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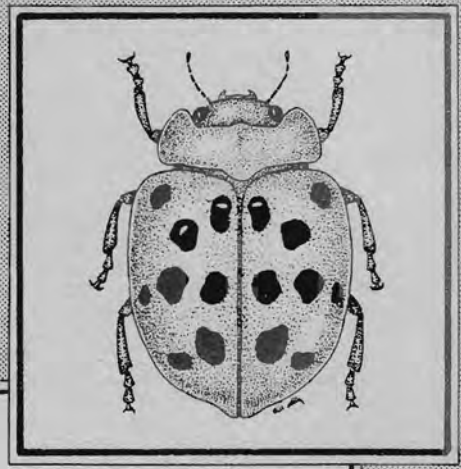

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United States Department of Agriculture

Otis Methods Development Center

Progress Report

Animal and Plant Health Inspection Service

October 1, 1980 - September 30, 1981

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

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Summary

A series of tests was conducted to evaluate methods for treating regulated articles. The contact toxicity of dust formulations of Diazinon, Pydrin, Imidan, the Pyrethroid NC6897, Permethrin, Pramex, Pyre-Kill, SBP-1382, SIR-8514 and Sumithion was evaluated and all insecticides were effective in killing larvae walking across dusted surfaces. Other tests showed, however, that many of these agents have very poor residual properties, and therefore, are not suitable for preventive treatments. It was also found that Ambush, Dimilin and SIR-8514 could be applied to soil surfaces and larvae walking over the treated areas received a lethal dose of these pesticides. This may be useful for creating an insecticidal barrier to protect against migrating large larvae. Egg masses treated with the detergents Top Job and Pine Scent, the fire extinguishing agent Light Water, and the insecticides Dylox, Dimilin, Resmethrin, Bioethanomethrin, Diazinon, Orthene and Sevin failed to hatch.

A large number of experiments were conducted with commercial formulations of Bacillus thuringiensis (Bt). The main emphasis of the work has been to improve the field performance of the formulations. Progress has been made in identifying suitable stickers which improve the deposit retention characteristics of the formulations when exposed to rainfall. Acrylocoat, RA-1990, RA-1645, Nu-Film 17 and Rhoplex B15 were all effective in improving the weathering behavior of the formulations. Similar studies show that those stickers can improve the deposit retention of pesticide sprays as well.

Sevin XLR and Sevin 4-oil were compared for weatherability, and were determined similar. It was also shown that Sevin XLR does not affect common automotive finishes significantly.

Bioassays of nursery stock treated with Carbaryl and DDT (micronized dust) showed that insufficient residue was deposited to effect significant reduction in hatch of eggs or control of 1st instar larvae.

Field tests conducted with SIR-8514, Bt and Gypchek in 1980 demonstrated that SIR-8514 effectively protected foliage and resulted in a substantial reduction in egg mass counts. The Bt treatments were effective in protecting foliage, but population reduction was not achieved. Significant problems were encountered during the application of Gypcheck and, consequently, conclusive evaluation data were not obtained. In 1981, field tests continued with SIR-8514 and Bt. In this case it was demonstrated that the growth regulator effectively protected foliage. Significantly, it was shown that a single application of 12 BIUs of Thuricide 16B, consistently achieved acceptable foliage protection. This indicates that the presently recommended 2 applications of 8 BIU can be changed to a single spray of 12 BIUs. Before recommending this spray schedule, however, confirmatory tests will be conducted in 1982.

In preparation for evaluating a field test of the inherited sterility approach, a series of studies on larval behavior have been conducted. Tests on hatch synchrony, dispersal and activity of 1st instar larvae, development and feeding rhythms of larvae, and density dependent stress factors were evaluated. In general, behavioral and developmental traits did not appear to carry over from one generation to the next. Of the density-dependent stress factors tested, only partial starvation caused measurable changes in both larval behavior and development.

Laboratory and field tests were conducted to evaluate the effects of internal marking, irradiation, packing, larval diet and pupal holding temperatures on male competitiveness. In addition, several sterile male release techniques were compared in field studies to compare relative efficiency.

Small scale trapping trials were conducted to determine the effects of traps on the incidence of mating of females. In simulated populations, mating occurred up to 4 days after males had been released into a mass trapped plot. Interestingly, very few males were captured in the traps 3 to 4 days following release, even though mating did occur. It was also demonstrated that the male:female sex ratio also greatly influences the degree to which mating can be prevented by trapping.

A demonstration project in Appleton, Wisconsin resulted in the elimination of a small infestation through the use of mass trapping. Another trial in Oconomowoc, Wisconsin, which employed a variety of control techniques (Gypchek, Sevin, mating disruption, mass trapping) has resulted in a substantial reduction in the size of the infested area. However, eradication has not yet been accomplished and additional mass trapping is planned for 1982. Small (1 ha) plots in Cotuit, Massachusetts were treated with disparlure at 5, 50 and 500 grams per hectare. These studies suggest that communication disruption is effected by chemical camouflage, and that even in very high density populations, mating can be suppressed. However, extraordinarily high airborne concentrations of disparlure are required to achieve this. Trap effectiveness in disparlure treated areas is dependent upon pheromone concentration within the trap. Males in the treated area apparently use other than chemical cues to locate and mate with females. In large scale mating disruption tests in Pennsylvania in 1980 it was shown that trap catch and mating of female moths could be reduced in areas containing low and medium egg mass densities. But, in all cases the post season egg mass counts increased. At the low population density, however, the rate of increase in the treated plots was substantially less than that in the control blocks. This suggests that the mating disruption technique may dampen population increase and have significance in slowing an outbreak.

A series of tests was conducted to evaluate Carboxide and methyl bromide as fumigants against gypsy moth egg masses. Results indicate that Carboxide can be effectively used as an ovicidal treatment against egg masses in varying stages of development. Concentration/time tables are presented.

A sustained flight wind tunnel was developed as a tool for monitoring pheromone mediated behavior of adult male gypsy moths, particularly as it relates to the competitiveness of laboratory reared sterile male insects. Tests showed that no significant differences exist between laboratory reared and field collected moths. However, it was noted that males held under cooler temperatures in the pupal stage are more prone to appetitive flight than males from warmer temperatures.

Tests concerning effects of irradiation on reproduction showed that the age at which pupae are irradiated influences the reproduction of the parental and F1 generations. Detailed observations on hatch rates and development of progeny of substerilized males has provided a basis for evaluating inherited sterility. Determining the optimum age and dose combination for inducing sterility in the F1 generation is underway.

The sterile male demonstration project in Berrien County, Michigan has shown that released males interacted with the native population and caused a reduction in the larval, adult and egg mass densities the following generation. These effects were corroborated by the observed reduction in the incidence of mating of females (by fertile males). Additional releases in 1982 will theoretically result in eradication through the use of the sterile male technique. Achieving a higher overflooding ratio in the first year of the project would have achieved that goal sooner.

In field studies and actograph (an automated activity monitoring device) tests it was shown that peak activity of laboratory reared males occurred later in the day than it did in field collected insects. By holding laboratory reared pupae at cooler temperatures, synchrony of activity of the 2 strains could be achieved. This phenomenon has been investigated to improve the competitiveness of sterile males by adjusting the activity periodicity through pupal holding conditions. In the course of these tests, gypsy moths were held under constant light from the time of hatch until adult eclosion. These insects showed a distinct circadian periodicity of activity and therefore, the circadian clock, if set from photoperiodic cues, must occur prior to hatch.

