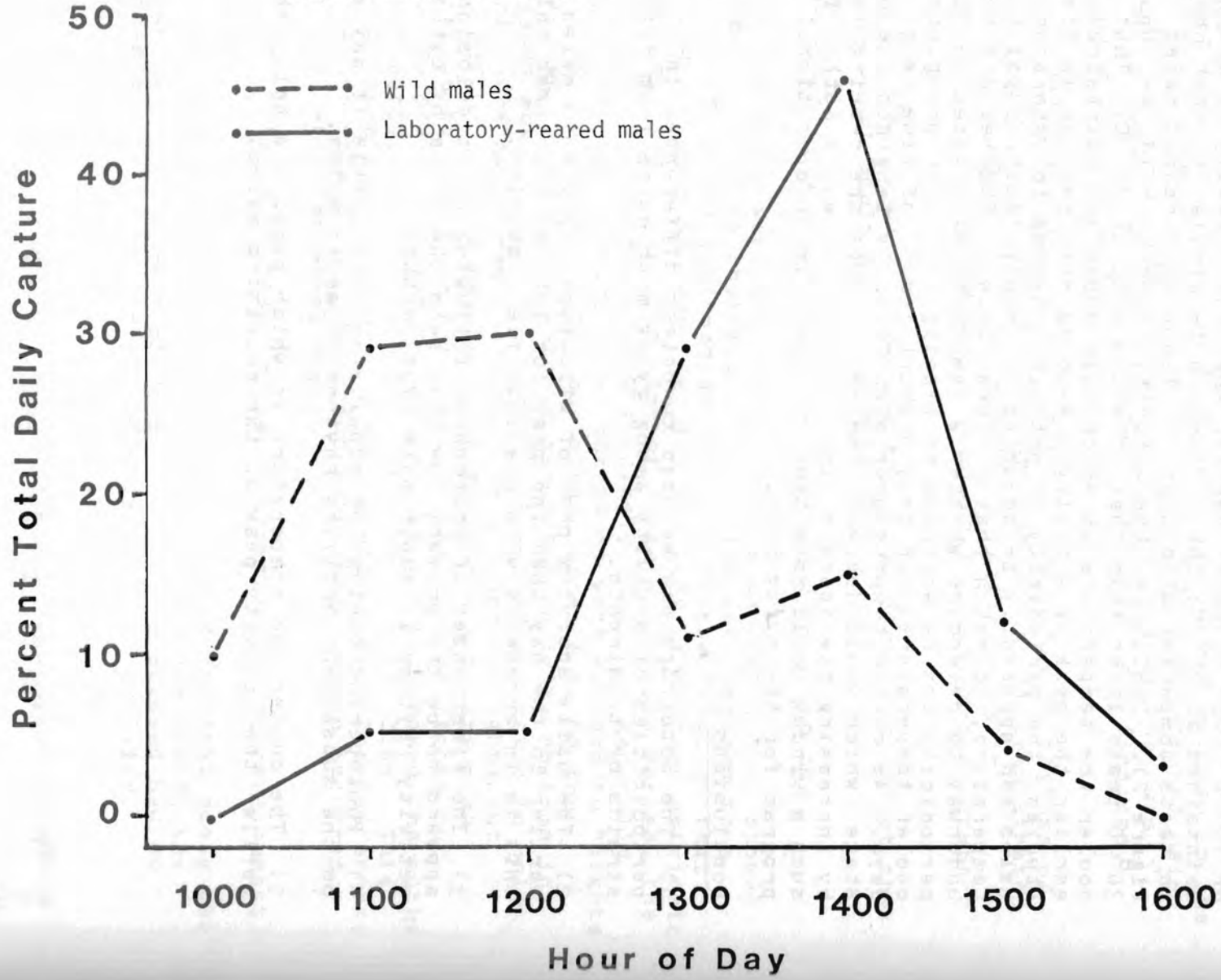
2) The daily activity peak of wild-type gypsy moth males occurs earlier in the day than the peak for laboratory-reared males; this is in agreement with previous field studies.

3) The first onset of scotophase following adult eclosion appears to be the primary cue that sets the phase of circadian activity rhythms in adult male gypsy moths.

4) Photoperiod during the pupal stage has little if any effect on the subsequent activity rhythms of adult males.

5) The cooler the temperature at which pupae are held, the earlier the activity peak of the resulting males.

Figure 1. Hourly percentages of the total daily capture of adult male gypsy moths (laboratory-reared vs. wild) which were caught in (+) disparlure baited traps. From 1977 data.



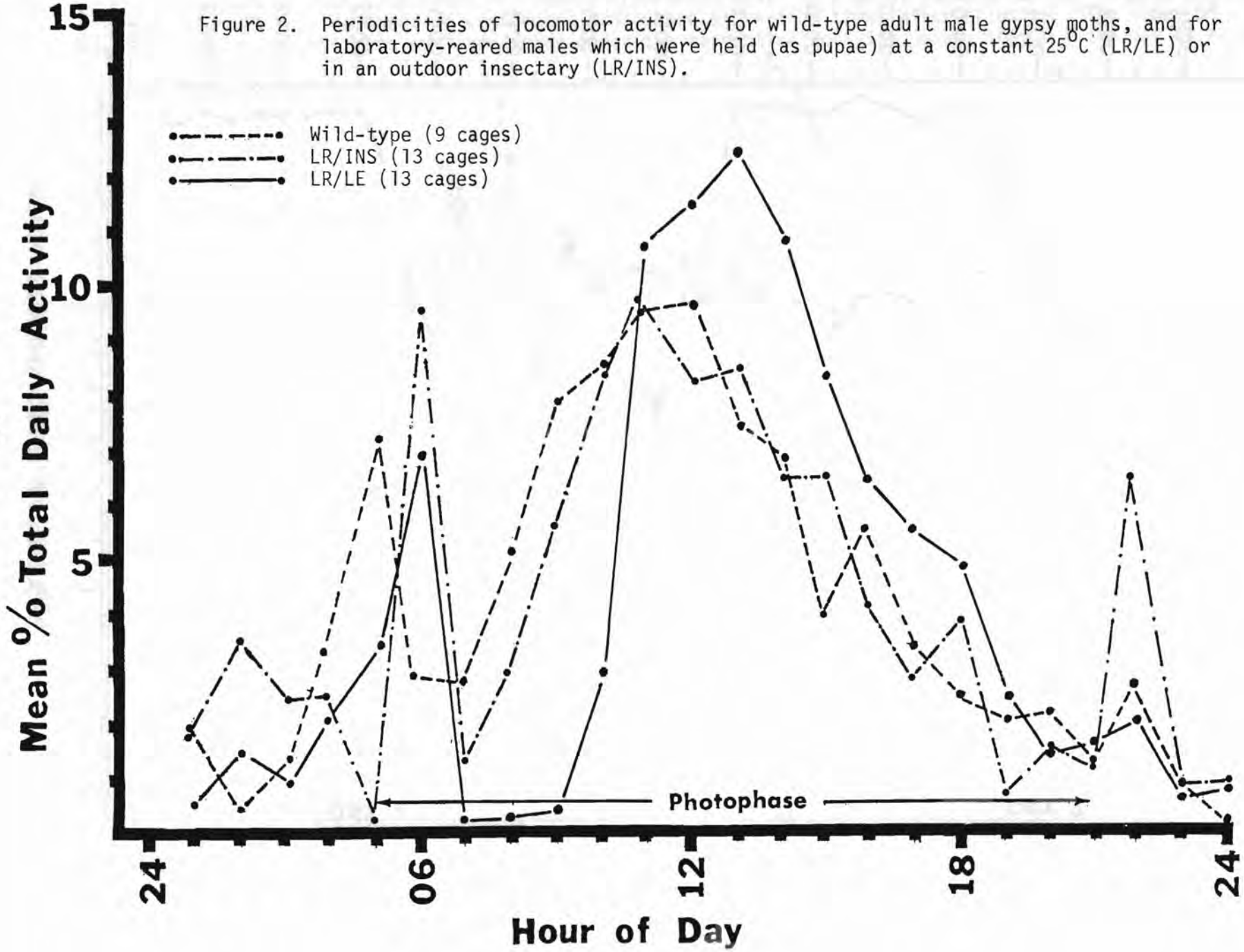
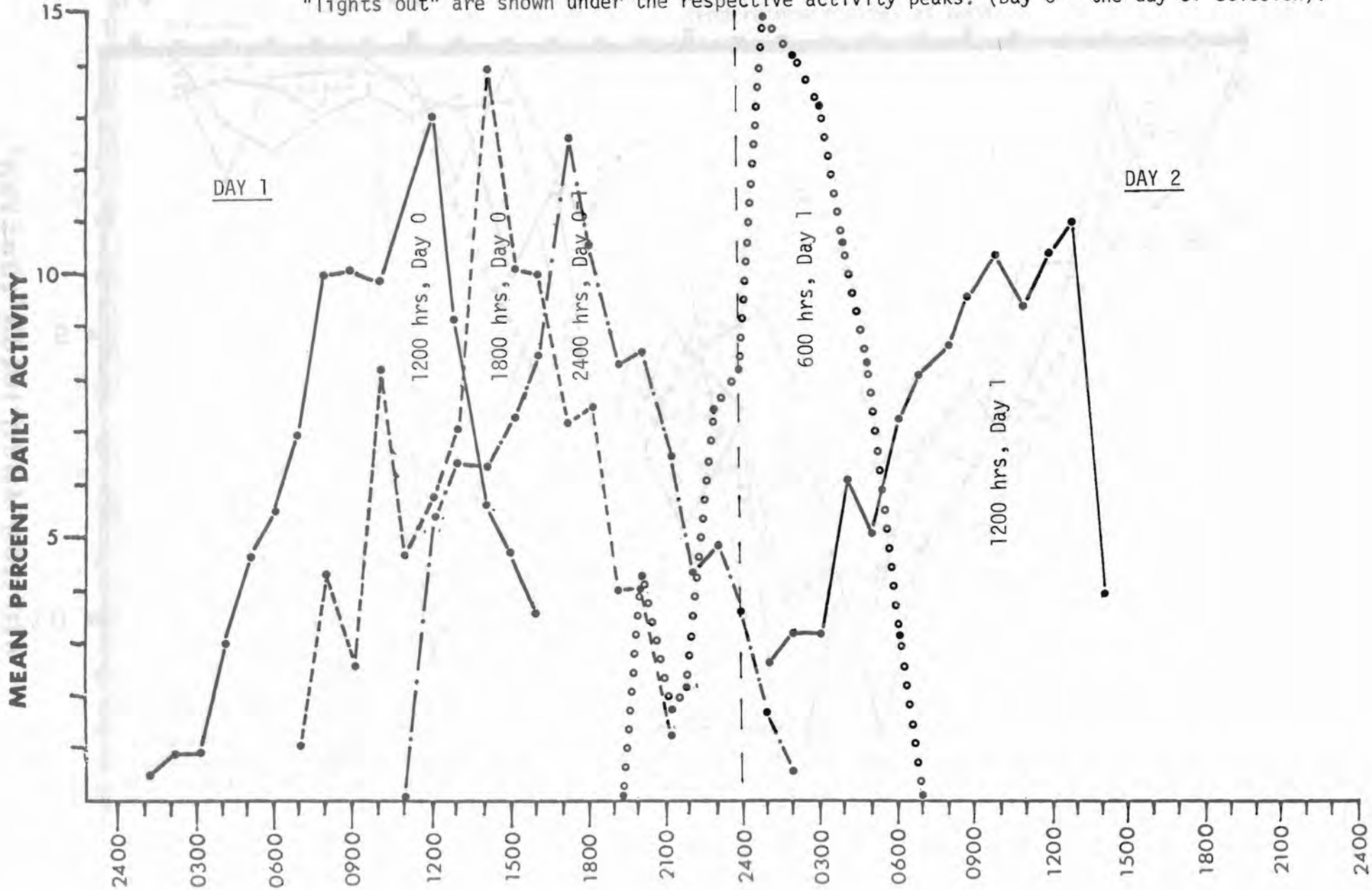


Figure 2. Periodicities of locomotor activity for wild-type adult male gypsy moths, and for laboratory-reared males which were held (as pupae) at a constant 25°C (LR/LE) or in an outdoor insectary (LR/INS).

Figure 3. Periodicities of activity for laboratory-reared adult male gypsy moths 1) under continuous light (broken line), and 2) under continuous dark (solid line). For the continuous scotophase, "light out" occurred at 2100 hrs on the day of eclosion (day 0).

Figure 4. Activity peaks for laboratory-reared adult male gypsy moths which were exposed to the onset of continuous scotophase at various times after eclosion. The times of "lights out" are shown under the respective activity peaks. (Day 0 = the day of eclosion).



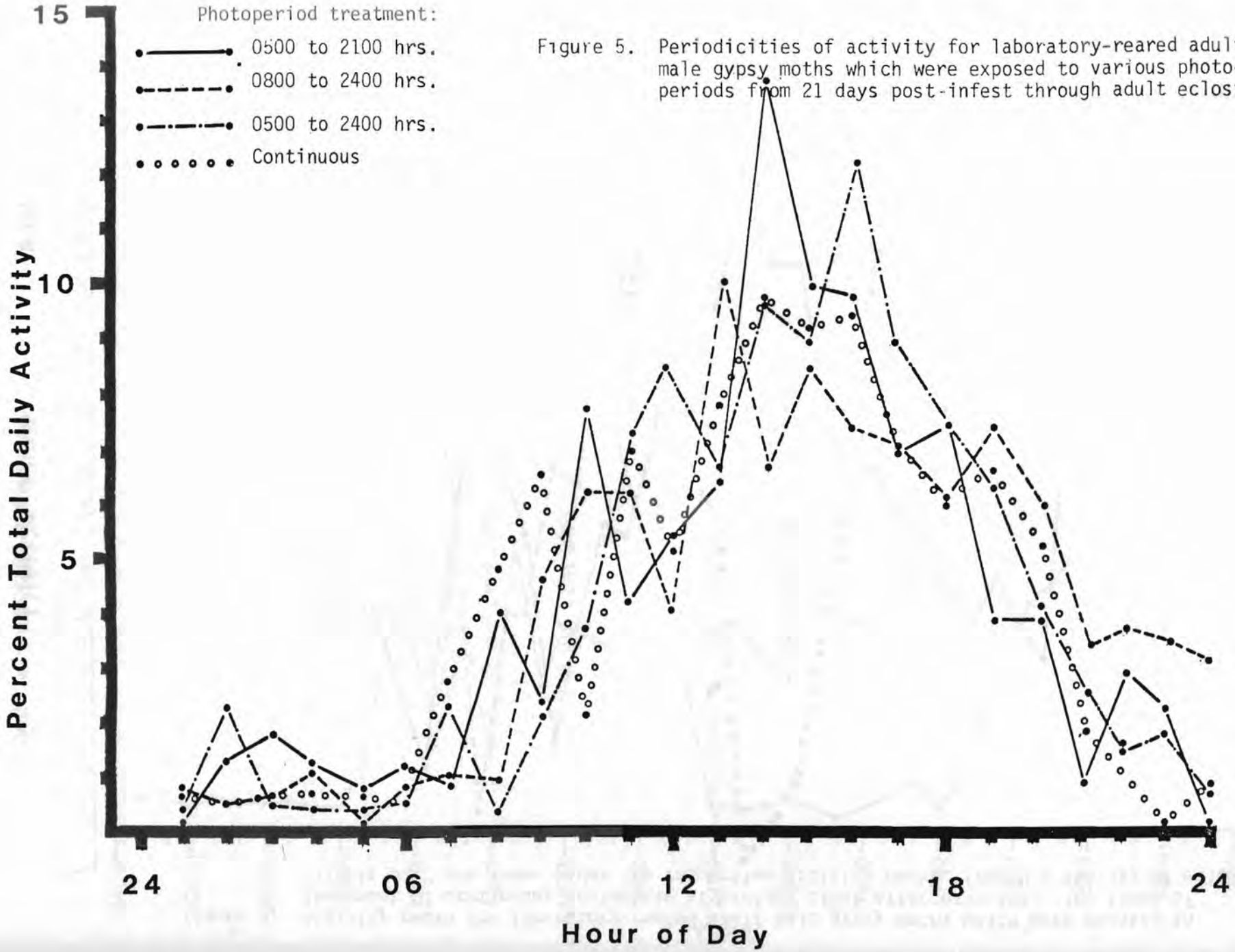


Figure 5. Periodicities of activity for laboratory-reared adult male gypsy moths which were exposed to various photoperiods from 21 days post-infest through adult eclosion.