Tests demonstrating that adult Pediobius foveolatus and parasitized mummies can be stored for certain periods of time without reducing the fecundity of the parasites. While host Mexican bean beetles can be stored at low temperatures, data indicate that insects thus stored are not suitable hosts. Other tests showed that the provision of adequate food source following parasitization is critical to parasite production efficiency. Laboratory tests directed at parasite host ratios, effect of parasite age on parasitism, and identification of alternate MBB food sources during and after parasitism have led to conclusions as to how rearing efficiency can be improved. Results indicate that snap bean foliage can be replaced with certain bean sprouts without affecting parasite yield. It was again demonstrated that it is extremely critical that suitable food be available for both parasites and hosts during the entire rearing procedure.

Extensive studies of various parasite release formulae in the Mid-atlantic MBB demonstration project provided information necessary for maximizing the productivity of available parasites for release. The data demonstrate that host density is the most significant factor in maximizing parasite yield from a nurse plot. In nurse plots with low host densities, increasing the number of parasites introduced, did not appreciably increase parasite yield. Consequently, optimum release ratios will vary according to the host population density.

Other experiments demonstrate that Mexican bean beetle can be controlled in truck farms and other small plantings of green beans with Pediobius foveolatus. Frequency of pesticide application was significantly reduced in fields receiving parasite release.

Several large capacity traps were evaluated and it was demonstrated that the standard USDA 1/2 gallon milk carton trap captured more moths than those commercially available. In other studies, the delta trap and milk carton traps were compared in a detection survey in Michigan and it was demonstrated that the delta trap is significantly superior to the large capacity trap for detection.

A number of commercially prepared pheromone dispensers and insecticide strips were evaluated as trap components. Preliminary data indicate that pheromone concentration in the dispenser can be reduced without compromising trap effectiveness. However, confirmatory tests are required before this significant change is adopted. No improvements over our present DDVP killing agent were identified. Additionally, several projects are reported which evaluated various commercial sources of (+) disparlure used in the operational survey program. These evaluations form the basis for selecting material suitable for program use. Presently there are 3 commercial suppliers of (+) disparlure.

Over 7 million neonates were infested onto diet during this reporting period. They were distributed to a variety of activities in the Center and to cooperators in numerous states to facilitate their research programs. During the course of production, routine monitoring of colony performance is conducted. Effects of modification of various rearing procedures are documented and a quality control baseline is maintained. Interestingly, we have observed a gradual lengthening of the developmental time which cannot be ascribed to any modifications in the rearing protocol. To lessen rearing costs, various sources of protein used in the diet were evaluated and alternatives identified. Thorough evaluation of a mechanical egg infestation procedure indicates that the costly operation of infesting larvae onto diet can be eliminated by implementing the use of this machine, without sacrificing developmental synchrony or insect quality.

A major project concerning the evaluation of the alfalfa weevil parasite redistribution project is reported and significant differences in alfalfa weevil density, parasite density and parasite species diversity were observed throughout the project area. Essentially there is an increase in alfalfa weevil density toward the west, and this is consistent with a significant increase in both parasite species diversity and overall alfalfa weevil parasitism. The number of fields sprayed for alfalfa weevil control also showed an east/west trend. In the east, no sites were treated, in the west, 13 out of 30 fields were sprayed for alfalfa weevil control. This is a very important project which will provide on-line evaluation of the biological significance of the redistribution of parasites of the alfalfa weevil.

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Project Number: GM 6.1.5
Project Title: Regulatory Treatments
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: W. H. McLane, J. A. Finney

The main objective of this work is the development of new and improved treatments for regulated items moving from regulated areas to non-infested locations. This project is primarily directed toward the development of treatments for recreational vehicles, mobile homes and outdoor furniture.

A number of insecticide dusts have shown promises in laboratory and field tests. Plastic petri dishes were treated with a light film of insecticide dust and laboratory reared, 2 instar gypsy moth larvae were allowed to walk on the treated surface for given amounts of time. Larvae were then placed on artificial diet and mortality readings were made for a number of days. Tests always included 5 replicates and 100 test insects were exposed to each dosage and/or rate. Dust formulations were diluted in talc and a check was included with each test.

Table 1. 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						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
25	0.1	100	100	100	100	100	100	100
25	0.05	100	100	98	100	100	100	100
25	0.02	86	88	88	80	82	100	88
25	0.01	14	4	10	20	32	18	44
25	0.005	22	34	4	4	2	2	0
2	1.0	100	98	100	98	100	100	100
2	0.5	100	100	100	100	100	100	100
2	0.25	74	70	84	72	92	92	100
2	0.1	0	0	6	26	8	12	36
2	0.05	0	2	0	4	4	14	4
1	1.0	100	100	100	100	100	100	100
1	0.5	100	100	100	100	100	100	100
1	0.25	56	64	100	92	92	100	100
1	0.1	8	46	28	86	34	84	90
1	0.05	0	4	2	6	18	26	4
Control	TALC							0

Table 2. Laboratory tests with Pydrin dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams Per Dish	Percent Mortality After 8 Days Time on Treated Surface						
		10 S	30 S	1M	2 M	3 M	4 M	5 M
10	0.5	100	100	100	100	100	100	100
10	0.25	100	100	100	100	100	100	100
10	0.12	100	100	100	100	100	100	100
10	0.06	100	100	100	100	100	100	100
10	0.03	100	100	100	100	100	100	100
Check	0.5	TALC ONLY						0

Table 3. Laboratory tests with Imidan dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Mortality After 8 Days — Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
25	0.5	100	100	100	100	100	100	100
25	0.25	100	0	100	100	100	100	100
25	0.12	100	100	100	100	100	100	100
Check	0.5	TALC ONLY						0
25	0.05	100	92	98	100	88	100	98
25	0.025	94	100	72	100	70	96	88
25	0.012	64	74	50	88	44	60	64
25	0.006	48	26	6	26	10	42	4
10	0.5	100	100	100	100	100	100	100
10	0.25	100	100	100	100	100	100	100
10	0.125	100	98	86	94	96	100	100
10	0.05	34	26	58	88	78	64	48
10	0.025	6	2	6	8	10	8	8
10	0.012	0	8	12	4	6	6	0
10	0.006	2	0	2	0	0	0	2
Check	0.5	TALC ONLY						0
5	0.5	100	100	100	100	100	100	100
5	0.25	100	100	100	100	100	100	100
5	0.12	100	100	100	98	100	100	98
5	0.05	56	30	42	48	50	64	44
5	0.025	6	4	2	8	8	16	2
5	0.012	2	8	2	4	0	2	0
5	0.006	6	0	0	0	0	0	0
Check								0
1	0.5	100	100	100	96	90	100	94
1	0.25	60	30	34	50	88	82	84
1	0.12	0	20	18	24	18	34	40
1	0.05	12	12	16	4	0	6	2
1	0.025	0	2	0	2	0	6	2
1	0.012	0	0	2	0	0	0	2
1	0.006	0	0	0	0	0	0	0
Check								0

Table 4. Laboratory tests with NC 6897 dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Mortality After 8 Days — Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
25	0.5	100	100	100	190	100	100	100
25	0.1	100	100	100	100	100	100	100
25	0.05	100	98	100	100	100	100	100
25	0.025	100	100	100	100	98	100	100
25	0.012	98	94	98	98	100	100	100
25	0.006	48	84	88	80	90	50	80
Check	0.5	TALC ONLY						0
Check	0.006	TALC ONLY						0
10	0.5	100	100	100	100	100	100	100
10	0.1	100	100	100	100	100	100	100
10	0.05	100	100	100	100	100	100	100
10	0.025	100	94	92	100	100	100	96
10	0.012	24	30	44	46	70	48	46
10	0.006	2	4	24	6	52	14	16
Check	0.5	TALC ONLY						0
Check	0.006	TALC ONLY						0
1	0.5	88	88	98	78	86	88	98
1	0.1	16	30	24	42	18	30	34
1	0.05	2	14	14	26	18	24	8
1	0.025	12	2	4	14	12	10	28
1	0.012	0	4	0	6	4	4	2
1	0.006	0	2	18	2	8	0	0
Check	0.1	TALC ONLY						2
Check	0.006	TALC ONLY						2

Table 5. Laboratory tests with Permethrin dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Mortality After 8 Days — Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
10	1.0	100	100	100	100	100	100	100
10	0.5	100	100	100	100	100	100	100
10	0.25	100	100	100	100	100	100	100
10	0.125	100	100	100	100	100	100	100
10	0.062	100	100	100	100	100	100	100
10	0.031	100	100	100	100	100	100	100
10	0.015	100	100	100	100	100	100	100
10	0.007	100	100	100	100	98	100	100
CHECK	1.0	TALC ONLY						0
5	1.0	100	100	100	100	100	100	100
5	0.5	100	100	100	100	100	100	100
5	0.25	100	100	100	100	100	100	100
5	0.12	100	100	100	100	100	100	100
5	0.06	100	100	100	100	100	100	100
5	0.03	100	100	100	100	100	100	100
5	0.015	100	100	100	100	100	100	100
5	0.07	100	100	100	100	100	100	100
CHECK	1.0	TALC ONLY						0
1	1.0	100	100	100	100	100	100	100
1	0.5	100	100	100	100	100	100	100
1	0.25	100	100	100	100	100	100	100
1	0.125	100	100	100	100	100	100	100
1	0.062	100	100	100	100	100	100	100
1	0.031	100	100	100	100	100	100	100
1	0.015	100	100	100	100	100	100	100
1	0.007	100	100	100	100	100	100	100
CHECK	1.0	TALC ONLY						2

Table 6. Laboratory tests with Permethrin dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Mortality After 8 Days — Time on Treated Surface: 10 S	30 S	1 M	2 M	3 M	4 M	5 M
0.5	1.0	100	100	100	100	100	100	100
0.5	0.5	100	100	100	100	100	100	100
0.5	0.25	100	100	100	100	100	100	100
0.5	0.12	100	100	100	100	100	100	100
0.5	0.06	100	100	100	100	100	100	100
0.5	0.03	100	100	100	100	100	100	100
0.05	0.015	90	100	100	100	98	09	94
Check	NO TALC							0
Check	1.0 TALC							0
0.25	1.0	100	100	100	100	100	100	100
0.25	0.5	100	100	100	100	100	100	100
0.25	0.25	100	100	100	100	100	100	100
0.25	0.12	100	100	100	100	100	100	100
0.25	0.06	100	100	100	100	100	100	100
0.25	0.03	16	14	52	36	46	66	36
Check		NO TALC						0
Check	1.0	TALC						0

Table 7. Laboratory tests with Pramex dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams Per Dish	Percent Mortality After 8 Days — Time on Treated Surface: 10 S	30 S	1 M	2 M	3 M	4 M	5 M
10	1.0	100	100	100	100	100	100	100
10	0.5	100	100	100	100	100	100	100
10	0.25	100	100	100	100	100	100	100
10	0.12	100	100	100	100	100	100	100
10	0.06	100	100	100	100	100	100	100
10	0.03	100	100	100	100	100	100	100
10	0.015	100	100	100	100	100	100	100
10	0.0075	100	100	100	100	100	100	100
10	0.0037	100	100	100	100	100	100	100
Check								12
5	1.0	100	100	100	100	100	100	100
5	0.5	100	100	100	100	100	100	100
5	0.25	100	100	100	100	100	100	100
Check								0

Table 8. Laboratory tests with Pramex dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Mortality After 8 Days Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
1.0	1.0	100	100	100	100	100	100	100
1.0	0.06	100	100	100	100	100	100	100
Check	1.0	TALC						0
Check		NO TALC						0

Table 9. Laboratory tests with Pyre-Kill dust (W/Dispersant) against 2nd instar gypsy moth larvae.

Percent Dust	Grams Per Dish	Percent Mortality After 8 Days Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
0.2	1.0	100	100	100	100	100	100	100
0.2	.5	100	100	100	100	100	100	100
0.2	.25	100	100	96	100	100	100	100
0.2	.125	90	80	96	88	92	98	98
0.2	.06	86	78	96	88	84	90	98
0.2	.03	24	50	22	32	42	48	34
Check								0

Table 10. Laboratory tests with Pyre-Kill dust (without Dispersant) against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Motrality After 8 Days Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
0.2	1.0	100	100	100	100	100	100	100
0.2	0.5	100	100	100	100	100	100	100
0.2	0.25	100	100	100	100	100	100	100
0.2	0.12	96	100	90	96	88	96	82
0.2	0.06	82	28	66	82	94	76	80
0.2	0.03	30	36	14	20	30	22	24
Check	1.0	TALC ONLY						0

Table 11. Laboratory tests with SBP-1382 dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Mortality After 8 Days — Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
10	1.0	100	100	100	100	100	100	100
10	0.5	100	100	100	100	100	100	100
10	0.25	100	100	100	100	100	100	100
10	0.12	100	100	100	100	100	100	100
10	0.06	100	100	100	100	100	100	100
10	0.03	100	100	100	100	100	100	100
Check	1.0	TALC ONLY						0
5	1.0	100	100	100	100	100	100	100
5	0.5	100	100	100	100	100	100	100
5	0.25	100	100	100	100	100	100	100
Check	1.0	TALC ONLY						0
2	1.0	100	100	100	100	100	100	100
2	0.5	100	100	100	100	100	100	100
2	0.25	100	100	100	100	100	100	100
2	0.06	100	100	100	98	100	100	100
2	0.03	84	86	86	74	98	90	96
Check	1.0	TALC ONLY						0
1	1.0	100	100	100	100	100	100	100
1	0.5	100	100	100	100	100	100	100
1	0.25	100	100	100	100	100	100	100
1	0.12	100	100	100	90	100	100	94
1	0.06	100	96	84	98	100	100	94
1	0.03	70	58	56	54	74	68	82
Check	1.0	TALC ONLY						0
0.5	1.0	100	100	100	100	100	100	100
0.5	0.5	100	100	100	100	100	100	100
0.5	0.25	100	100	100	100	100	100	100
0.5	0.12	100	100	100	100	98	94	100
0.5	0.06	86	94	98	86	90	96	94
0.5	0.03	48	28	52	50	64	32	48
Check	1.0	TALC ONLY						1
0.25	1.0	100	100	100	100	100	100	100
0.25	0.5	100	100	100	100	100	100	100
0.25	0.25	98	100	98	100	96	100	100
0.25	0.12	92	96	98	100	100	100	96
0.25	0.06	32	38	36	38	28	38	42
0.25	0.03	12	26	32	24	42	26	24
0.25	0.015	8	28	24	12	10	0	38
Check	NO TALC							0
Check	1.0 g TALC							0