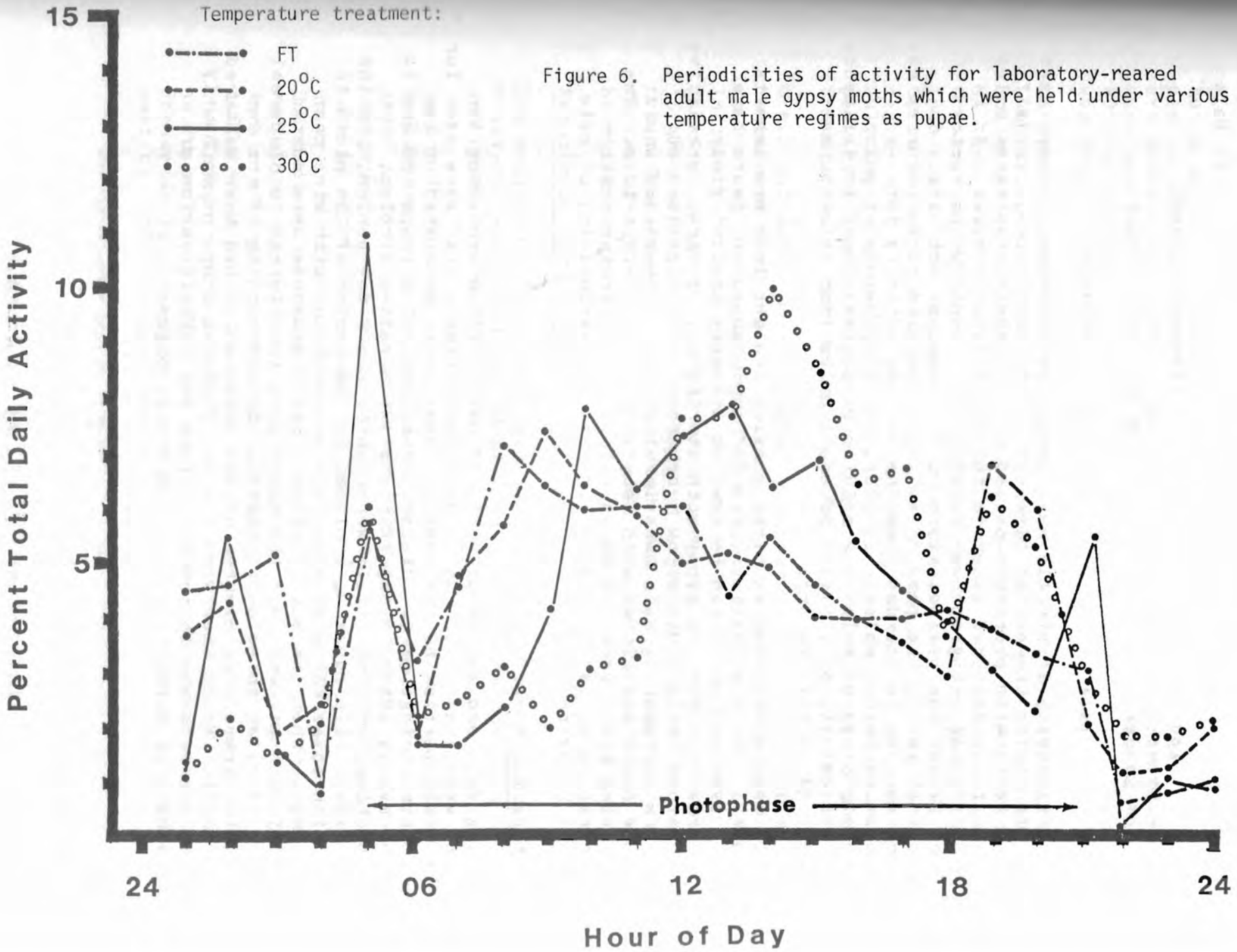


Figure 6. Periodicities of activity for laboratory-reared adult male gypsy moths which were held under various temperature regimes as pupae.

Project Number: GM 0.2.8B
Project Title: Factors Affecting Sexual Periodicity in Male Gypsy Moths
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: D.R. Lance, V.C. Mastro, T.M. ODell

Section B: Relating actograph data to field data

The periodicity of sterile male sexual behavior may be important in determining the competitiveness of released males. In 1977 field tests with pheromone-baited traps, laboratory-reared males tended to be captured later in the day than wild males. In 1978-1979, an actograph was developed to identify the factors which affect the periodicity of male locomotor activity. In actograph tests, the peak of activity for wild males occurred ca. 2 hrs earlier in the day than the peak of activity for laboratory-reared males. Further, the temperature at which laboratory-reared males are held as pupae, was found to influence the periodicity of activity of adult males (for details, see GM 0.2.8A in this report).

While laboratory studies often provide insight into how insects behave in field situations, few (if any) laboratory tests have been developed which can be used to directly predict field performance. For the gypsy moth sterile male program, predictive laboratory tests would allow year around investigations on factors influencing male behavioral competitiveness and would reduce costs associated with labor-intensive field trials. The following tests were run as an attempt to determine whether actograph data can be used to predict the periodicity of male sexual behavior.

Methods and Materials

During July and August of 1980, 17 paired field and actograph tests were conducted. Circular "competition plots" were used for the field portion of the tests. These plots consisted of two concentric rings of (+) disparlure-baited delta traps (22 and 44 traps evenly spaced along 70 m and 140 m radius circles, respectively). The trials were run over a 2 day period. On the first day, male pupae were placed in the center of the plots in eclosion boxes (60 x 60 x 30 cm h wooden boxes with wire mesh bottoms). When the moths eclosed, their antennae were dusted with Dayglo pigment, and the males were transferred to dispersal boxes (similar to eclosion boxes). On the morning of the 2nd day, the traps were cleared of any moths which had been captured overnight. Moths then were removed from the traps on an hourly basis and returned to the laboratory for identification of treatment or strain (by color of Dayglo pigment).

Adult males for actograph tests, were collected from the field plot eclosion boxes. The actograph and actograph test procedures are described in GM 0.2.5A in this report. For this study, tests were started at 2100 hrs on the day of eclosion and concluded at 2000 hrs on the following day. The photophase in the actograph was 0500 hrs to 2100 hrs EDT.

In total, tests were run using wild males from 3 locations and laboratory-reared males from 7 pupal treatments. Wild males were collected from beneath burlap bands in Sandwich, MA, Bridgewater, MA, and East Brewster, MA. For 5 of the laboratory-male treatments, NJS males were harvested as prepupae and were placed into one of the following temperature regimes: 20° C, 25° C, 30° C, an outdoor insectary (INS), or a fluctuating temperature regime (FT). The FT regime was designed to simulate an "average" July day (high-28° C; low 17° C). One to two days before the peak of eclosion, pupae were taken to the field plots and transferred to the eclosion boxes.

Other NJS males were internally marked (i.e., reared on a diet containing 0.15 g/l red Calco dye). These males were harvested as 0-4 day old pupae and placed into the 20° C chamber (20° IM/IRR) or the FT chamber (FT/IM/IRR). On the day before the start of eclosion, the internally marked pupae were irradiated at 15 krad and placed into a chilled styrofoam cooler for ca. 18 hrs. The pupae then were placed into field eclosion boxes. This series of treatments was designed to simulate treatment and handling which could be used in a sterile male release program.

Results and Discussion

The analysis of the data from these tests is not yet complete; however, the periodicities of activity and trap capture are shown (for most of the treatments) in Fig. 1-6.

In the field tests, the periodicity of recapture was strongly influenced by ambient temperature. For example, the "control peak" (= the hour in which the highest number of 25° C males was recaptured) occurred as early as 1100 hrs on warmer days and as late as 1500 hrs on cooler days. If periodicity data from several field tests had been combined using EDT as the independent variable for time, a good deal of temperature-related variation would have been included in each treatment's (or strain's) data; this variation could have obscured the differences in periodicity among treatments. As an alternative the independent variable for time was converted (from EDT to hours before or after the control peak. The data which are presented here include only the 13 tests in which at least 16 control (25° C) males were recaptured.

Figs. 1 and 2 show the periodicities of recapture and activity, respectively for tests of 20°, 25°, 30° and FT males. In the field, males from the cooler treatments tended to be recaptured earlier in the day than males from the warmer treatments. The results of the actograph tests (Fig. 2) are similar to those in GM 0.2.5A (except for the FT males - a warmer FT temperature regime was used in the present study). The 20° C males were active early in the morning and remained active into the afternoon; 12±1% of their activity occurred during each hour from 0900 to 1300 hrs. For the 25° C males, activity was low in the early morning and peaked at noon. Similarly, the peak recapture of 20° males occurred 2 hrs ahead of the control peak. For the 30° males the peaks of both activity and trap capture occurred 1 hr after the rspective peaks for the 25° C males.

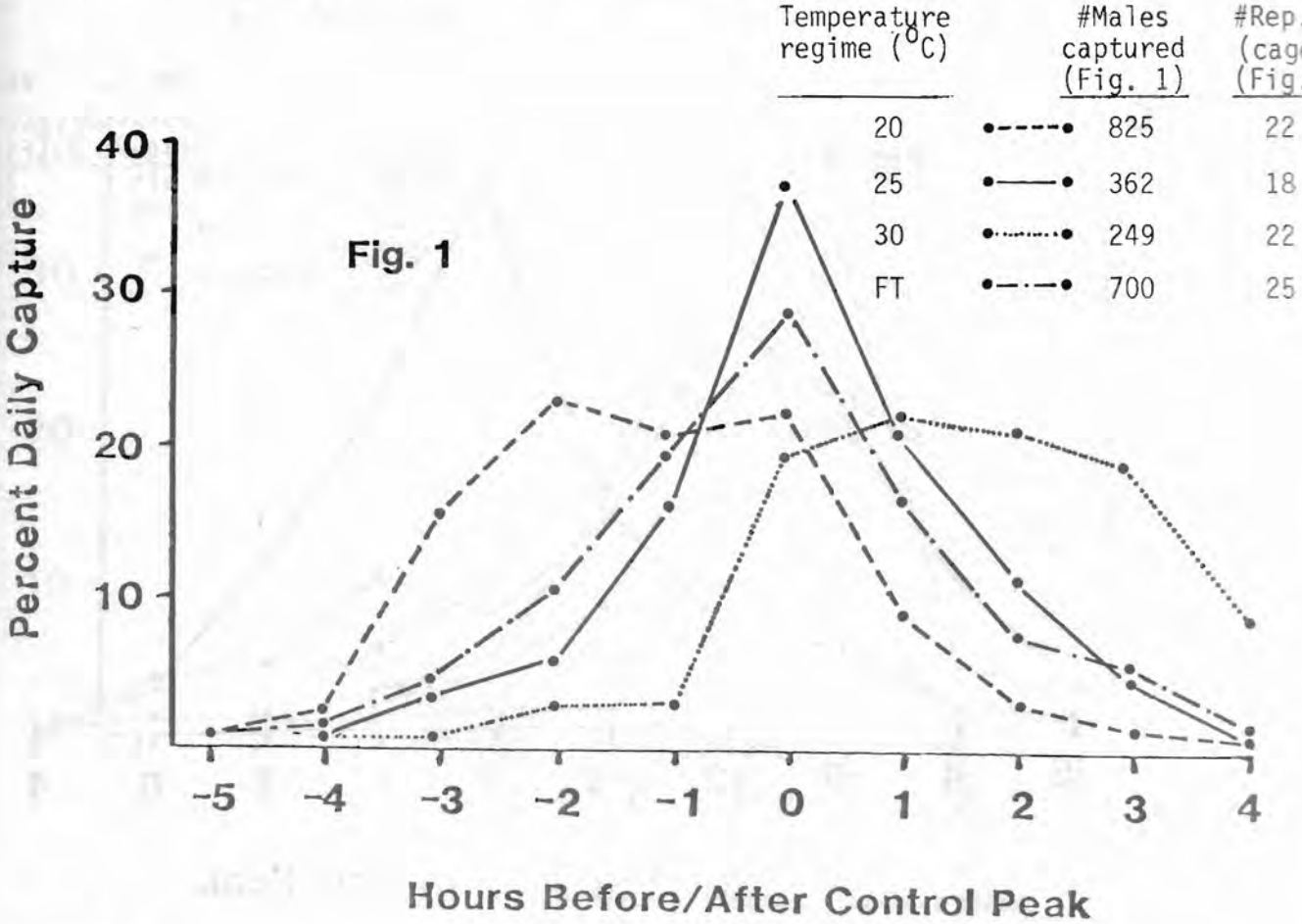
For this report, all wild male data were pooled, and no distinction was made among the geographic strains. As in previous tests, the wild males tended both to be recaptured and active earlier in the day than the control males (Figs. 3 and 4). However, the periodicities of the 20° C males were similar to those of the wild males.

In 1978, INS males showed a periodicity of trap capture similar to that of wild males. This year, the periodicities of INS male recapture and activity were later than those of the wild males and intermediate between those of the 25° and 30° males. However, in 1978, the wild males and INS males were held in the same insectary and were flown concurrently. This season, the wild males were held in a shaded insectary of mosquito netting, and the INS males were held in an insectary which was exposed to sunlight and partially shielded with sheets of polyethylene. Further, the wild males were held in their insectary early in the season (7/5 - 7/19) when the weather was generally cooler than during the holding period for INS males (7/15 - 8/25). Thus, INS pupae were exposed to warmer temperatures than wild pupae. This could have caused the differences in wild male vs. INS male periodicities.

Conclusions

Throughout these tests, if males from 2 treatments had different periodicities of recapture at pheromone sources, then they also had similarly different periodicities of activity in the actograph. Because of this, we are confident that actograph data can be used to predict male sexual periodicity. Further, the sexual periodicity of NJS males can be manipulated by adjusting the temperature at which they are held as pupae. We soon should be capable of producing sterile males whose sexual periodicity is in synchrony with the sexual periodicity of males from target populations.

Figures 1 and 2. Periodicity of capture at pheromone sources in the field (Fig. 1) and periodicity of locomotor activity in the actograph (Fig. 2) for laboratory-reared adult male gypsy moths which were subjected to various temperature regimes as pupae.