Table 12. Laboratory tests with SIR-8514 dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Mortality After 8 Days — Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
10	0.1	100	100	100	100	100	100	100
10	0.05	100	100	100	100	100	100	100
10	0.025	100	100	100	96	100	100	100
10	0.01	100	100	98	100	100	100	100
10	0.005	90	96	96	98	100	100	100
10	0.002	44	58	50	68	60	26	78
10	0.001	10	12	0	8	6	2	8
Check	0.1	TALC ONLY						2
5	0.1	100	100	100	100	100	100	100
5	0.05	100	100	100	100	100	100	100
5	0.025	100	100	100	100	100	100	100
5	0.01	100	100	100	100	100	100	100
5	0.005	94	94	100	100	100	100	100
5	0.002	66	66	78	86	92	100	100
5	0.001	2	28	12	0	42	26	18
Check	0.1	TALC ONLY						0
1	0.5	100	98	100	100	100	100	100
1	0.1	100	100	100	100	98	100	100
1	0.05	96	98	86	84	86	96	98
1	0.025	96	30	20	82	44	36	72
1	0.01	2	6	18	4	16	22	18
1	0.005	6	8	2	4	2	0	16
1	0.002	4	24	18	18	22	22	6
1	0.001	4	40	48	2	56	8	36
Check								8

Table 13. Laboratory tests with Sumithion dust against 2nd instar gypsy moth larvae.

Percent Dust	Grams per Dish	Percent Mortality After 8 Days — Time on Treated Surface						
		10 S	30 S	1 M	2 M	3 M	4 M	5 M
10	1.0	100	100	100	100	100	100	100
10	0.5	100	100	100	100	100	100	100
10	0.25	96	96	98	100	100	100	100
Check	1.0	TALC ONLY						0
5	1.0	100	100	100	100	100	100	100
5	0.5	100	100	100	100	100	100	100
5	0.25	100	100	100	100	100	100	100
5	0.12	96	88	88	98	98	92	98
5	0.06	82	50	56	74	68	76	68
5	0.03	22	54	18	48	24	12	10
Check	1.0	TALC ONLY						0
1	1.0	100	100	100	100	100	100	100
1	0.5	84	88	92	88	94	94	88
1	0.25	84	70	74	62	54	86	90
1	0.12	36	12	6	24	32	30	42
1	0.06	2	2	2	2	6	4	2
Check	1.0	TALC ONLY						

Residual properties of 14 compounds were tested under field conditions. Two-hundred petri dishes were treated with 0.5g of each formulation, inverted and stored outside in trays. A plastic bag was placed over each stack to protect dishes from wind and rain. Dishes used for checks were also stored outside. On a weekly schedule, 10 dishes from each treatment and check were bioasayed in the laboratory.

Newly molted 2nd instar larvae were exposed to the treated surface for periods of 1 or 5 minutes. One-hundred test insects were exposed to each treatment. Following treatment, larvae were placed on artificial diet. Mortality readings were made over a period of days.

Table 14. Materials tested for residue.

Material	Dosage	Dust	Spray
Ambush	0.06 lbs./gal/sol		X
Baygon	10 %	X	
Diazinon	4 %	X	
Dimilin	5 %	X	
Dimilin	0.06 lbs./gal/sol		X
Dylox	10 %	X	
Imidan	10 %	X	
NC - 6897	1 %	X	
Orthene	10 %	X	
Pramex	5%	X	
SBP - 1382	10 %	X	
Sevin	10 %	X	
SIR-8514	5 %	X	
SIR-8514	0.06 lbs./gal/sol		X
Check			

Table 15. Residue test results with 14 formulations.

Material	Minutes Exposure	Percent Mortality After 4 Days — Days of Outside Aging: 0	7	14	21	28	35	42	49	56	63	70	77
Ambush 1/	1	100	100	100	99	100	100	98	94	96	67	100	97
	5	100	100	100	100	100	100	100	100	100	100	100	92
Baygon	1	3	0	0	0	0	0	1	3	3	0	3	1
	5	6	0	0	0	0	0	1	0	1	0	3	0
Diazinon	1	0	0	1	0	0	0	0	0	0	0	2	0
	5	0	0	2	0	0	0	8	0	0	0	3	1
Dimilin 2/	1	85	88	99	97	98	100	100	100	100	100	100	98
	5	97	99	99	100	100	100	100	100	100	100	100	100
Dimilin 1/	1	87	85	66	99	100	100	100	100	100	90	100	100
	5	73	87	96	100	100	100	100	100	100	100	100	100
Dylox	1	1	0	0	0	1	0	70	2	0	4	0	0
	5	0	1	1	0	0	0	5	0	0	4	1	0
Imidan	1	22	4	3	0	1	3	0	1	16	0	5	3
	5	18	15	1	19	4	6	11	20	28	5	14	13
NC-6897	1	24	12	1	3	0	0	1	1	3	0	0	2
	5	84	9	4	6	7	3	1	12	7	0	3	11
Orthene	1	7	1	6	8	6	25	27	12	3	2	65	20
	5	3	1	45	38	3	71	39	75	51	45	21	51
Pramex	1	100	46	11	56	41	37	42	70	10	31	9	43
	5	100	77	81	54	54	100	90	93	65	52	68	48
SBP-1382	1	4	0	0	0	0	0	0	0	0	0	1	7
	5	5	0	0	0	0	2	0	2	1	0	2	8
Sevin	1	50	35	6	55	22	39	44	63	43	47	5	72
	5	83	67	15	80	80	74	2	83	68	85	2	65
SIR-8514 2/	1	99	81	99	100	100	99	97	98	100	100	96	100
	5	100	98	98	100	100	100	100	100	100	100	100	100
SIR-8514 1/	1	38	71	83	99	70	98	100	100	91	93	94	72
	5	68	86	100	100	93	99	100	100	100	96	100	84
Check	1	0	0	0	80	0	0	0	1	2	0	0	1
	5	2	0	0	15	0	0	0	0	2	0	15	5

Dimilin and SIR-8514 readings were after 14 days.
1/ Spray treatment
2/ Dust treatment

Based on the above tests with Ambush, Dimilin and SIR-8514, effective larvicidal treatment of RV's and house trailers can be accomplished.

Another approach to the regulatory problem is to apply treatments to the ground around wheels, post stands and steps. This type of treatment might prevent late instar larvae from surviving movement over the treated surface and onto the RV. Observations at a number of infested campsites indicated that large larvae move onto vehicles by going across the ground and then up a tire or other object in contact with the ground.

Three foot diameter circles were treated with 7 materials to test for contact effect on 6th instar larvae. Three locations were selected to run the tests. At location #1 treatments were applied to sandy soil; at location #2 treatments were made in wooded area and at location #3 treatments were applied to gravel.

Table 16. Materials applied to regulatory ground plots.

Material	Mix used
Ambush	0.06 lbs. AI/gal
Diazinon	1.0 lbs. AI/gal
Dimilin	0.06 lbs. AI/gal
Dylox	1.0 lbs. AI/gal
Orthene	1.0 lbs. AI/gal
Sevin	1.0 lbs. AI/gal
SIR-8514	0.06 lbs. AI/gal
Check	

All materials were applied at 0.333 gallons per plot with a hand held pump sprayer. On a weekly basis, sixth instar, laboratory reared, larvae were exposed to treatment areas. Exposure times were 1, 5 and 10 minutes. After exposure, larvae were placed on artificial diet and held in the laboratory. Mortality readings were made for a given number of days and tests were terminated when mortality dropped off. Because of the mode of action of the materials, larvae exposed to Dimilin and SIR-8514 plots were reared through to adults.

Table 17. Test results with Ambush against 6th instar larvae.

		Percent Mortality with Days of Aging									
Soil Type	Minutes Exposure	0	7	14	17	22	28	35	42	49	56
1	1	100	15	20	-	-	-	-	-		
1	5	100	65	45	-	-	-	-	-		
1	10	100	100	60	75	30	-	-	-		
2	1	95	95	75	90	90	80	75	95	85	40
2	5	100	95	80	90	100	85	40	85	95	45
2	10	100	85	90	60	100	95	55	100	95	5
3	1	100	65	75	70	-	-	-	-		
3	5	100	85	55	65	-	-	-	-		
3	10	100	40	95	70	-	-	-	-		
Check	10	0	0	0	0	0	0	0	0	0	0

Table 18. Test results with Diazinon against 6th instar larvae.

Soil Type	Minutes Exposure	Percent Mortality with Days of Aging 0	1	5	9	13	20	27	35	41	48	55
1	1	100	100	65								
1	5	100	95	80								
1	10	100	100									
2	1	100	90	100	95	90	60					
2	5	100	100	100	90	85	75					
2	10	100	100	-	100	100	90	100	90	15	75	50
3	1	100	95	95	40	85						
3	5	100	95	100	80	85						
3	10	100	100	-	95	70						
Check	10	0	0	0	0	0	0	0	0	0	0	0

Table 19. Test results with Dimilin against 6th instar larvae.

Soil Type	Min. Exp.	Percent Mortality with Days of Aging 0	2	5	9	10	17	24	30	35	41	48	55	62
1	1	100	100	100	100	80	80	75	100	85	85	60	50	65
1	5	100	100	100	100	95	100	100	100	65	95	35	30	100
1	10	100	100	100	100	95	100	100	100	80	100	95	75	100
2	1	100	100	100	100	100	100	100	100	80	100	100	100	100
2	5	100	100	100	100	100	100	100	100	100	100	100	100	100
2	10	100	100	100	100	100	100	100	100	100	100	100	100	100
3	1	100	100	100	100	100	95	100	100	55	100	75	95	100
3	5	100	100	100	100	100	100	100	100	95	100	95	100	100
3	10	100	100	100	100	100	100	100	100	100	100	85	100	100
Check	10	0	0	0	0	0	0	0	0	0	0	0	0	9

Table 20. Results of treatments with Dylox against 6th instar larvae.

Soil Type	Minutes Exposure	Percent Mortality with Days of Aging 0	2	5
1	1	100	100	70
1	5	100	100	60
1	10	100	100	-
2	1	100	100	75
2	5	100	100	100
2	10	100	100	-
3	1	100	95	75
3	5	100	100	70
3	10	100	100	-
Check	10	0	0	0

Table 21. Test results with Orthene against 6th instar larvae.

Soil Type	Minutes Exposure	Percent Mortality with Days of Aging 0	2	9	11	13
1	1	100	100	80	-	-
1	5	100	100	75	-	-
1	10	100	100	-	-	-
2	1	100	100	70	100	50
2	5	100	100	80	95	55
2	10	100	100	-	95	35
3	1	100	100	100	55	-
3	5	100	100	90	40	-
3	10	100	100	-	30	-
Check		0	0	0	0	0

Table 22. Test results with Sevin against 6th instar larvae.

Soil Type	Minutes Exposure	Percent Mortality with Days of Aging 0	2	5	9	13
1	1	100	100	55	-	-
1	5	100	100	45	-	-
1	10	100	100	-	-	-
2	1	100	100	100	85	25
2	5	100	100	90	85	95
2	10	100	100	-	70	90
3	1	100	100	85	-	-
3	5	100	100	65	-	-
3	10	100	100	-	-	-
Check						

Table 23. Test results with SIR-8514 against 6th instar larvae.

Soil Type	Min. Exp.	Percent Mortality with Days of Aging 0	2	5	9	13	20	30	35	41	48	55	62
1	1	100	100	100	100	100	100	100	100	80	95	75	95
1	5	100	100	100	100	100	100	100	95	100	80	80	100
1	10	100	100	100	100	100	100	100	100	100	100	100	100
2	1	100	100	100	100	100	100	100	100	100	100	100	100
2	5	100	100	100	100	100	100	100	100	100	100	100	100
2	10	100	100	100	100	100	100	100	100	100	100	100	100
3	1	100	100	100	100	100	100	100	100	95	70	70	100
3	5	100	100	100	100	100	100	100	100	100	90	100	100
3	10	100	100	100	100	100	100	100	100	100	95	100	100
Check	10	0	0	0	0	0	0	0	0	0	0	0	0

Dimilin and SIR-8514 continued to give high degrees of mortality of larvae after treatments had aged and weathered for 62 days. In most cases mortality took place in the pupal and/or adult stage. Larvae were not able to completely pupate, and adults did not emerge properly. The tests show Dimilin and SIR-8514 to be very effective for long periods when applied by hand sprayer to the ground. It should therefore be possible to apply effective treatments of the materials around RV wheels, post stands and steps. When larvae cross this small treated area to climb up and pupate on wheels or the underside of an RV, mortality will take place before spread is accomplished. A potential problem with this type of treatment would be the distribution of pesticides in areas frequented by people. Although other materials tested gave good results for shorter periods of time, multiple application of the materials would be necessary to protect an area for an extended period of time.

Ready to hatch, field collected, gypsy moth egg masses were treated with 5 insecticides as potential ovicides. Some formulations were also tested with emulsifiers. Some egg masses were exposed to a water wash at various times following treatment. Insecticides used were Sevin 80S, Orthene 75S, Dylox 80S, Sevin XLR and Diazinon. Triton X-180 was used as an emulsifier. Five egg masses were treated with each formulation to the point of complete saturation (3.0 ml of a 1.0 pound per gallon solution). Treatments were made with a small hand held sprayer.

Table 24. Results of egg mass treatments with liquid insectide formulations.