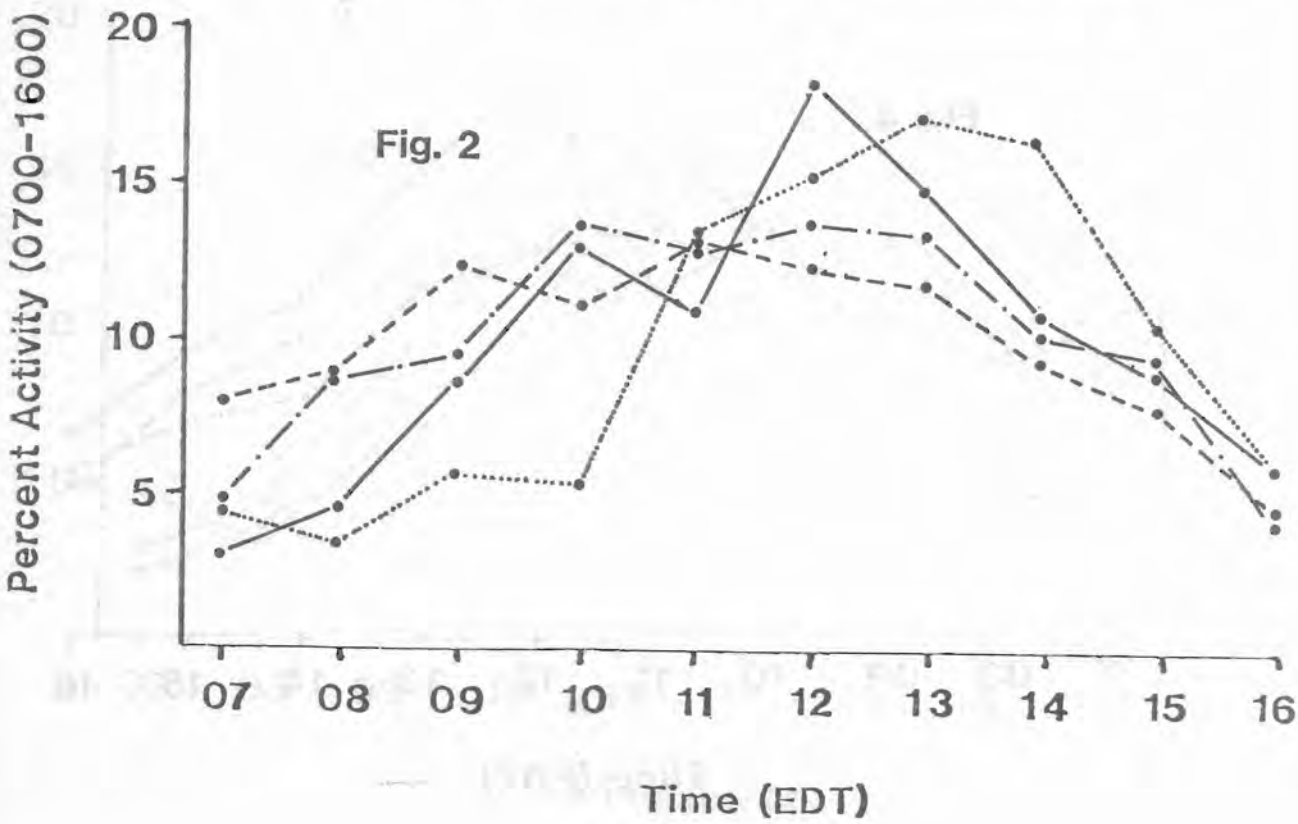
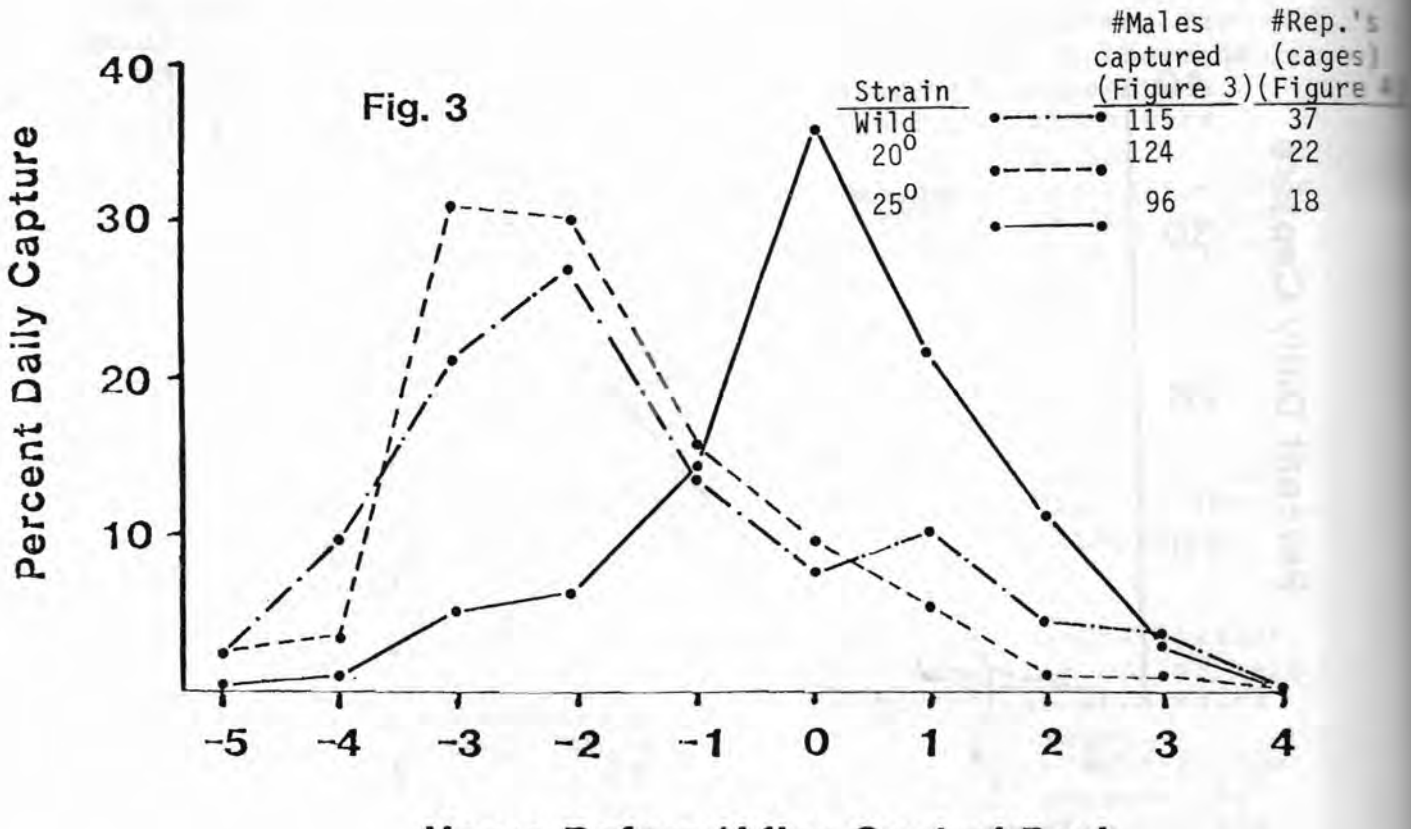
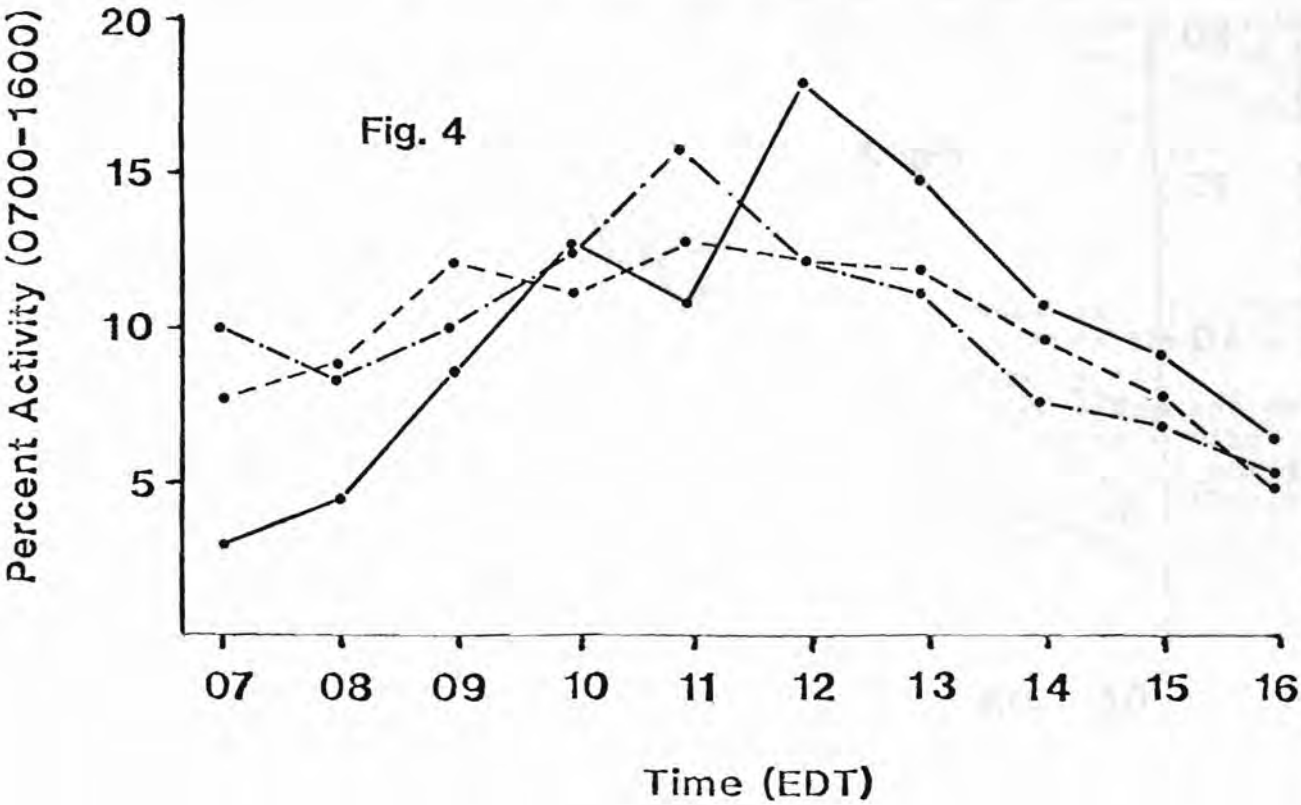
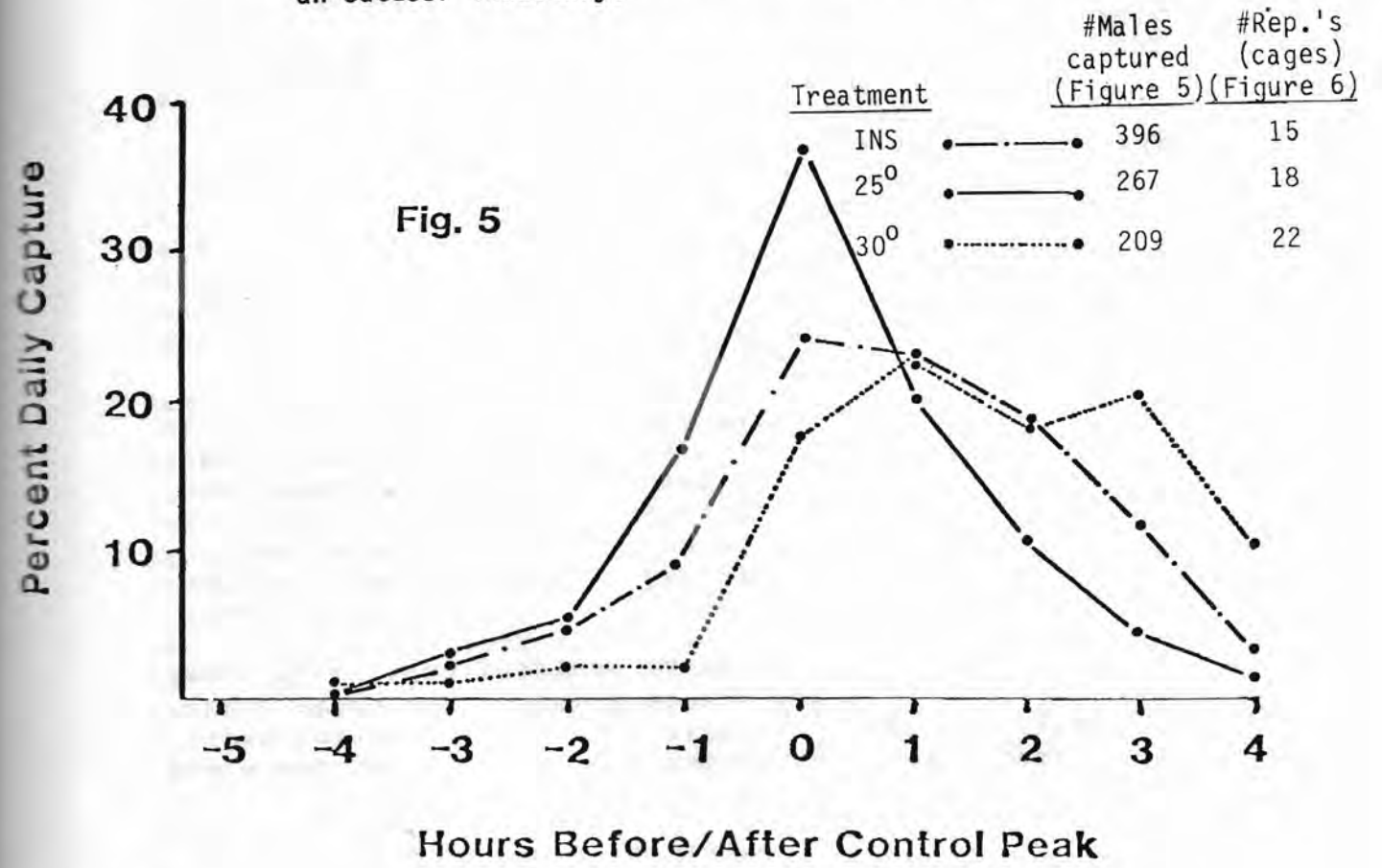


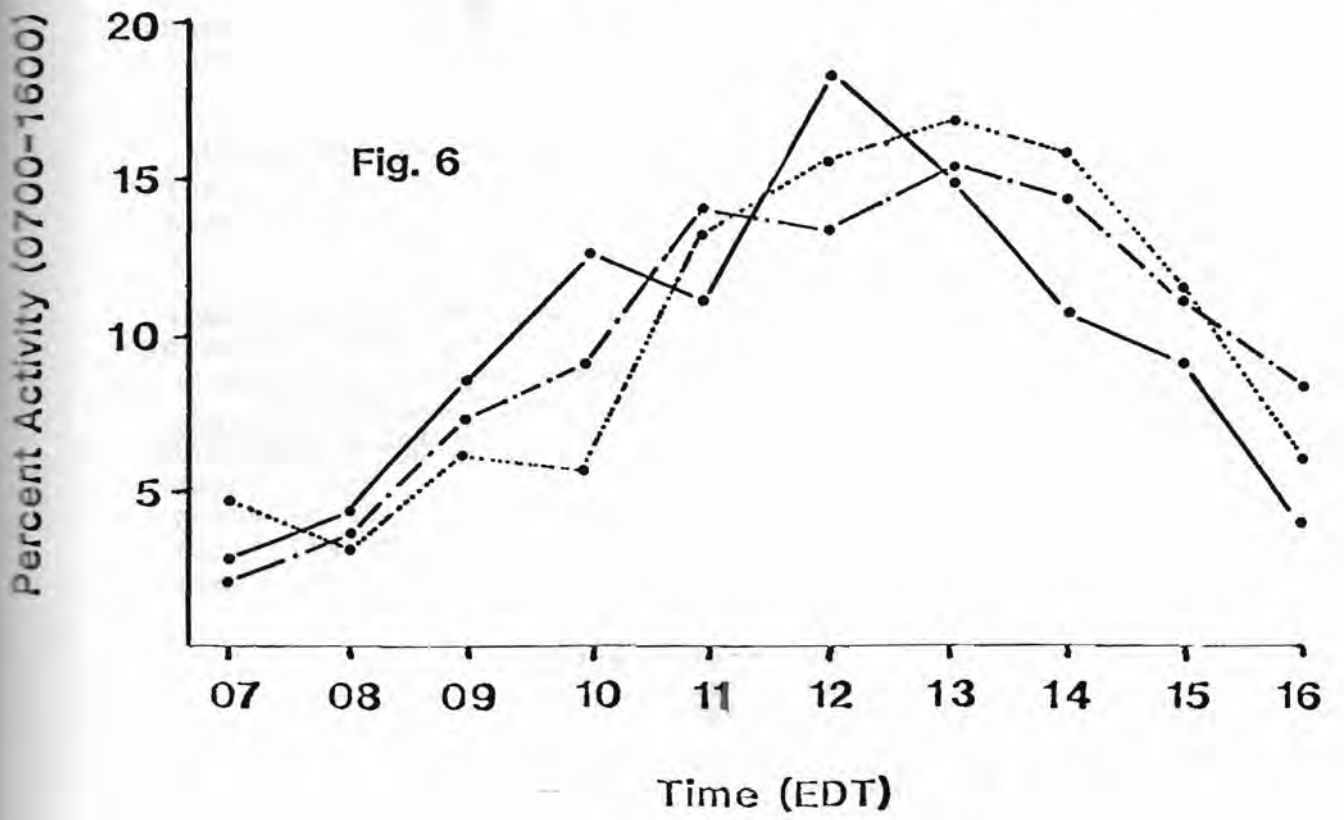
Figure 3 and 4. Periodicity of capture at pheromone sources in the field (Figure 3) and periodicity of locomotor activity in the actograph (Figure 4) for wild male gypsy moths vs laboratory-reared males which were held at 20°C or 25°C as pupae.





Figures 5 and 6. Periodicity of capture at pheromone sources in the field (Figure 5) and periodicity of locomotor activity in the actograph (Figure 6) for laboratory-reared adult male gypsy moths which were held (as pupae) at 25°, 30°C, or in an outdoor insectary.





Project Number: GM.0.2.9
Project Title: The Effect of Temperature on the Duration of the Pupal Stage
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: D. R. Lance and V. C. Mastro

Introduction

To produce sterile gypsy moth males which are behaviorally competitive with feral males, it may be necessary to hold laboratory-reared male pupae in a temperature regime which differs from the standard rearing temperature (i.e., 25° C). In actograph studies, daily locomotor activity of field-collected males peaked ca. 2 hours earlier than that of males which were reared under standard laboratory rearing conditions (SLRC); however, activity for males which were held as pupae at cool temperatures (e.g. 20° C) also peaked ca. 2 hours ahead of the peak for males which were held at 25° C throughout development.

A change in pupal-holding temperature would affect, among other aspects of gypsy moth biology, the duration of the pupal stage. As current gypsy moth SMT plans call for the irradiation and shipment of pupae rather than adults, the duration of the pupal stage will affect:

1) The age at which pupae should be irradiated
2) The age at which pupae are shipped
3) The capacity of the rearing facility (in terms of moths/day)

Accordingly, studies were undertaken to determine the relationship between temperature and the duration of the pupal stage.

Materials and Methods

Gypsy moth larvae (NJS) were maintained under standard rearing conditions (25° C, 50-60% RH, 0500 to 2100 hrs Pp) until becoming pre-pupae (spins). They were then harvested from the rearing containers and placed into 480 ml squat cups (50 males/cup). Squat cups were then placed into one of the following temperature regimes:

1) Constant 20° C, 50% RH (75 cups = 3750 moths).
2) Constant 25° C, 60% RH (SLRC); 84 cups = 4200 moths).
3) Constant 30° C, 40% RH (42 cups = 2100 moths).
4) A fluctuating temperature regime with a high of 25° C in the early afternoon, and a low of 12° C in the early morning; 60% RH (47 cups = 2350 moths).

The cups were checked daily, and any males which had eclosed were counted and removed. For each day, the percentage of males which eclosed was computed for each cup, and overall means and standard deviations were computed on a percentage of eclosion per cup per day basis.

In a second series of tests, individuals within a more narrow age class were used. Spins were allowed to pupate, and green pupae (i.e., individuals from 0-3 hours after pupal ecdysis) were held, 50 per 480 ml squat cup. A cup of green pupae was placed in each of the 20° C, 25° C, and fluctuating temperature (FT) chambers. The cups were checked daily, and eclosion was recorded.

Results and Discussion

Figure 1 shows adult eclosion curves for gypsy moth males which were placed, as spins, into the various temperature regimes. For individuals held at 20°, 25°, 30° C, and FT, peak adult eclosion occurred on days 20, 14, 11 and 23, respectively (day 0 = the day on which pupation was expected).

When green pupae were placed into the various temperature regimes (see Fig. 2), the eclosion of males which were held at 20°, 25 C, and FT peaked on days 20, 15 and 22, respectively. The differences in day of peak eclosion in the two tests may have been due to either the small sample size in the green pupae test, or differences in the techniques used. In the test using spins (prepupae), the day of harvest was assumed to be the day before pupation. At 25°, a large proportion of the males may have pupated on the day of harvest rather than on the day after harvest. Similarly, the FT regime may have delayed most of the pupation until 2 days after harvest.

In general, the duration of the pupal period increased as holding temperatures decreased. If cool pupal holding temperatures prove necessary to adjust the periodicity of sterile males, extra space and/or a reduction in rearing capacity may be required to accommodate the insects for the ca. 1 week longer period than they are now held.

Another important consideration is that the temperature-mediated change in the rate of development may differ from tissue to tissue. If so, a linear relationship between the length of the pupal stage and the optimal age range for the irradiation of male pupae would not apply. Accordingly, competitiveness and fertility studies on the effects of pupal irradiation at various times and doses, would have to be redone.

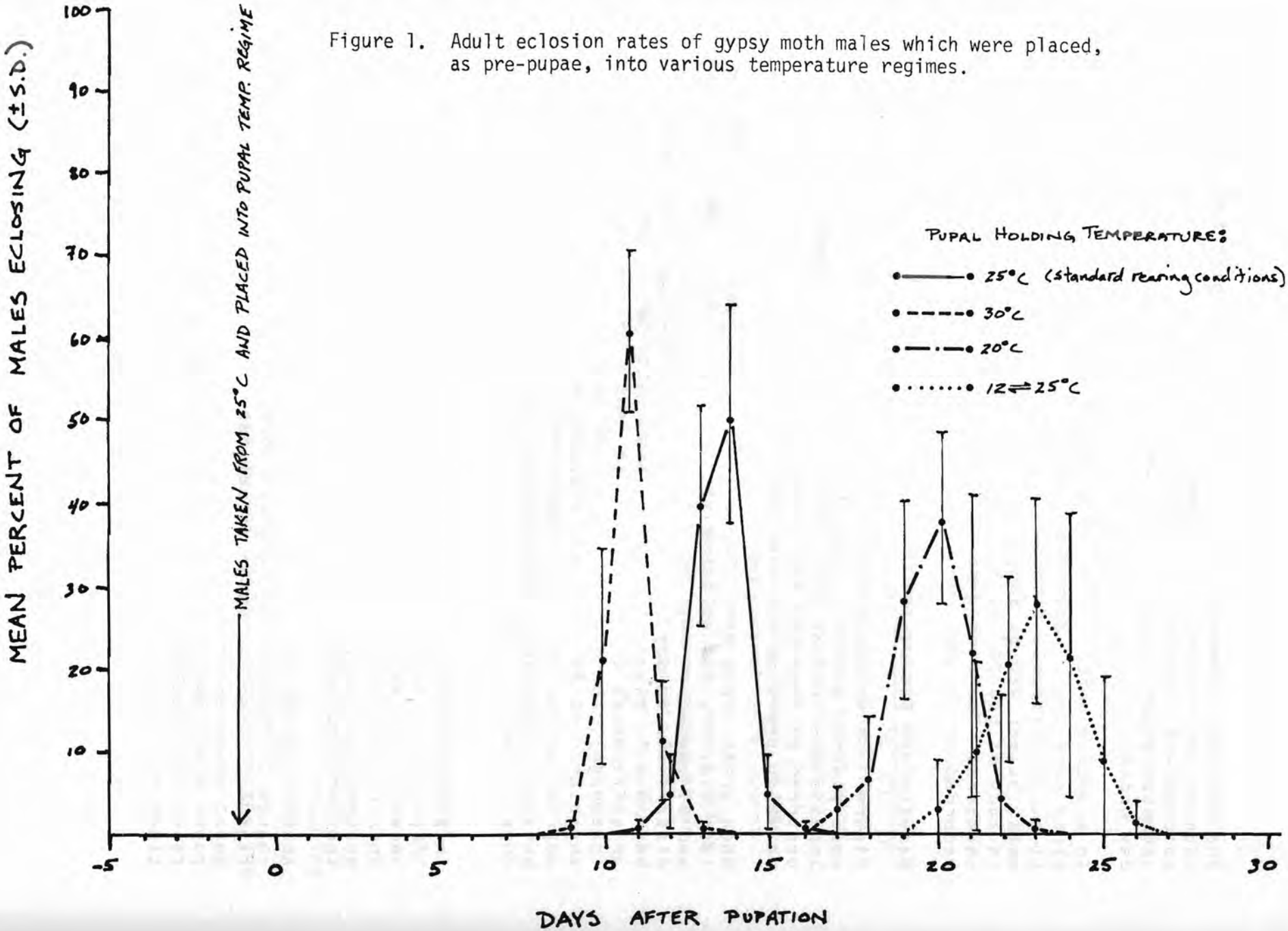
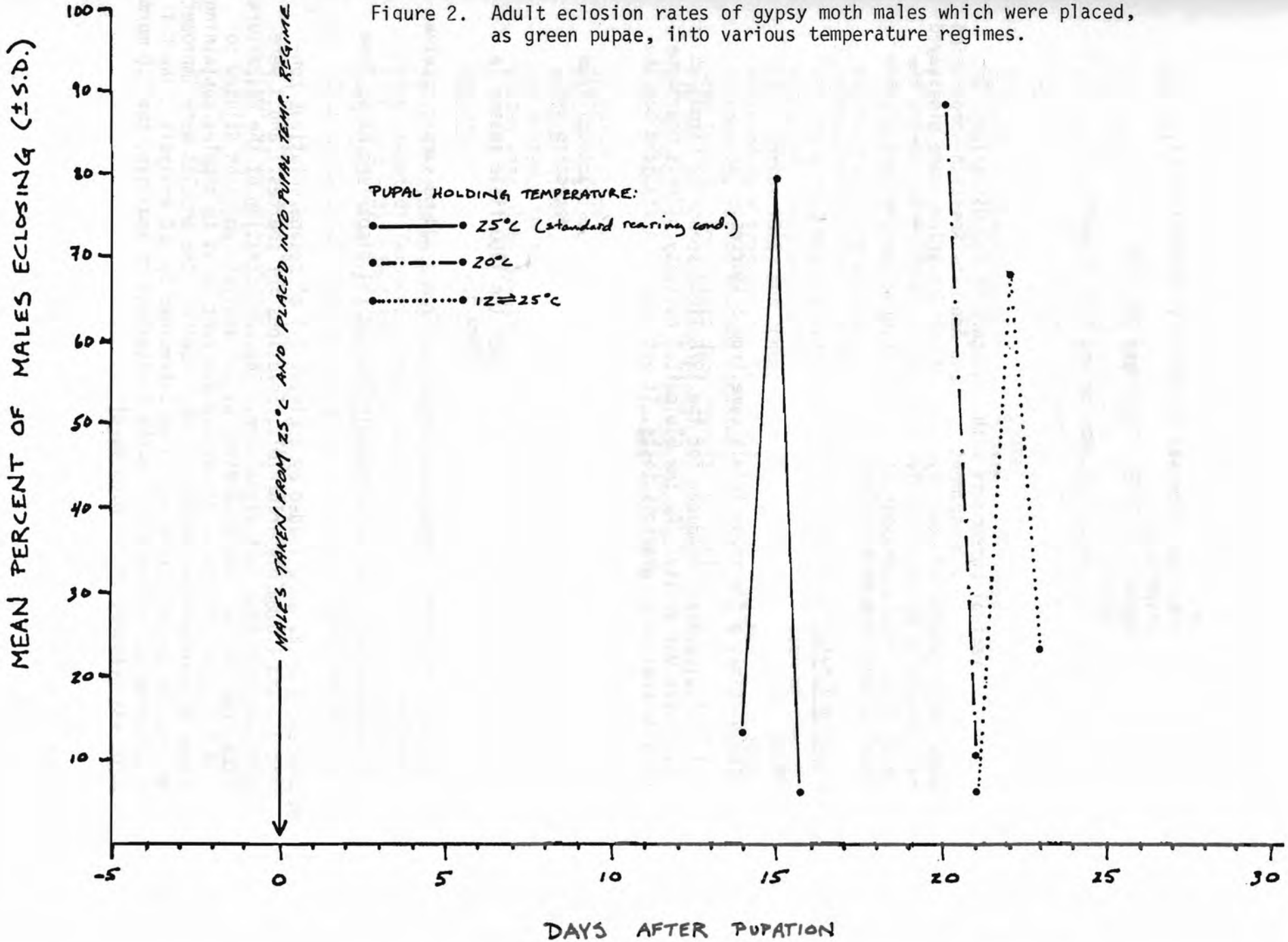


Figure 1. Adult eclosion rates of gypsy moth males which were placed, as pre-pupae, into various temperature regimes.

Figure 2. Adult eclosion rates of gypsy moth males which were placed, as green pupae, into various temperature regimes.



Project Number: GM 0.2.10
Project Title: Preseason Bioassay of Recently Synthesized (+) Disparlure
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: D.R. Lance, V.C. Mastro and C.P. Schwalbe

Introduction

To satisfy contractual agreements with producers of (+) disparlure (Dr. Donald Farnum, Chemistry Department, MSU and Andrulis Research Corporation, Bethesda, MD), samples of newly synthesized (+) disparlure were bioassayed in field trials at Brownsville, Texas in March, 1980. Also, during the course of these tests, possible effects of internal dye markers on male response to pheromone were evaluated.

Materials and Methods

Pheromone

The disparlure for these trials came from 5 sources:

1) (+) disparlure produced for the 1978 field season by Farnum at Michigan State University. As the biological activity of this pheromone had been determined in previous tests, it was used as a standard for the present trials.

2) (+) disparlure which was reclaimed (by hexane extraction) from Hercon wicks which were used in the field for the 1979 detection and delimitation program.

3) (+) disparlure produced by Farnum for the 1980 field season (a pooled sample of 10 µl from each of lots 1 thru 5).

4) (+) disparlure produced by Andrulis, from a 300 mg sample received for testing.

5) Unused Hercon wicks, containing 500 µg (+) disparlure each, from the 1979 field season.

Cotton dental wicks were loaded by applying 0.1 ml hexane solutions containing 0.1 and 1.0 mg disparlure/ml. Solutions for sources 1 and 3 were prepared directly from neat disparlure. The concentration of the disparlure solution from source 2 was determined by GLC analysis and then diluted to 0.1 and 1.0 mg/ml. Andrulis disparlure was sent to us in ampules containing solutions of appropriate concentrations. However, the ampules were improperly sealed and the concentration was later determined by GLC analysis. The 0.1 mg/ml solution was accurate within the limitations of analysis; the 1.0 mg/ml solution was measured at ca. 0.85 mg/ml.