Formulation	Inches Rain	Hrs. After Treatment Washed	Percent Hatch
Sevin 80S	-		0
Sevin 80S + 10% X-180	-		0
Sevin 80S	0.5	1.0	20 *
Sevin 80S	0.5	3.0	0
Sevin 80S + 10% X-180	0.5	3.0	0
Sevin 80S	0.5	4.0	0
Sevin XLR			0
Sevin XLR + 10% X-180		1.0	0
Sevin XLR	0.5	1.0	0
Sevin XLR	0.5	3.0	0
Sevin XLR + 10% X-180	0.5	3.0	0
Sevin XLR	0.5	4.0	0
Diazinon AG 500			0
Diazinon AG 500 + 10 % X-180			0
Diazinon AG 500	0.5	1.0	0
Diazinon AG 500	0.5	3.0	0
Diazinon AG 500 + 10% X-180	0.5	3.0	0
Diazinon AG 500	0.5	4.0	0
Dylox 80S			0
Dylox 80S + 10% X 180			0
Dylox 80S	0.5	1.0	0
Dylox 80S	0.5	3.0	0
Dylox 80S + 10% X 180	0.5	3.0	0
Dylox 80S	0.5	4.0	0
Orthene 75S			20 *
Orthene 75S	0.5	1.0	0
Orthene 75S	0.5	3.0	0
Orthene 75S + 10% X 180	0.5	3.0	0
Orthene 75S	0.5	4.0	0
Check (No Treatment)			100
Check (10% X-180)			20

* Larvae that emerged died shortly thereafter.

Excellent control of hatch was obtained with all treatments. The emulsifier, Triton X-180, gave control as good as some insecticide treatments. It was noted that insecticides mixed with Triton X-180 penetrated the egg mass much faster and more thoroughly than mixes without.

These data indicate hatch of spring egg masses can be controlled with this type of treatment. However, most treatments of this nature employed in camp areas will be conducted during late summer and early fall against newly deposited egg masses. Efficacy against eggs in that stage of development is not known, but we expect similar results.

Laboratory produced, ready to hatch, egg masses were treated with 9 experimental formulations supplied by J. T. Baker Chemical Company on 8/24/81. Using a 10cc syringe, egg masses were treated with 1 or 2 cc of material directly into the center of the mass. Five masses were treated with each dosage and 5 were used as controls.

Table 25. Gypsy moth egg masses treated with 2cc of formulation.

Formulation	Total larvae hatched from 5 masses
8/7/01	1
8/7/02	0
8/7/03	0
8/7/04	0
8/7/05	0
8/7/06	0
8/7/07	157
8/7/09	0
Check	2,366

Table 26. Gypsy moth egg masses treated with 1cc of formulation.

Formulation	Total larvae hatched from 5 masses
8/7/01	0
8/7/02	0
8/7/03	0
8/7/04	0
8/7/05	0
8/7/06	0
8/7/07	470
8/7/08	0
8/7/09	0
Check	1,887

Test samples were stored in a freezer at -15°C for a number of days. All samples were very fluid and flowable at the low temperature.

Field tests were conducted at Nickerson State Forest, Brewster, MA, with a number of materials against late instar larvae in an extremly heavy population of gypsy moths. Five gallon and 55 gallon cans, with each end cut out, were treated with dust and liquid formulations of insecticides. Untreated cans were used as checks. Cans were placed under trees at 12 foot intervals. The objectives were to assess mortality of larvae crawling on the treated surfaces and to determine viability of eggs laid on the surfaces.

Table 27. Materials with which cans were treated.

Material	Dosage	Spray 1/	Dust 2/
Ambush	0.06 lbs./gal	X	
Diazinon	1.0 lbs./gal	X	
Diazinon	4%		X
Dimilin	0.06 lbs./gal	X	
Dimilin	25%		X
Dimilin	5%		X
Dylox	1.0 lbs./gal	X	
Dylox	80%		X
Dylox	20%		X
Orthene	1.0 lbs./gal	X	
Orthene	75%		X
Orthene	25%		X
Sevin	80%		X
Sevin	20%		X
SIR-8514	0.06 lbs./gal	X	
SIR-8514	25%		X
SIR-8514	5%		X
Check	-	-	-

1/ Spray was applied with a hand sprayer to the point of run-off.
2/ Dust was applied with a hand duster until good coverage was obtained.

On a daily basis following treatment, larvae were collected from the cans and reared on artificial diet. All treatments gave excellent control of the large larvae for a period of 2 weeks. Because of virus disease and pupation, larvae were not available after that time.

Because pupae were rare in the treated cans, we collected male and female pupae and hand placed them in cans so that we would get egg masses deposited. A screen was placed over the ends of each can to keep the adults in. So much adult mortality took place presumeably,because of the insecticides, that we were unable to acquire egg masses on the treated surfaces.

A 24 foot trailer was also parked at the campsite near the treated cans. The ground around the wheels, steps and post stand was treated with Ambush insecticide. After all pupation had taken place, the trailer was inspected and no pupae were found on the vehicle.

During general observations large larvae were observed going along the ground in large numbers. Some were seen going onto campers by way of tires, steps and stand post. Other large larvae were also seen dropping onto the camper roof from trees above. This was at a very heavy infestation where 100 percent defoliation had occurred.

The movement of gypsy moth life stages on recreational vehicles, house trailers and outdoor furniture has long been a problem in the movement and potential establishment of new infestations. With the large increase in populations throughout the northeast during the past year, the potential for movement of life stages has increased greatly.

Until now we have depended upon inspection of vehicles and insecticide treatments of campgrounds to halt the movement from infested areas. This has proved to be costly and time consuming and in some cases ineffective. We are therefore, in need of new techniques that can be used to reduce the risk of movement.

There are basically 3 forms of control that show promise: mechanical control, ovicidal treatments and larval treatments. These are discussed briefly below.

MECHANICAL CONTROL

A. Inspection and manual removal of observed life stages should continue to be the first step in certification of regulated articles. Life stages should be destroyed once removed. This can be done by burning or soaking in a solution such as kerosene.

B. Double-sided tape (such as carpet tape or Repel'M) can be placed around the tires and stand posts of RV's while parked in hazardous campgrounds. This tape will keep larvae from moving onto RV's where they may pupate and subsequently oviposit as adults.

C. Removal of tree limbs and bushes that come in contact or extend near parked RV's will prevent the movement of larvae from such objects onto RV's where they may pupate.

OVICIDAL TREATMENTS

A. In laboratory tests some detergents have been effective when sprayed and/or brushed onto egg masses. Top Job, Pine Scent and Light Water may effectively prevent hatch of gypsy moth egg masses on RV's, house trailers, outdoor furniture and other regulated articles. In order to be effective, materials must be applied until the egg mass is completely saturated. It has still not been proven that such treatments are effective throughout the egg stage.

Table 28. Test results with detergents on gypsy moth egg masses.

Material	Percent Concentration	Total Hatch in 5 Samples Treatment	Check
Top Job	100	4	1.858
	50	0	995
Pine Scent	100	0	1,209
	50	0	1,192
Light Water	10	0	794
	5	1,032	1,584

B. There are a number of insecticides that have proven to be effective against gypsy moth egg masses in laboratory tests. The direct application of Dylox, Dimilin, Resmethrin Bioethanomethrin, Orthene, Sevin and Diazinon to egg masses reduces hatch. However, these pesticides are not registered for this use.

Table 29. Results of tests in which insecticides were applied to gypsy moth egg masses.