Insects

Male gypsy moths used in the test were reared at the Otis Methods Development Center on the B4 production diet. A portion of the males were marked internally by adding 0.3 g/L of either solvent red Calco dye or blue No. 35 dye (both in 3 ml linseed oil carrier) to the B4 diet. Male pupae were transported, in biomailers, as airline luggage, and were eclosed in the Brownsville Methods Development Center (at night) and in the field (during the day). Before release, undyed males were marked by dusting their antennae with Day-glo powder.

Plot and trial design

In a grapefruit orchard in Brownsville, two concentric rings of Delta traps baited with (+)-disparlure, were placed at 70 m and 140 m from the central release point. The 70 m ring was divided into 3 - 120° arcs, or sectors; the 140 m ring was divided into 6 - 60° sectors. Traps were evenly spaced along the rings, and, within a sector, each combination of pheromone source (e.g. Farnum 1978) and concentration (e.g. 10 or 100 μg) was randomly assigned to a trap point. In Trials 1 and 2, 7 pheromone treatments (Farnum 78, Farnum 80, and reclaimed, each at 10 and 100 μg, and Hercon wicks) were tested. For Trials 3-5, Andrulis disparlure was included as 2 treatments (10 and 100 μg) and Hercon wicks were dropped. Within sectors, the positions of pheromone treatments were rerandomized before each trial, except before Trial 5, when only the 70 m line was rerandomized.

In Trial 5, males marked with internal dyes were tested against undyed males. On the first day of Trial 5, undyed moths were dusted with blue Day-glo pigment (previously released males had been dusted yellow), and dyed males were not dusted. On the second release day, all males, dyed and undyed, were dusted with pink Day-glo powder.

Results

In total, 2788 males were released and 853 (30.6%) were recaptured. No significant differences in moth catches were observed among pheromone sources (Tables 1 - 3). In Trials 1 and 2, collectively, (but not in Trials 3-5), traps containing 100 μg wicks caught significantly ($p < 0.05$) more males (89.5 males/Trial) than traps baited with 10 μg wicks (44.0 males/Trial).

In general, the Farnum 1980 (+) disparlure appears to be of high quality. Traps baited with Andrulis disparlure did not catch significantly fewer moths than traps baited with disparlure from other sources (Table 3); however, Andrulis captures were the lowest, and as Andrulis is a new producer, further testing of their product is warranted.

For males released on the first day (but not the second day) of Trial 5, blue-dyed males were recaptured in a significantly lower proportion than either red-dyed or undyed males (Table 4). The low proportion of recapture of the dyed males which were released on day 1, and the extremely high proportion of dyed day-2 males which were recaptured, leads us to suspect that some of the (undusted) dyed day-1 males may have been "accidentally dusted" while in the traps (they would have been subsequently counted as day 2 males). A high proportion of the recaptured males which were released on day 2 were very heavily dusted. In the traps, some of their Day-glo powder may have been transferred to undusted, dyed males, effectively marking them. Further studies (investigating both dye type and concentration) are required before any conclusions can be made concerning the competitiveness of internally dyed males.

Table 1. Total numbers of laboratory-reared male gypsy moths captured by (+) disparlure-baited Delta traps in a circular plot in Brownsville, Texas (March 1980).

		TRIAL 1		TRIAL 2		TOTAL 3	
Pheromone source	Pheromone on wick (μg)	70m[1]	140m[2]	70m	140m	Treat-ment	Source
Farnum 1978	10	13	2	5	2	22	81
(Standard)	100	33	6	17	3	59	
Farnum 1980	10	16	2	7	4	29	93
	100	25	6	29	4	64	
Reclaimed 1979	10	25	2	6	4	37	93
	100	31	5	18	2	56	

1 3 traps per treatment

2 6 traps per treatment

3 No significant differences, as determined by ANOVA, were found among pheromone sources, however, 10 μg traps caught significantly ($p<0.05$) fewer moths than 100 μg traps.

Table 2. Total numbers of laboratory-reared male gypsy moths captured by (+) disparlure-baited Delta traps in a circular plot in Brownsville, Texas (March 1980).

Pheromone source	Pheromone on wick (μg)	TRIAL 1		TRIAL 2		TOTAL 3
		70m[1]	140m[2]	70m	140m	
Farnum 1978	100	33	6	17	3	59
Farnum 1980	100	25	6	29	4	64
Reclaimed 1979	100	31	5	18	2	56
Hercon wick 1979	50	32	16	18	5	71

1 3 traps per treatment

2 6 traps per treatment

3 ANOVA detected no significant differences in trap captures among pheromone sources.

Table 3. Total numbers of laboratory-reared male gypsy moths captured by (+) disparlure-baited Delta traps in a circular plot in Brownsville, Texas (March 1980).

Pheromone Source	TRIAL 3		TRIAL 4		TRIAL 5		TOTAL	
	70m[1]	140m[2]	70m	140m	70m	140m	Treatment	Source[3]
Farnum 1978	15	12	3	1	9	3	43	119
	19	7	6	4	27	13	76	
Farnum 1980	18	7	1	2	31	5	64	153
	28	4	3	5	40	9	89	
Reclaimed 1979	16	3	3	2	47	7	78	140
	23	6	2	3	27	4	62	
Andrulis	7	8	0	1	7	4	27	104
	23	8	5	4	26	10	77	

1 3 traps per treatment

2 6 traps per treatment

3 No significant differences, as determined by ANOVA, were found among pheromone sources, however, 10 μg traps caught significantly ($p<0.05$) fewer moths than 100 μg traps.

Table 4. Numbers of internally dyed and undyed male gypsy moths which were released, and the proportion which were recaptured in (+) disparlure-baited Delta traps, in a circular plot in Brownsville, Texas (March 1980).

	Moths released on day 1			Moths released on day 2		
Type of dye	No. released	No. recaptured	Percent recapture[1]	No. released	No. recaptured	Percent recapture[1]
Calco red	118	31	26.3 a	51	31	60.8 a
Blue No. 35	85	12	14.1 b	44	24	54.5 a
Undyed	134	47	35.1 a	237	122	51.5 a

1 Within a column, percentages not followed by the same letter represent proportions which are significantly different at the $p<0.05$ level (for analysis, actual numbers, not proportions, were used in multiple uncorrected 2 x 2 contingency table comparisons).

Project Number: GM 0.2.11
Project Title: Mass Trapping of Male Gypsy Moths
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: D. H. Simser, C. P. Schwalbe

Introduction

Grids of gypsy moth traps (Delta or milk carton design) are currently employed to determine the distribution of small, isolated infestations. High densities of traps may capture a large proportion of the male moth population, thereby efficiently removing potential mates of virgin female moths and resulting in a subsequent decrease in population density the following season. This technique of mass trapping male moths may eliminate sparse populations in isolated sites; however, documentation of this potential control measure has not been recorded.

In this post-season study, milk carton traps were arrayed in five grid distributions, ranging from 0 to 25 traps/ha. Daily releases of male moths, and placement and recovery of virgin female moths within these sites resulted in a range of mating of female moths. Trap catch of males and mating reduction of females (numbers of moths mated/numbers recovered) in plots provided evidence that trap density influences male moth capture and female moth mating.

Our objectives were: 1) to ascertain the effectiveness of trap distributions in preventing mating through male moth capture; 2) to evaluate the degree of mating reductiton as influenced by trap distribution and subsequent male moth capture; and 3) to compare the proportion of male moths captured within each trap array.

Methods and Materials

Five study sites were established in early September on or near Otis Air Base, MA. Forest composition of all sites was similar, predominently scrub oak with understory, and sites were separated by at least 1 km to minimize dispersal of released male moths into other study areas. Within each site, a 9 ha treatment plot (1 ha subplots arranged 3x3) was established.

Five trap densities were compared:

Site	# traps/ha	ITD (meters)	Total # traps/plot
A	0	-	0
B	0.75	115.0	16
C	2.5	52.6	36
D	7.5	36.5	100
E	25.0	16.7	225

Prior to their placement, milk carton traps were baited with a Hercon wick containing 500 ug (+) disparlure; a 2" strip of Zodiac Pet Products flea collar (DDVP) was stapled within as a moth killing agent. Traps were suspended ca 1.5 m above ground level, away from obstructive branches or foliage.

In each 1 ha subplot, 10 marked male moths (48 hr old NJF_{20}) were released near the 50 m mid-point by opening the 8 ounce holding container, and placing it on cleared ground, away from direct sunlight. Liberated males dispersed within 30 minutes. Females held singly in 2 ounce plastic cups were allowed to crawl into a wooden bark flap (teepee). After a period of acclimation, a female settled into calling position, and the teepee was then suspended ca 1.5 m from ground level on a tree bole. Two teepees were placed 180° apart, and 25 m from the male release point, in a subplot.

This procedure of release and placement was repeated in each of 9 subplots. Thus, in a replicate, a total of 90 males and 18 females were utilized per treatment. Four consecutive replicates were conducted at 24 hour intervals. During this test period, weather conditions remained uniform, marked by temperatures peaking near 24°C, partly sunny skies, and negligible wind speed (<2mph).

Prior to placement of a "fresh" female, a teepee was examined for a placed female, egg mass, or both. Recoveries were collected, and the teepee returned to another randomly selected tree bole within the proximity. Collections were held in 2 ounce plastic cups, and placed in an environmental chamber (25 ± 3°C, 50% RH, 16:8=L:D) until female mortality or oviposition occurred. All egg masses or spewed eggs were collected and incubated at the above described conditions fpr 21 days, and examined for embryonation. Such eggs appeared darkened, and were readily discriminated from pale, unembryonated eggs. Females which were successful in mating could thus be identified, and their condition recorded.

Following the final female collection, all traps were collected and examined. Captured males were removed and examined by ultraviolet light in the laboratory. Moth color was discernable, and the number of moths captured was recorded.

Results and Discussion

Mating reduction can be achieved through mass trapping only if male moths are effectively removed prior to mating. Increasing the density of traps should likewise increase the probability that a searching male will encounter, and be captured by, a trap prior to female moth encounter.

In this study, the percentage of captured moths increased slightly with increased densities of traps; however, only the 25 traps/ha grid removed more than 50% of the male moth population.

Trap	% Males Captured
0	-
0.75	32.5
2.5	36.1
7.5	41.1
25.0	65.6

Extrapolation of these data indicate that a trap density of 38/ha is necessary to capture 100% of a male moth population.

A moth may encounter a trap, such as a milk carton or other design, and even enter, only to escape and resume searching. Such a phenomenon has been observed, and data are required to determine the efficiency of traps in attracting and capturing male moths. Clearly, the success of mass trapping sparse populations to achieve mating elimination is contingent upon the success of individual traps in capturing male moths.

Factors inherent in a post season release and placement study contribute to a high mortality of moths prior to mate location and mating. These include predation, inclement weather, insect responsiveness, and other causes. Accordingly, a low incidence of mating was expected. Recoveries of placed female moths were high in all plots, and 73% of recovered females in the control plot (0 traps) were mated; thus, male moths are capable of dispersing and locating calling females within a 24 hour period.

Mating was not eliminated by mass trapping in any treatment plot, although a 5 fold mating reduction was apparent in the highest concentration tested (25 traps/ha) versus the control. Corrected percentages of mating were very high, averaging ca 50%, in trap distributions of 7.5//ha or lower.

Trap Density	% Recovered 1/	% Mated 2/	Corrected % Mated 3/
0	92.0	73.0	100
0.75	79.0	45.6	62.4
2.5	65.0	36.2	49.5
7.5	86.0	30.6	41.9
25.0	90.0	13.3	18.2

1/ # of females or egg massees recovered/total # females placed x 100.
2/ # of mated females or embryonated egg masses/# females recovered x 100.
3/ Corrected to control (=100%)

The locations of male release and female placement may have favored successful mating. The lower trap densities may have provided direct routes between released male moths and placed females (as would be the case in natural infestations) without trap interception.

Linearity of mating reduction with increasing numbers of males captured suggest a correlation between these variables, and regression analysis of % mated females vs % males captured reinforces this assumption ($r^2 = 0.64$). Similarly, a high correlation between percent males captured and trap distribution is apparent ($r^2 = 0.94$). These results do not confirm correlation between these variables, but strengthen the likelihood that a relationship may exist. Analysis of percent mated females vs trap density indicates a good correlation ($r^2 = 0.66$). However, mating reduction is not correlated to trap density, of course, but is influenced by the number of males removed from an area. This example demonstrates a problem in dependence on regression analysis to confirm correlation between variables. Additional testing and observations are required to test statistical significance.

In summary, mating is apparently reduced as greater numbers of males are removed from a population. Since complete elimination of mating was not observed, increased trap density may be required for eradication. Multiyear trapping may also prove fruitful. Commercial application of male moth traps may serve to heighten public awareness of the gypsy moth problem, but is unlikely to exert any control on the target pest population, especially those at high density.

Project Number: GM 6.3.6
Project Title: Establishing Standards
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: John Allen Tanner and Bernadine P. Weeks

The purpose of this project is to measure the quality of the insect produced by the rearing facility, and to identify the effects of altering rearing conditions on quality. Data are collected for each strain/generation and are used to develop life tables and to detect changes from normal development so that corrective action can be taken.

Developmental and reproductive data are collected with the modified Tanner-Houle procedure (Tanner and Buck 1979a and 1979b). Daily production is compared with standards developed from data collected in early 1979 (Tanner and Buck 1979b). Significant variations indicate changes in colony development.

Sampling Procedures Modifications

Since the last report, the only modification in the sampling procedure concerns the way the pupation rate is determined. Previously, five cups were selected on the post infest day 20 (PID) (calculated male pupae DT_{50}) and on the PID 30 (calculated female pupae DT_{50})with the corresponding percentage of male and female pupae determined. These percentages were compared to those of the standard. Significant differences would indicate that a change in the DT_{50}(s) had occurred. This procedure is less time consuming than examining each sample cup daily. However, it was not possible to tell the magnitude of the change nor what the actual DT_{50} was. In some programs, particularly the sterile male program, there is a need to know the actual DT_{50} in order to determine when to harvest the pupae for radiation.

With this in mind, the following procedure was established for determining DT_{50}s. Rearing cups are randomly selected until there are 6 cups with two or more male larvae and 6 cups with two or more female larvae. One cup may be used to measure both male and female DT_{50}s. Each cup is checked daily until 50% pupation has occurred. DT_{50}s are calculated for each cup. A mean DT_{50} is then calculated and compared with the standard.

When necessary, a pupation curve can be extrapolated from the data by assuming that the curve is symmetrical around the DT_{50}.

The FY 1980 rearing program saw some changes made in the techniques used in the rearing facility. Some of these changes were made quickly in response to problems with larval development, while others were improvements based upon experimental evidence.

The major changes and problems arising during the past rearing year are listed in chronological order in Table 1. Commencing October 2, 1979, rearing cups (6 oz. plastic cups) were placed in a single layer (non-stacked) on each rearing tray. Prior to that date, the cups were stacked in two layers. Stacking the cups resulted in an environment conducive to fungal growth in the lower level cup (which subsequently had to be discarded). The loss in insects often made it difficult to provide the necessary number of pupae during the 1979 sterile male program.

Table 1. Chronological order of problems encountered and changes made in the rearing facility during FY 1980.

October 2, 1979	Insect rearing cups placed non-stacked on rearing trays.
November 26, 1979	Poor larval development commences.
January 24, 1980	Colony started with neonates that hatched from eggs chilled 200 day. Poor larval development ceases.
February 27, 1980	Poor larval development commences with 200 day chilled eggs. Eggs chilled 120 days also produce larvae which developed poorly
March 13, 1980	Colony started with 2nd instar larvae. Reduces incidence of poor larval development.
May 26, 1980	Incidence of poor larval development ceases. Colony started with neonates hatching from eggs chilled 120 days.
June 7, 1980	Colony started with neonates hatching from eggs chilled 150 to 180 days.