Material	Formulation	Hatch per Egg Mass	Mortality of Neonates
Dylox 80S	40% Dust	0	
Dimilin 255W	12.5% Dust	100%	100%
Resmethrin	Liquid		
	0.597/g/10 ml	0	
	0.147/g/10 ml	0	
Bioethanomethrin	Liquid		
	0.597/g/10 ml	0	
	0.147/g/10 ml	0	
	0.035/g/10 ml	0	
Diazinon	1.0 lb/g/3 ml	0	
Orthene	1.0 lb/g/3 ml	0	
Sevin	1.0 lb/g/3 ml	0	
Check		100%	

LARVAL TREATMENT

A. During the time of larval activity, a treatment of Sevin 80S or 4 flowable to the undersides of RV's and house trailers will kill larvae contacting the treated surfaces. This can be done with a small hand sprayer at the infested location.

B. Ongoing tests indicate there are a number of insecticide dusts that can be used to treat surfaces that gypsy moth larvae may contact.

Table 30. Test results of 6 insecticide dust formulations against 2nd instar gypsy moth larvae.

Materials	Dosage	Days Aging	Percent Mortality	
			1 Min. Exp.	5 Min. Exp.
NC-6897 10%	0.05 gm	76	56	50
Bay SIR-8514 1 %	0.5 gm	76	100	100
Pramex 5%	0.25 gm	76	100	100
Resmethrin 1%	0.25 gm	76	100	96
Diazinon 1%	1.0 gm	76	84	100
Dimilin 25W 1%	0.5 gm	76	100	100
Check	-	76		0

If campers from outside the quarantined area that have visited infested areas during July and August could be identified, potentially infested vehicles could be dusted at destination during winter and spring. Such treatment would give control of any neonates resulting from hitch-hiking egg masses. When eggs hatch and neonates move across treated surfaces, mortality will occur in a short period of time.

C. Insecticides such as Ambush, Diazinon, Dimilin, Dylox, Orthene, Sevin and SIR-8514 can control large instar larvae when sprayed onto ground areas where larvae are active. Ground areas treated with Dimilin or SIR-8514 continued to kill large instar larvae 62 days following treatment. Materials could be applied to the ground area around RV wheels, post stands and steps to kill larvae selecting such locations to migrate onto RV's and/or house trailers.

Pilot testing needs to be done before we can prove that such treatments will meet with success. All data presented are from laboratory work only. However, we feel that the principles have been established and that, with proper application, the treatments described will reduce the risk of establishment of isolated infestations.

Project Number: GM 8.1.1
Project Title: Evaluation of Ultraviolet Screening Agents for Pesticides and Microbials.
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: W. H. McLane and J. A. Finney

No work was conducted with ultraviolet screening agents during this reporting period. In the future this type of work will be reported under "Laboratory Screening of Candidate Pesticides against the Gypsy Moth."

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

The objectives of this laboratory screening project are to collect and evaluate mortality data on experimental and registered compounds potentially useful for gypsy moth control, and to select materials for field studies and further development. These tests are designed to identify new materials that are active against various life stages of the gypsy moth and increase effectiveness of registered projects.

Unless otherwise stated, all tests have been conducted with our standard red oak seedling technique. Test insects are of the New Jersey strain and have been laboratory reared on artificial diet.

As the use of Bacillus thuringiensis becomes more common for gypsy moth control, scientists develop new strains and formulations in an attempt to improve its effectiveness. This may involve the development of cheaper and more effective stickers and/or ultraviolet screens or more potent formulations. During the past year we have devoted a large percentage of our time to the improvement of Bacillus thuringiensis. The following tables summarize that work.

Table 1. San 410, San 412 and HPC seedling test data.

	Dosage	Percent Mortality			
Formulation	BIU/gal	2 day	4 day	5 day	6 day
HPC	8	2	18	20	30
HPC	4	1	10	12	18
HPC	2	0	1	4	5
San-410	8	2	7	26	36
San-410	4	0	2	7	12
San-410	2	1	2	4	8
San-412	8	1	4	9	11
San-412	4	1	2	2	3
San-412	2	0	0	0	0
Check	-	-	-	-	-

Table 2. San 410, San 412 and HPC seedling test data.

Formulation	Dosage BIU/gal	Rain	Percent Mortality 2 day	4 day	5 day	6 day
HPC	8	-	34	60	71	82
HPC	8	-	10	23	56	
HPC	8	1"	1	1	1	
HPC	4	-	27	51	66	83
HPC	2	-	14	20	26	31
San-410	8	-	9	39	69	92
San-410	8	-	6	31	70	
San-410	8	1"	0	5	11	
San-410	4	-	11	44	57	81
San-410	2	-	1	20	44	66
San-412	8	-	8	22	48	72
San-412	8	-	0	7	19	
San-412	8	1"	0	1	1	
San-412	4	-	5	12	18	26
San-412	2	-	4	7	9	9
Check	-	-	0	0	0	0

Table 3. Tests with 32 BY-PH 4.5 at 8.0 BIU/acre

Rate	Rain	1% Sticker	Percent Mortality 2 Day	4 Day	6 Day
1 QT	-	-	5	59	92
1 QT	1"	-	1	7	24
2 Qts	-	-	9	47	89
2 Qts	1"	-	0	7	20
1 Gal	-	-	13	69	99
1 Gal	1"	-	0	4	20
1 Gal	-	-	19	44	99
1 Gal	1"	-	1	15	34
1 Gal	-	Acrylocoat	23	80	99
1 Gal	1"	Acrylocoat	4	22	59
1 Gal	-	RA - 1990	14	58	97
1 Gal	1'	RA - 1990	21	66	94
1 Gal	-	-	6	25	63
1 Gal	1"	-	0	1	20
1 Gal	-	TS - 30	9	37	88
1 Gal	1"	TS - 30	2	6	36
1 Gal	-	TS - 85	11	47	88
1 Gal	1"	TS - 85	2	7	36
1 Gal	-	TS - 100	8	59	87
1 Gal	1"	TS - 100	1	19	52
Check	-	-	0	0	0

Table 4. Tests with 32 BY/AS 2.5 at 8.0 BIU/acre

Rate	Rain	Sticker 1%	Percent Mortality 2 Day	4 Day	6 Day
1 QT	-	-	10	32	63
1 QT	1"	-	1	4	18
2 QTS	-	-	7	32	77
2 QTS	1"	-	0	2	26
1 Gal	-	-	19	61	80
1 Gal	1"	-	2	18	41
1 Gal	-	-	15	63	96
1 Gal	1"	-	1	8	33
1 Gal	-	Acrylocoat	21	54	96
1 Gal	1"	Acrylocoat	5	45	93
1 Gal	-	RA - 1990	14	53	86
1 Gal	1"	RA - 1990	8	49	96
1 Gal	-	-	3	29	78
1 Gal	1"	-	2	9	32
1 Gal	-	TS - 30	9	43	82
1 Gal	1"	TS - 30	1	4	33
1 Gal	-	TS - 85	14	49	83
1 Gal	1"	TS - 85	1	12	42
1 Gal	-	TS - 100	7	43	88
1 Gal	1"	TS - 100	4	11	29
Check	-	-	0	0	0

Table 5. Test with 16B at 8.0 BIU/gallon/acre

Rain	Percent Mortality 2 Day	4 Day	5 Day	6 Day
--	20	91	98	100
1"	1	16	17	18
Check	0	0	0	0

Tests were conducted with Thuricide 16B and Dipel 4L to determine feeding repellency. Figures in Table 6 represent percentage of feeding that occurred on oak leaf discs over a given period of time. Controls received no treatment.