Table 2 compares the developmental and reproductive data of colony insects reared in non-stacked and stacked cups and Table 3 lists the major developmental and reproductive differences observed. Insects reared in the non-stacked mode generally had lower pupal weights and shorter adult DT_{50}'s. Less adult deformity, greater insect survival, and better hatch in the eggs deposited are also benefits of non-stacked rearing. All other developmental and reproductive data were generally similar to those from insects reared in stacked cups.

Table 2. Developmental and reproductive data of colony insects reared in non-stacked and stacked modes.

Larval Development (Mean Larval stage)	Non-stacked 1/	Stacked 2/
7 days	2.1 ± .2	2.0 ± .3
14 days	3.9 ± .1	3.7 ± .2
21 days	5.3 ± .2	5.3 ± .2
Pupal Weights		
Males (gms)	0.68 ± .04	0.71 ± .03
Females (gms)	2.23 ± .14	2.37 ± .1
(DT_{50} Days)		
% Pupal Deformity		
Males	3.6 ± 3.1	4.8 ± 4.0
Females	76.4 ±11.0	86.6 ± 8.1
% Pupal Harvested	86.3 ± 5.6	80.3 ± 7.8
Adult DT_{50}		
Males	41.4 ± 0.9	44.5 ± .9
Females	40.8 ± 1.0	43.2 ± .7
% Adult Emergence		
Male	98.9 ± 1.6	97.8 ± 5.0
Female	96.8 ± 3.4	92.1 ± 8.2
% Adult Deformity		
Males	0.4 ± 0.8	1.5 ± 2.6
Females	4.0 ± 4.6	8.0 ± 5.4
Sex Ratio (M:F)	52:48 ±6.9	55:45 ± 8
% Survival to Adult)		
Males	85.4 ± 5.5	78.6 ± 9.0
Females	83.6 ± 6.2	74.3 ± 9.2
% Mating Pairs	96.9 ± 4.5	92.3 ± 7.6
Eggs laid/female	1035 ± 77.3	1055 ± 122.9
% Hatch		
120 day chilled eggs	92.6 ±5.4	86.0 ± 10.8

1/ New Jersey F_{20} - Larval Infest Dates October 2, 1979 to November 25, 1979.

2/ New Jersey F_{19} - Standard Larval Infest Dates January 31, 1979 to April 10, 1979.

Table 3. Major developmental and reproductive differences between the New Jersey colony reared in non-stacked and stacked cups.

Rearing New Jersey colony in non-stacked cups resulted in:

1) Lower pupal weights.
2) Reduced DT_{50} for adult males and females.
3) Less adult deformity.
4) Increased survival from neonate to adult.
5) Increased hatch rates.

Poor larval development began with neonates infested on November 26, 1979 and was characterized by the failure of many larvae to develop past the first or second instar. Individual rearing containers were observed to contain both early instar larvae and pupae. We termed this phenomenon "straggling". The proportion of larvae which failed to develop often reached 40 - 50 percent of the larvae infested. The reason for the poor larval development is presently unknown. Table 4 compares the developmental and reproductive data of colony insects started during the poor larval developmental period with colony insects started immediately prior to this period. Both groups of insects were reared in non-stacked cups. Table 5 lists the major developmental and reproductive differences between these groups.

Table 4. Developmental and reproductive data of colony insects infested during the poor larval developmental period and colony insects infested prior to this period.

	Colony Insects Reared During the Straggling Period 1/	Pre-straggling Period 2/
Larval Development (Mean Larval Stage)		
7 days	1.6 ± 0.2	2.1 ± 0.2
14 days	3.2 ± 0.5	3.9 ± 0.1
21 days	4.5 ± 0.7	5.3 ± 0.2
Pupal Weights		
Males (gms)	0.76 ± 0.05	0.68 ± 0.04
Females (gms)	2.61 ± 0.23	2.23 ± 0.14
% Pupal deformity		
Males	3.2 ± 3.3	3.6 ± 3.1
Females	81.7 ± 12.1	76.4 ± 11.0
% Pupal Harvested	56.6 ± 13.5	86.3 ± 5.6
Developmental Time to 50% Adult emergence (DT_{50} days)		
Males	42.5 ± 1.1	41.4 ± 0.9
Females	41.7 ± 1.0	40.8 ± 1.0
% Adult Emergence		
Male	98.7 ± 2.1	98.9 ± 1.6
Female	93.2 ± 5.8	96.8 ± 3.4
% Adult deformity		
Males	0.2 ± 0.7	0.4 ± 0.8
Females	5.1 ± 4.5	4.0 ± 4.6
Sex Ratio (% Males: % Females)	57:43 ± 9.0	52:48 ± 6.9
% Survival (Neonate to adult)		
Males	57.9 ± 14.0	85.4 ± 5.5
Females	54.8 ± 14.3	83.6 ± 6.2
% Mating Pairs	97.7 ± 4.1	96.9 ± 4.5
Eggs laid/female	1119.6 ± 127.9	1035 ± 77.3
% Hatch		
120 day chilled eggs	92.5 ± 7.1 3	92.6 ± 5.4

1/ New Jersey F_{20} - Larval infest dates November 26, 1979 to January 23, 1980 and February 27, 1980 to March 2, 1980.
2/ New Jersey F_{20} - Larval infest dates October 2, 1979 to November 25, 1979.
3/ The data are only from the November 26, 1979 to January 23, 1980 larval infest dates.

As would be expected, insects started during the poor larval developmental period had lower mean larval stages on day 7, 14 and 21 PID compared with insects started prior to this period. The percentage of neonates surviving to adults was lower during the poor larval developmental period. Most of the insects were lost during the larval stage as indicated by the low percentage of pupae harvested. Adult emergence was similar for both groups.

Pupal weights were higher and a greater number of eggs were deposited during the poor larval developmental period. This was the result f the reduced number of larvae developing per rearing container. Lower larval densities results in heavier pupae (Leonard 1968) and heavier female pupae produce adult females with a higher fecundity (Maksimovic 1958, Campbell 1978).

Table 5. Major developmental and reproductive differences between the New Jersey colony reared during the poor larval development period and prior to this period.

1) Lower mean larval stages on the 7, 14 and 21 PID.
2) Greater pupal weights (M + F)
3) Fewer pupae harvested.
4) A lower percent survival neonate to adult (M + F).
5) A greater number of eggs deposited/female.

The poor larval developmental period actually encompassed two separate periods; November 26, 1979 to January 23, 1980 and February 27, 1980 to March 2, 1980. Larval development was normal from January 24 to February 26, 1980 (Table 6). This period of normal development was accomplished by utilizing the remaining egg masses from the group of masses used prior to the masses which produce larvae that developed poorly. These masses had been chilled 200 days or 80 days longer than the normal 120 days.

Table 6 compares the developmental and reproductive data of the colony insects started from the 200 day chilled eggs with colony insects started from the same eggs after they were chilled only 120 days. Pupal weights were higher when the insects came from 200 day chilled eggs. However, unlike the results observed by Maksimovic 1958, Campbell 1978, the heavier female pupae did not lay more eggs. Insects from 200 day chilled eggs took a slightly shorter period of time to complete development but they also had a slightly lower percent survival.

Table 6. Developmental and reproductive date of colony insects selected from 200 day chilled eggs and colony insects selected from 120 day chilled eggs (standard).

	Length of Egg Chilling	
	200 days 1/	120 days 2/
Larval Development (Mean larval stage)		
7 days	2.1 ± 0.3	2.1 ± 0.2
14 days	3.7 ± 0.6	3.9 ± 0.1
21 days	5.2 ± 0.6	5.3 ± 0.2
Pupal Weights		
Males (gms)	.77 ± 0.05	0.68 ± 0.04
Females (gms)	2.53 ± 0.23	2.23 ± 0.14
% Pupal Deformity		
Males	3.1 ± 2.5	3.6 ± 3.1
Females	74.0 ±12.3	76.4 ± 11.0
% Pupal Harvested	75.9 ± 22.1	86.3 ± 5.6
Developmental Time to 50% Adult emergence (DT_{50} days)		
Males	40.5 ± 1.0	41.4 ± 0.9
Females	40.0 ± 1.2	40.8 ± 1.0
% Adult Emergency		
Male	98.2 ± 2.0	98.9 ±1.6
Female	90.6 ± 6.7	96.8 ±3.4
% Adult deformity		
Males	0.2 ± 0.6	0.4 ± 0.8
Females	5.5 ± 4.8	4.0 ± 4.6
Sex ratio (% Males: % Females)	50:50 ± 9.2	52:48 ± 6.9
% Survival (Neonate to adult)		
Males	74.5 ± 21.5	85.4 ± 5.5
Females	69.7 ± 23.0	83.6 ± 6.2
% Mating Pairs	97.7 ± 3.1	96.9 ± 4.5
Eggs laid/female	1066 ± 125.1	1035 ± 77.3
% Hatch 120 day chilled eggs	91.0 ± 8.4	92.6 ± 5.4

1/ New Jersey F_{20} - Larval infest date - January 24 to February 26, 1980.

2/ New Jersey F_{20} - Larval infest date - October 2, 1979 to November 25, 1979.

On February 27, 1980, the only 200 day chilled eggs available were eggs that had produced larvae which developed poorly. Larvae from these eggs still produced this phenomenon. Eggs which had just completed 120 days of chilling also produced larvae which developed poorly. A new method was needed for selecting larvae for use in the rearing program.

The major problem with the poor larval development was the low pupal harvest rate (Table 4). Only slightly more than 50% of the larvae infested were harvested as pupae. This caused a considerable waste of time, money and space. It also jeopardized the summer sterile male program. With this low rate of pupal harvest, it would have been impossible to supply the appropriate number of male pupae the program needed. To provide the sterile male program with an appropriate number of male pupae, a new method was developed for selecting larvae for use in the rearing program. This new method was based on the observation that a large percentage of larvae which entered into the 2nd instar, after a normal developmental period, also completed development normally (Forrester, personal communication).

This new method consisted of allowing newly hatched neonates to develop to 2nd instar in large petri dishes (150 x 15 mm) containing a thin layer of diet. Dishes were inverted and placed on bread trays. Those neonates which developed normally into the 2nd instar were removed and placed into the normal 6 oz. plastic rearing cups and used in the rearing program. The remaining insects were discarded.

Table 7 compares the developmental and reproductive data of insects selected for colony after reaching the 2nd instar to insects that were selected for colony as newly hatched neonates before the poor larval development period began. No major differences between each group could be observed. Selecting 2nd instar larvae for rearing resulted in a normal percentage of larvae being harvested as pupae. There also was a reduction in the percentage of deformed pupae.

Table 7. Developmental and reproductive data of colony insects selected during their second instar and colony insect selected as neonates.

	Colony Insects Selected as Second Instar Larvae 1/	Colony Insects Selected as Neonates 2/
Larval Development (Mean larval stage)		
7 days	2.0 ± 0.1	2.1 ± 0.2
14 days	3.9 ± 0.1	3.9 ± 0.1
21 days	5.2 ± 0.1	5.3 ± 0.2
Pupal Weights		
Males (gms)	0.63 ± .04	0.68 ± 0.04
Females (gms)	2.03 ± .21	2.23 ± 0.14
Developmental Time to 50% Pupation (DT_{50} days)		
Males	29.1 ± 0.9	- -
Females	31.2 ± 1.2	- -
% Pupal deformity		
Males	0.9 ± 2.1	3.6 ± 3.1
Females	48.9 ± 13.8	76.4 ± 11.0
% Pupal Harvested	78.5 ± 13.9	86.3 ± 5.6
Developmental Time to 50% Adult emergence (DT_{50} days)		
Males	42.5 ± 0.9	41.4 ± 0.9
Females	41.7 ± 1.3	40.8 ± 1.0
% Adult Emergence		
Male	98.9 ± 1.4	98.9 ± 1.6
Female	95.1 ± 4.9	96.8 ± 3.4
% Adult deformity		
Males	0.2 ± 0.7	0.4 ± 0.8
Females	6.2 ± 5.2	4.0 ± 4.6
Sex Ratio (% Males: % Females)	51:49 ± 4.8	52:48 ± 6.9
% Survival (Neonate to Adult)		
Males	76.8 ± 14.4	85.4 ± 5.5
Females	73.9 ± 13.9	83.6 ± 6.2
% Mating Pairs	97.6 ± 3.0	96.9 ± 4.5
Eggs laid/female	939.4 ± 134.7	1035 ± 77.3
% Hatch		
120 day chilled eggs	- -	92.6 ± 5.4

1/ New Jersey F_{20} - Larval infest date - March 13, 1980 to May 26, 1980.

2/ New Jersey F_{20} - Larval infest date - October 2, 1979 to November 25, 1979.

Starting the rearing program with 2nd instar larvae was much more time consuming than with neonates. It was very difficult to infest the number of larvae needed for the sterile male program. Fortunately, the poor larval development ceased to be a problem and neonates are again used to start each rearing program.

Neonates presently used in the rearing program are from eggs chilled 180 days. Chilling eggs 180 days result in quicker, more uniform hatch than eggs chilled only 120 days.

Project Number: GM 8.3.2
Project Title: Rearing Technique and Production Costs
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leaders: O. T. Forrester, L. F. Kennedy, J. J. Baker

Work on this project has been terminated.

Project Number: GM 8.3.4
Project Title: Evaluating the Sampling Techniques used to Establish Standards and Monitor Production
I. Egg Sampling
II. Pupal Development
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: John Allen Tanner and Bernadine P. Weeks

Introduction

A quality control program must have sampling techniques which accurately estimate the true population mean. This requires careful evaluation of each sampling technique before it is accepted for use in a quality control program. In this and future reports, sampling techniques proposed for use in our quality control program will be evaluated for accuracy in expressing the true population mean. Unreliable or inaccurate techniques will be modified or discarded.

I. Egg Sampling

A reliable hatch test is necessary to determine the effects of alternate rearing methods on gypsy moth reproduction. Also, hatch tests are needed to determine the number of eggs that must be incubated for production. Dehaired samples are preferred, since sample size and composition can be controlled.

Tests by Tanner and Buck (1979) have shown that the hatch of dehaired eggs decreases when incubated directly in an environmental room, (25.5° C, 50-55% RH). Further testing has shown that this reduction was due to low humidity. Consequently, a high humidity egg chamber was developed for the incubation of a large number of egg samples. A test was designed to determine if the percent eclosion of egg samples incubated in this chamber were comparable to the percent eclosion of egg samples incubated in a desiccator containing water (saturated atmosphere).

Ten egg samples were removed from the cores of 5 New Jersey F_{19} egg masses (120 day chilled). One half of the samples were dehaired and placed into individual petri dishes (50 x 9 mm). The non dehaired egg samples were also placed into individual petri dishes. One dehaired and one non-dehaired sample was placed into each of the following locations:

1) Directly in the environmental room (25.5° C, 50-70% RH)
2) A desiccator containing water (100% RH) located within the environmental room.
3) On shelf 2, 7 and 13 within a high humidity chamber (100% RH) located in the environmental room.

Five replications were conducted over a period of time. Results are summarized below.

Table 1. Hatch of gypsy moth eggs incubated in a prototype high humidity chamber.

Location of Eggs	Percent hatch 1/			
	Dehaired Eggs		Non-dehaired Eggs	
High Humidity Chamber				
Shelf 2	83.1	a	83.4	a
7	83.1	a	84.0	a
13	81.6	a	85.3	a
Environmental Room				
Desiccator	84.3	a	83.9	a
Shelf	59.1	b	84.0	a

1/ Means within a column not followed by the same letter are significantly different at the 5% level (Duncan's new multiple range test).

There were no differences in percent hatch between eggs incubated in the high humidity chamber (100% RH) and eggs incubated in the desiccator (100% RH). However, at 100% RH there was a problem with fungus growing on the surface of eggs. When the humidity in the chamber was dropped to 80-85%, the incidence of fungal contamination declined but the egg hatch also seemed to decline (personal observation). A second test was conducted to determine if there was a significant reduction in egg hatch when the relative humidity was decreased from 100 to 80-85%. This test was also designed to determine the optimum humidity level for hatch.

Egg samples were removed from the core of New Jersey F_{20} egg masses and dehaired. Five twenty egg samples were placed into individual petri dishes (50 x 9 mm) and incubated at one of the following humidities:

1) Ambient humidity: 50 to 60% RH (Environmental room)
2) 80 to 85% RH (Humidity chamber)
3) 100% RH (desiccator - 0 gms KOH/100 gms H_2O)
4) 92% RH (desiccator - 11 gms KOH/100 gms H_2O)
5) 85% RH (desiccator - 19 gms KOH/100 gms H_2O)

Five masses were used/replication and five replications were conducted at the rate of one/week.

Egg hatch tended to decrease as the relative humidity decreased (Table 2). However, the hatch rate of eggs incubated in the high humidity chamber was not significantly lower than the rates observed in any of the desiccators.

The standard deviation of the mean increased as the humidity decreased (Table 2). This indicates that response of individual samples to humidity became more variable as the humidity decreased.

Table 2. Percent hatch of gypsy moth eggs incubated under various relative humidities.

Relative Humidity (%)	Percent Hatch 1/		
100 - desiccator	90.8	12.2	a
92 - desiccator	88.8	13.9	a
85 - desiccator	79.3	17.5	a b
80-85 - Humidity chamber	77.6	20.0	a b
50-60 - Environmental room	58.3	23.6	b

1/ Means followed by the same letter are not significant at the 5% level (Duncan's multiple range test)

II. Pupal Development

Male and female pupal DT_{50} is predicted for each larval infest date by randomly selecting six rearing cups which contain at least two larvae of each sex. The six cup sample size is used regardless of the number of rearing cups originally infested. The following test was conducted to determine the number of rearing cups that must be sampled to provide a standard error that is within 20% of the sample mean.

Two trays (60 cups) of colony insects were selected for each of three replications. Each cup was numbered. A cup was used to determine the pupal DT_{50} of a sex as long as it contained at least two larvae of that sex. A DT_{50} was calculated for each sex/cup. A mean DT_{50} standard deviation, and standard error of the mean was calculated for each sex/replication. The number of sample units needed to ensure a sample error within 20% of the sample mean was determined by the following formula for each replication.

$$\sqrt{n} = \frac{t.05S}{s\bar{x}}$$

n = number of sample units
t.05 = student's at the 5% level of probability
s = standard deviation of the mean DT_{50}
$s\bar{x}$ = standard error (20% or .2)

Pupal DT_{50}s (♂+♀) did not vary substantially within a replication (Table 3). This actually resulted in sample units of less than one cup. For a more concise expression of each sample unit, each unit was converted to express the maximum number of cups from which only one sample cup needs to be selected (Table 4). For males, the maximum number of cups varied from 96 to 270 with a mean of 181 ± 87. For females, it was 163 to 285 with a mean of 238 ± 65.

Table 3. The sample unit size needed to insure a sample error within 20% of the mean pupal DT_{50}. 1/

Replication	Mean DT_{50} (Days) ♂	Mean DT_{50} (Days) ♀	Total cups used to determine DT_{50} ♂	Total cups used to determine DT_{50} ♀	Sample unit size (cup) 2/ ♂	Sample unit size (cup) 2/ ♀
1	28.4 ± 1.5	30.3 ± 1.9	48	49	0.5	0.3
2	29.3 ± 1.2	30.3 ± 1.8	53	57	0.3	0.2
3	30.4 ± 1.4	31.9 ± 1.5	54	53	0.2	0.2
			$\bar{x}$ = 51.7±3.2	53.0±4.0	0.33±.15	0.23±.06

1/ New Jersey F_{20} laboratory strain

2/ Formula used to compute sample unit size (n):

$$\sqrt{n} = \frac{t.05S}{s\bar{x}}$$

n = sample unit size (cup)
t.05 = Student's at the 5% level of probability
s = Standard deviation of the mean DT_{50}
$s\bar{x}$ = Standard error (20% or .2)

Table 4. The maximum number of cups from which a randomly selected cup will provide a sample error within 20% of the mean pupal DT_{50}.

	Sample Unit Size (cup)		Maximum Number (cups) 1/ 2/	
Replication	♂	♀	♂	♀
1	0.5	0.3	96	163
2	0.3	0.2	177	285
3	0.2	0.2	270	265
	$\bar{x}$ = 0.33±.15	0.23±.06	181±87	238±65

1/ The following formula was used to convert each sample unit size into the maximum number of cups from which only one sample cup needs to be selected.

$$\text{Maximum number} = \frac{\text{Total cups used to determine } DT_{50} \text{ (Table 2)}}{\text{Sample unit size}}$$

2/ Figures have been rounded

During the 1979-80 rearing period, daily colony pupal DT_{50}s (♂+♀) were calculated from six randomly selected rearing cups. The daily total of rearing cups infested with neonates was 120 or 375 cups. This resulted in one sample cup being selected for every 20 or 62 cups infested. Sample units of these size would result in sample errors that are well within 20% of the mean pupal DT_{50}.

Project Number: GM 9.3.2
Project Title: Silk Removal from Gypsy Moth Pupae
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leader: O. T. Forrester

Work on this project has been terminated.

Project Number: GM 9.3.5
Project Title: Comparison and Evaluation of Rearing Techniques
Report Period: October 1, 1979 - September 30, 1980
Report Type: Final
Project Leader: O. T. Forrester

Work on this project has been terminated.

Project Number: GM 9.3.6
Project Title: Substituting Supro-610 for Casein in the Gypsy Moth Diet (B-4) Under Mass Rearing Conditions
Report Period: October 1, 1979 - September 30, 1980
Report Type: Interim
Project Leaders: John Allen Tanner and Bernadine P. Weeks

Introduction

Casein is the major source of protein in the high wheat germ diet (B-4). The cost of casein is increasing rapidly and future supplies may be scarce. Supro-610, a modified soybean protein, has been substituted for casein in small research tests with no adverse effects on gypsy moth growth and development (Bell: personal communication). Supro-610 currently costs $.30/lb less than casein, and should therefore be considered as a possible substitute for casein in the mass rearing diet. Before Supro-610 is substituted for casein it must be tested with the techniques and conditions in the mass rearing program. This will determine if problems may arise when Supro-610 is used on a large scale.

Objectives

1) To determine the effects of Supro-610 on the consistency of the high wheat germ diet when large volume mixing techniques are utilized.
2) To determine the effects of Supro-610 on the growth and development of mass reared gypsy moth.

Methods and Materials:

Two 60 l batches of high wheat germ diet (B-4) were made on three consecutive days. One batch contained 1500 gms of casein, the other batch contained 1500 gms of Supro-610. Each batch was mixed and dispensed following the techniques described by Bell et al. 1980. Individual diet batches were mixed for a standard 8 minutes and visually checked for consistency. Mixing was continued, if necessary, until the diet ingredients were completely mixed. Approximat Upon solidification and cooling of the diet, 8 neonates were infested into each cup. All the cups were stored on trays (30 cups/tray) supported by a rearing rack (24 trays/rack). One rack was used for each treatment/replication.

The insects were allowed to develop to the pupal stage within a growth chamber programmed for 25.5° C, 50% RH, and a 15L:9D photoperiod. The pupae were held in another growth chamber programmed for 25.0° C, 50% RH, and a 15L:9D photoperiod where adult emergence occurred. Both growth chambers were known to have a 2 to 3° C temperature difference between the upper and lower level during the summer rearing program. To minimize this effect, each rack was divided into three levels (upper, middle, and lower). Each level contained 8 trays (240 cups). Complete insect developmental and reproductive data were taken for each level. Ten egg masses were obtained from each level/treatment/replication, and embryonated 28 days. All the masses are currently being chilled for 120 days. After completing chilling, developmental and reproductive data will be taken on the F_1 generation.

The developmental and reproductive data taken for both the parent generation and the F_1 generation consists of the following measurements:

1) The mean larval stage of the 7, 14, and 21 post infest date (PID). This will be calculated for each cup sampled by the following formula:

$$\frac{\#\ \text{larvae/developmental instar} \times \text{developmental instar (i.e. 1-6)}}{\text{total}\ \#\ \text{larvae}}$$

2) The mean male and female DT_{50}.
3) The mean male and female pupal weight on the 35th PID.
4) The percent male and female pupal deformity.
5) Percent survival of neonate to pupae.
6) The mean male and female adult DT_{50}.
7) The percent male and female pupal deformity.
8) The percent male and female adult recovery.
9) The percent survival of neonate to adult.
10) The sex ratio.
11) The percentage of mating pairs.
12) The mean number of eggs laid/female.
13) The percent hatch of eggs chilled 120 days.

The methods used to obtain these data were originally described by Tanner and Houle (1978) and modified by Tanner and Buck (1979 a & b) and Tanner and Weeks (1980).

Results

The initial generation of insects reared on B-4 diet containing Supro-610 had similar developmental and reproductive data as those insects reared on B-4 diet containing casein (Table 1). The location of the rearing tray on the rearing rack had no effect on the development and reproduction of the insects. Only slight temperature differences were observed between the upper and lower levels of each rearing rack (Table 2). This is contrary to the 2 to 3° C temperature difference observed during the summer rearing program. During that program, both chambers were full of rearing racks. This was not the case during the above test. The packing of the chamber with rearing racks may have reduced the air flow causing the temperature discrepancy between the upper and lower levels of a rearing rack.

Table 1. Development and reproduction of gypsy moth under mass rearing conditions as affected by the substitution of Casein with Supro-610 in the B-4 diet.

Parameter	Casein	Supro-610
$\bar{x}$ larval stage		
7 (PID)	2.0 ± .1	1.9 ± .1
14 (PID)	3.9 ± .1	3.8 ± .2
21 (PID)	5.4 ± .2	5.3 ± .2
DT_{50} Pupae		
♂	27.8 ± 1.10	28.5 ± 1.3
♀	29.9 ± 1.20	30.6 ± 1.6
Pupal Wts (gms)		
♂	0.69 ± .02	0.70 ± .03
♀	2.28 ± .11	2.31 ± .13
% Pupal deformity		
♂	11.3 ± 3.5	11.3 ± 4.6
♀	82.7 ± 5.8	81.5 ± 6.9
% Survival neonate to pupae	93.8 ± 2.5	89.6 ± 1.0
DT_{50} Adults		
♂	41.5 ± 1.1	42.1 ± 0.7
♀	41.0 ± 0.5	41.3 ± 0.8
% Adult deformity		
♂	0.7 ± 1.0	0.0 -
♀	15.4 ± 6.8	18.6 ± 7.9
% Adult emergency		
♂	99.6 ± 0.9	98.9 ± 1.4
♀	92.4 ± 5.0	93.2 ± 5.5
% Survival neonate to adult		
♂	92.6 ± 2.7	88.6 ± 2.1
♀	88.3 ± 5.6	84.5 ± 5.7
Sex ratio		
♂:♀	50:50 ± 2.3	52:48 ± 2.5
% Mating pairs	96.5 ± 4.2	97.3 ± 2.8
# Eggs/mass	1061.9 ± 75.0	1102.9 ± 83.7
Diet contamination:		
% of cups with:		
Virus	0 -	0 -
Bacteria	0 -	0 -
Mold	0.1 ± 0.2	0.4 ± 0.5

Table 2. The mean temperature (C^o) observed in the upper, middle and lower portion of the rearing racks in environmental chambers.

Chamber 1/	Insect Stage present	Upper	Middle	Lower
II	Larvae, pupae	25.6 ± 0.4	25.1 ± 0.4	25.1 ± 0.5
III	Pupae, adult	25.9 ± 0.2	25.1 ± 0.2	25.1 ± 0.2

1/ Chamber II - small holding room - 25.5° C ± 1°C, 49.3 ± 3.8% RH and 15L:9D photoperiod.

Chamber III - large holding room - 25.0° C ± 1° C, 47.8 ± 5.3% RH and 15L:9D photoperiod.

Conclusions

Based on data from one generation, Supro-610 can be successfully substituted for casein, in the B-4 diet, under mass rearing conditions. However, before making such a substitution, it is strongly suggested that development and reproduction of the offspring be examined.

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For Publication in Environmental Entomology

A Comparison of Laboratory-Reared and Wild-Type Gypsy Moth *Lymantria dispar* L. Males

by

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ABSTRACT

Two strains of laboratory-reared male gypsy moths responded to pheromone sources (calling female moths and synthetic (+)-disparlure sources) in proportions as great as wild-type males. Ages at the time of capture were also similar for laboratory-reared and wild-type males except that a larger proportion of wild males were captured on the day they eclosed. Sterilization of a laboratory strain by exposure in a ^{60}CO irradiator (15 krads) affects male competitiveness least when males are treated as 10 and 11 day old pupae. Irradiation of younger (8 and 9 days old) pupae resulted in a decreased proportion of males responding to pheromone sources. Males treated in the older age class were similar to wild-type and untreated laboratory-reared males in the proportions released males, recovered.

INTRODUCTION

Use of the sterile male technique (SMT) for control of the gypsy moth, *Lymantria dispar* L., was proposed as early as 1955 (Knipling 1969). Studies carried out between 1957 and 1973 investigated the effects of gamma radiation and chemical sterilants on male fertility (reviewed by Mastro et al., 1981). These early studies were hampered by the want of reliable rearing techniques. Experimental insects were often field collected as pupae, or reared in the laboratory using a variety of diets and holding conditions. Low survival and inconsistent male quality contributed to either incomplete tests or poor field performance of both sterilized and control insects. Even after an artificial diet had been developed (Leonard and Doane 1966) laboratory reared males were found to be less "sexually aggressive" than their wild counterparts (Richerson and Cameron 1974). Recent improvements in rearing technology (reviewed by Bell et al. 1981) have renewed interest in developing the sterile male technique. The studies reported here are a portion of a program that has the overall objective of evaluating the feasibility of using the SMT for treatment of sparse, isolated gypsy moth infestations. Specifically, these studies were designed to compare the quality of laboratory-reared (radiologically sterilized and untreated) males with feral males.

METHODS AND MATERIALS

Feral males were collected as pupae from wooded sites in Massachusetts, New York and Pennsylvania in 1977 and from Massachusetts, Pennsylvania and New Jersey in 1978. A laboratory strain (NJ) which had been in colony for 16 generations was used for 1977 field trials. The same NJ strain (in the 17th generation) and a second laboratory strain, YC-P_1 (reared from field collected eggs) were evaluated in 1978. Males were either irradiated 8-9 or 10-11 days after pupation. These two age classes were chosen on the basis of results of unpublished laboratory studies, which demonstrated that males exposed to 15 krads 8 days or later after pupation show no obvious morphological abnormalities. When irradiated males were mated with untreated females, the resulting egg masses show a very low percent hatch and a low incidence of larval survival. Insects were treated in a ^{60}CO irradiator with a source strength of 3120 C: and a dose rate of 2.17 krad/min. (July 1978).

Field comparisons of male strains were carried out during June, July and August in 1977 and 1978 near Otis AB, MA. The test area had a sparse native population. Native males were not captured until the first week of August of either year. To facilitate release, pupae were placed in open-top plywood boxes (61 cm^2 x 30 cm h) with wire mesh (.32 cm^2) bottoms, located in the center of test plots. As males eclosed, they normally crawled to and up the sides of boxes from where they were gently transferred to a similar box. When male wing expansion progressed to the point where wings were held vertically over the abdomen, each male was dusted with fluorescent dust [17]. Several different colors were used to distinguish among days of release and male strains.

Eclosion and dispersal (flight from release boxes) were monitored continuously from 6 a.m. to 10 p.m. in 1977 tests and from 9 a.m. to 5 p.m. in 1978 tests. During the first year's trials, males were released on 2 or 3 consecutive days. In the second year, males were released on a single day; however, if for any strain the number of males eclosed was judged insufficient to obtain adequate numbers for analysis, all strains in that trial were released for a second day.

Study plots in 1977 consisted of a 6 x 6 square grid of stations (50 m between station spacing). Each station in the grid was randomly assigned one of 6 trap treatments; treatments were randomized for each test. Five of these treatments consisted of a mesh (12 mm^2) screen trap (30 cm L x 25 cm W) coated with tack trap and stapled to the bole of a tree. A small wire (6 mm^2 mesh - 7 cm dia x 6 cm) bait container was hung in the center of each screen trap. The trap treatments were baited with the following: a feral female, a YC-P_1 female, a NJ-F_{16} female or a cotton dental wick (ca. 11 mm dia x 6 mm) treated with 100 ug (+) disparlure. The fifth trap treatment had an empty bait container hung in the center of a screen trap. A feral female placed on the bole of a tree served as the sixth treatment. All treatments were placed on trees at 1.5 m in height. Females used in 1977 and 1978 trials were virgin and one day old (the day following eclosion) when placed in the field for monitoring and were replaced daily for the length of the test. Disparlure baited wicks however, were charged on the first day and not replaced throughout each test. Traps were checked hourly (9 a.m. - 4 p.m.) and all captured males were placed in labeled cups for later laboratory determination of male treatment group. An observer was stationed by each free feral female (treatment 6) and any males attempting to mate with a female were collected and placed in labeled cups. Female moths that were observed mating were replaced by virgin females.

To evaluate male competitiveness in a situation that was comparable to a proposed target population, 1978 plots were designed to provide a female density of ca 11/ha. Sixty-six monitoring stations were located 20 m apart along the circumference of two concentric circles (70 m and 140 m radii). Virgin one day old female moths, in small wooden A-frame structures, were placed at stations, alternately on the bole of a tree (1.5 m height) or on the ground (1 m from the base of a tree). Female placement was designed to simulate the hidden niches (i.e. under back flaps and rock and stumps) in which females are found in sparse populations (Campbell et al., 1975). Females were checked at one-half hour intervals from 9:30 a.m. to 4:00 p.m. Schwalbe (personal communication) found in the laboratory the average length of mating for 1 day old females was 65.6 minutes and the minimum mating time was 44 minutes; therefore, it was assumed few mating pairs would not be detected. When a mating pair was found at a monitoring station, the female was collected and replaced with another virgin female. Mating males were placed in cups which were labeled with the time of capture and the monitoring station position. With the exception of two replicates, monitoring was carried out in all 1978 plots in the manner described above. Plots 1 and 2 (7/31 and 8/7, Table 3) were monitored with Delta traps baited with (+) disparlure instead of females. As personnel and weather constraints prevented the continuous monitoring of these plots, age of capture comparisons cannot be made.

During both years of study, wind speed and direction were monitored constantly with the aid of a recording anemometer (Weather Measure Model 103A - 3 cup and Model W104-2 vane). Temperature and relative humidity were also recorded using a hygrothermograph (Belfort Instrument Co. Model 5594). In 1977, both instruments were placed in the center of each plot when male activity was monitored. During 1978 trials, the instruments were located in a wooded area central to all of the plots. During both years, a pyroheliometer recorder (Airojet-General Corporation, Model R-401) was located in a grassy field within 2 km of the test plots to record solar radiation.

Each release date was treated as a replicate for statistical analysis. The proportion of males recovered from a particular male treatment group was computed by dividing the number of males captured by the number of males leaving a pupal box (dispersing). The number of males eclosing would not have provided an accurate comparison of male recovery because avian predation in the pupal boxes prior to male flight was common and not quantified. Male recoveries were compared using chi^2 analysis techniques.

RESULTS AND DISCUSSION

Male moth recovery varied dramatically throughout the test period. For example, NJ-F_{16} males were recovered in proportions ranging from 5 to 77 percent of the released population in all 1977 studies. Plot differences apparently did not account for this variability, e.g. various release dates on the same plot resulted in recovery rates of from ca. 4 to 40 percent for the same laboratory-reared strain (Table 1). This variability in recovery was observed in both 1977 and 1978 studies (Tables 2 & 3). Environmental variables, especially temperature, appear to be related to recovery rates (Elkington and Carde, 1980, In press). Periods of cool weather generally coincided with low male recovery rates.

Comparison of recoveries among strains for the same release date indicates that laboratory-reared males locate synthetic or natural pheromone sources as well as do field collected males. In 1977 studies, the percentages of NJ-F_{16} males recovered was as great as, or significantly greater than that for feral males for all releases, except the first three release days. The reasons for the differences between these strains on the first three release dates are unclear. Daytime temperattures were cool during this period, and possibly feral males are better able to respond to pheromone or fly at low temperatures. Both laboratory strains located, placed females as readily as all the feral strains tested (Table 2). In the first two 1978 trials, the YC-P_1 laboratory strain performed significantly better than both the NJ-F_{17} and feral strains possibly due to retention of characteristics that allow this strain to respond to pheromone signals under a wider range of environmental conditions.

$NJ-F_{17}$ males, irradiated as 10-11 day old pupae, were captured as frequently as laboratory-reared untreated strains ($NJ-F_{17}$ and $YC-P_1$) at female sites (Table 3). However, when the $NJ-F_{17}$ males were irradiated as 8-9 day old pupae (trials 2 and 3, Table 3) a significantly smaller proportion of those released were recovered. Even though males irradiated as 8-9 day old pupae appear to be at a competitive disadvantage in these two trials, they did not exhibit any obvious signs of morphological abnormalities or differences in eclosion rates or dispersal behavior.

Adult male longevity in the field could affect competitiveness of a strain. Lengthening the time that sterile males actively search for possible mates would benefit any sterile male release program. Tables 4 (1977) and 5 (1978) present the age distribution of captured laboratory-reared and feral males. Because males were captured and removed from the population, the results do not provide an accurate comparison of potential longevity in a field situation; however, moth age at the time of capture does provide a comparison of how males of different strains mature and become sexually active. During both seasons of testing a larger proportion of feral strains than laboratory strains were captured on the day of eclosion (day 0) or on the first full day of adult life (day 1). The distribution of capture ages varied for all strains between tests. Environmental conditions may influence the length of time required for a male to begin actively searching for females or to respond to pheromone cues. When temperatures were high in the morning and remained high throughout the day, increased captures of 0 day old males were observed.

From observation at eclosion sites, it was found that males eclosing in the morning hours usually initiated flight several hours post-eclosion, while males eclosing in late afternoon do not initiate flight until sunset (ODell and Mastro, 1980). Observations indicate that maiden flights are usually of short duration after which males generally seek sheltered resting sites (e.g. under leaves or branches). Males exhibiting this behavior late in the day normally remain at the resting site to the following morning. Eclosion and dispersal of laboratory reared and feral males from eclosion sites is synchronous (unpublished data). Description of dispersal from resting sites, used by males after their first flight, was not attempted. Perhaps laboratory reared males remain in these resting sites longer than wild males and therefore a smaller proportion of them are captured on the day they eclose. Another possible explanation is that dispersal characteristics are similar but that feral males become receptive to pheromone cues earlier in their adult life. Either case could explain the smaller proportion of laboratory-reared moths captured on the day they eclose.

These field tests have demonstrated that laboratory-reared strains, including F_{16} and F_{17} and P_1 generations, responded to pheromone sources at least as well as did feral strains. Theoretically, it is advantageous to use near-wild strains (P), in a sterile male program, but we have observed that growth and development of wild-type strains is less synchronous than that of strains that have been reared for several generations. Thus far, advertant and inadvertant selection due to laboratory rearing does noot appear to have substantially decreased the competitiveness of the NJ strain.

Laboratory males could be predisposed to predation, since on the day of eclosion they are less effective in mate finding. More laboratory males would be removed before mating assuming equal predation rates on any given day. However, this does not appear to be occurring. In our tests, larger proportions of laboratory-reared males are responding on the days following eclosion.

Sterilization of the NJ strain by irradiation of 10-11 day old pupae does not appear to markedly affect male searching ability, survival, or eclosion and dispersal patterns. Considering field performance within the parameters measured, release of sterile laboratory-rared males appears to be a prospect for treatment of sparse gypsy moth infestation. Other behavioral characteristics (i.e. periodicity of activity and propensity to disperse), which if different from feral males may impact on the overall competitiveness of laboratory-reared are being studied. In addition, quality control laboratory techniques are being developed with the intent that they can predict field performance. If feasible, an operational program will require development of new techniques in many areas including; mass rearing, release, and monitoring the impact of a release on a native population.

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Table 1. Proportions of released male gypsy moths recovered from field plots - 1977

Date Eclosed	NJ F_{16} No.[1/]	NJ F_{16} Proportion	Feral[2/] No.[1/]	Feral[2/] Proportion	Chi^2
7/5	71	39.4 b	40	75.0 a[3/]	11.5838
7/6	375	15.2 b	321	26.2 a	12.2097
7/7	471	8.3 b	758	15.0 a	11.5648
7/8	185	4.9 a	245	9.4 a	2.5082
7/11	120	67.5 a	195	66.2 a	0.01514
7/13	288	68.4 a	211	52.1 b	12.9394
7/14	243	70.4 a	213	39.9 b	41.5538
7/18	698	66.0 a	257	48.2 b	24.3236
7/19	571	58.1 a	344	48.0 b	8.5574
7/20	479	53.4 a	353	45.6 b	4.6825
7/25	174	46.6 a	221	34.4 b	5.5161
7/26	116	49.1 a	101	53.5 a	0.2499
7/27	130	77.7 a	54	55.6 b	8.0691

1/ Number of males dispersing from eclosion boxes.

2/ Source of feral males released: 7/5 - 7/8 Bushkill, PA; 7/11 - 7/14 Dighton, MA; 7/18 - 7/20 Abington, MA; 7/25 - 7/27 Fern Lake, NY.

3/ Horizontally, values followed by the same letter are not different at the 5% level of significance according to Chi^2 comparison using Yates correction factor.

Table 2. Proportions of male gypsy moths recovered from simulated low density field plots - 1978

	Trial No. (Release date)							
	1 (6/27)		2 (6/28)		3 (7/18)		4 (7/24)	
Male Strain	No.[1/]	Percent Recovered[2/]	No.	Percent Recovered	No.	Percent Recovered	No.	Percent Recovered
NJ F_{17}	116	47.4 b	98	28.6 b	221	54.8 a	514	45.3 a
YC P_1	50	66.0 a	42	47.6 a	161	54.0 a	440	39.5 a
Feral[3/]	222	35.1 c	159	16.5 b	207	28.0 b	189	29.5 b
Feral	-	-	-	-	85	25.0 b	132	27.5 b

1/ Number of males dispersing from eclosion boxes

2/ Vertically, percentages followed by the same letter are not different at the 5% level of significance according to pair wise Chi^2 Analysis with Yates correction factor

3/ Feral strains collected as pupae from natural populations:
Yards Creek, NJ - Trial 1 & 2; Dighton MA (207) and Abington, Ma (85) - Trial 3: and Bridgewater, Ma (189) and Abington, MA (132) - Trial 4

Table 3. Proportions of Irradiated and untreated male gypsy moths recovered from simulated low density field plots - 1978

Male Treatment	Trial No. (Release date) 1 (7/31)		2 (8/7)		3 (8/16)		4 (8/21)	
	No.[1/]	Percent Recovered	No.	Percent Recovered	No.	Percent Recovered	No.	Percent Recovered
NJ F_{17}	367	28.6 a	60	43.1 a	108	54.6 a	94	26.6 a
YC P_1	398	24.6 a	351	53.6 a	269	55.8 a	254	22.8 a
NJ (S8-9)	85	25.9 a	161	23.0 b	112	27.7 b	91	17.6 a
NJ (S10-11)	0	0	202	47.2 a	124	45.1 a	94	20.2 a

1/ Numbers of males dispersing from eclosion boxes

2/ Vertically, percentages followed by the same letter are not different at the 5% level of significance, according to pair wise Chi^2 comparisons with Yates correction factor

Table 4. Proportions of males captured on days following release - 1977

Age in Days	Trial No. 1 (7/5-7/8)		2 (7/11-7/14)		3 (7/18-7/20)		4 (7/25-7/27)	
	NJ-F_{16}	Feral[1/]	NJ-F_{16}	Feral	NJ-F_{16}	Feral	NJ-F_{16}	Feral
0	16.3	13.9	8.0	15.6	3.2	14.1	4.4	15.0
1	45.0	60.9	70.0	67.6	80.6	71.9	73.7	78.6
2	32.6	21.8	18.2	14.0	14.4	12.5	18.6	4.6
3	6.2	3.4	3.7	2.8	1.9	1.6	3.3	1.7
Total[2/]	n=129	n=238	n=537	n=435	n=1044	n=448	n=274	n=173
	X^2=9.479		X^2=15.746		X^2=61.939		X^2=31.154	

1/ Feral males released: 7/5-7/8 - Bushkill, PA; 7/11-7/14 Dighton, MA: 7/18-7/20 Abington, MA; 7/25-7/27 - Fern Lake, NY

2/ Total number of males recovered

Table 5. Proportions of males captured on days following release - 1978.

Age in days	Trial No. 1 & 2: NJ-F_{17}	YC-P_1	Feral[1/]	Trial No. 3: NJ-F_{17}	YC-P_1	Feral[1/]	Feral[1/]	Trial No. 4: NJ-F_{17}	YC-P_1	Feral[1/]	Feral[1/]
0	0.0 a[2/]	1.9 a	5.5 b	0.0 a	1.1 a	10.3 b	33.3 c	32.2 b	23.6 a	48.1 bc	38.5 c
1	53.0	52.8	61.5	57.0	57.5	55.2	38.1	35.2	30.5	30.8	41.0
2	43.4	35.8	32.1	42.1	41.4	34.5	28.6	30.5	42.5	9.6	20.5
3	3.6	9.4	0.9	-	-	-	-	2.1	3.4	11.5	0.0
n=[3/]	83	53	109	121	87	58	21	233	174	52	39

1/ Feral strains released in trial 1 - Yards Creek, NJ, trial 2 - left to right: Dighton, MA and Abington, MA and trial 3 - left to right, Bridgewater, MA and Abington, MA.

2/ Horizontally, columns followed by the same letter are not different according to Chi^2 at the 5% level of significance.

3/ Number of males recovered.

Table 6. Proportions of males captured on days following release - 1978

	Trial No.							
	3 (8/16)				4 (8/21)			
Age in days	$NJ-F_{17}$	$YC-P_1$	$NJ-F_{17}$ (S8 + 9)	$NJ-F_{17}$ (S10 + 11)	$NJ-F_{17}$	$YC-P_1$	$NJ-F_{17}$ (S8 + 9)	$NJ-F_{17}$ (S10 + 11)
	a 1/	a	ab	b	a	a	a	b
0	8.5	14.7	25.8	28.6	4.0	10.3	0.0	0.0
1	86.4	81.3	71.0	64.3	12.0	13.8	18.8	57.9
2	5.1	4.0	3.2	7.1	84.0	75.9	81.3	42.1
n = 2/	59	150	31	50	25	58	16	19

1/ Horizontally columns followed by the same letter are not significantly different according to Chi^2

2/ Number of males recovered

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