Table 6. Feeding responses of 2nd instar gypsy moth larvae exposed to paired leaf discs treated with formulations of BT.

	T-16B	D-4L	Control	T-16B	D-4L	Control
Spray tower application	17.5	12.4		63.1	41.4	
Spray tower application	21.2		38.6	72.1		84.8
Spray tower application		15.0	65.9		70.9	98.8
Brush application	.25	.9		1.9	1.5	
Brush application	.25		12.3	5.2		32.6
Brush application		2.0	20.2		3.7	59.2

Table 7. Weathering tests with Thuricide 16B, 32B, Dipel 4L with and without stickers. Material was applied at 8 BIU/a. Amount of sticker was 3% of total volume of solution.

Material	Sticker	Rain	Percent Larval Mortality					
			24H	48H	72H	96H	120 H	144 H
Thuricide 16B	-	-	-	9	29	60	86	96
"	-	1"	-	0	0	4	12	17
"	Acrylocoat	"	-	0	3	8	19	24
"	RA -1990	"	-	0	4	12	21	27
Thuricide 32B	-	-	-	8	18	50	75	93
"	-	1"	-	3	5	20	40	49
"	Acrylocoat	"	-	2	22	73	84	99
"	RA -1990	"	-	10	29	66	84	93
Dipel 4L	-	-	-	14	23	60	80	90
"	-	1"	-	0	0	0	0	0
"	Acrylocoat	"	-	1	2	9	20	27
"	RA - 1990	"	-	3	11	25	40	59
Control	-	-	-	0	0	1	1	1
"	-	1"	-	0	0	0	0	1

Table 8. Weathering tests with Thuricide 16B and Dipel 4L with and without stickers. Material was applied at 8 BIU/a. Stickers comprised 3% of the volume of solution.

Material	Sticker	Rain	24H	48H	72H	96H	120 H	144 H
Dipel 4L	-	-	-	21	36	53	66	79
"	-	1"	-	0	1	2	4	6
"	Acrylocoat	-	-	11	26	53	70	90
"	"	1"	-	7	7	30	49	67
"	RA-1645	-	-	11	24	43	62	82
"	"	1"	-	1	6	16	26	42
"	RA-1990	-	-	20	42	70	88	94
"	"	1"	-	3	13	33	50	64
Thuricide 16B	-	-	-	27	37	65	89	95
"	-	1"	-	0	0	2	3	3
"	Acrylocoat	-	-	16	29	57	83	92
"	"	1"	-	1	4	12	36	56
"	RA-1645	-	-	15	31	49	76	89
"	"	1"	-	0	2	3	12	22
"	RA-1990	-	-	20	29	65	92	99
"	"	1"	-	0	1	3	12	20
Control	-	-	-	0	0	0	0	0
	-	1"	-	0	0	0	0	0

Table 9. Weathering tests with Thuricide 16B, 32B and Dipel 4L without stickers. Material was applied at 8 BIU/a and allowed to dry 120 min. before exposure to rain.

Material	Rain	PERCENT LARVAL MORTALITY					
		24 Hr	48 Hr	72 Hr	96 Hr	120 Hr	144 Hr
Dipel 4L	-	2	6	16	61	84	94
	.5"	0	0	1	9	20	42
	1"	0	0	2	5	12	36
	2"	0	0	2	3	11	24
Thuricide 32B	-	2	17	39	65	81	97
	.5"	0	4	8	26	60	89
	1"	0	4	13	37	62	92
	2"	0	0	2	14	46	27
Thuricide 16B	-	0	7	18	72	91	100
	.5"	0	0	1	13	31	76
	1"	0	1	5	28	54	82
	2"	0	0	2	17	43	70
Control	-	0	0	0	0	1	5

Table 10. Weathering tests with 8 BIU Thuricide 32B and 16B, and Dipel 4L applied at various rates.

Material	Rate qt/a	Drying Time(HR)	Rain	Percent Larval Mortality After 24 Hr	48 Hr	72 Hr	96 Hr	120Hr	144Hr
Thuricide 32B	4	-	-	1	10	28	70	-	98
Dipel 4L	4	-	-	2	8	28	52	-	96
Thuricide 16B	4	-	-	2	13	34	64	-	98
	4	2	1"	3	16	-	99	-	100
	4	2	2"	0	0	4	15	-	62
	4	2	3"	0	0		4	-	12
	4	24	3"	-	1	-	-	1	1
	4	24	3"	0	0	-	-	7	9
Thuricide 32B	2	-	-	0	9	-	-	91	99
Dipel 4L	2	-	-	2	14	-	-	85	95
Thuricide 16B	2	-	-	-	18	32	81	-	98
Thuricide 32B	2	2	1"	0	0	1	4	-	18
Dipel 4L	2	2	1"	0	0	0	0	-	0
Thuricide 16B	2	2	1"	0	-	-	5	-	6
Thuricide 32B	2	2	0	0	-	2	-	18	
Dipel 4L	2	2	2"	0	0	-	0	-	1
Thuricide 16B	2	2	2"	-	-	3	5	12	14
Thuricide 32B	2	2	3"	-	0	-	-	4	4
Dipel 4L	2	2	3"	-	1	-	-	1	1
Thuricide 16B	2	2	3"	0	-	3	-	-	9
Thuricide 32B	2	24	3"	0	1	-	-	5	5
Dipel 4L	2	24	3"	0	1	-	-	5	6
Thuricide 16B	2	24	3"	0	0	0	-	-	4
Thuricide 32B	1	-	-	-	21	34	64	-	97
Dipel 4L	1	-	-	-	7	22	53	-	
Thuricide 32B	1	2	1"	0	-	-	5	-	17
Dipel 4L	1	2	1"	0	-	-	1	-	3
Thuricide 32B	1	2	2"	-	-	2	4	8	13
Dipel 4L	1	2	2"	-	-	0	0	0	0
Thuricide 32B	1	2	3"	-	-	1	-	-	2
Dipel 4L	1	2	3"	0	-	1	-	-	2
Thuricide 32B	1	24	3"	0	1	1	-	-	2
Dipel 4L	1	24	3"	0	0	0	-	-	2
Control	-	-	-	0	0	0	0	0	0

Table 11. Weathering tests with Thuricide 32B and Dipel 4L with various stickers. Treatments were applied at 8 BIU/a and dried for a minimum of 1.5 hours. Sticker was 3% of total volume of material.

			Percent Larval Mortality After					
Material	Sticker	Rain	24 Hr	48 Hr	72 Hr	96 Hr	120 Hr	144 Hr
Thuricide 32B	-	-	0	5	15	53	71	84
	-	-	0	1	7	50	77	92
	-	1"	0	0	1	9	14	17
	-	2"	0	0	0	7	19	21
	RA-1990	1"	1	6	16	51	77	96
	RA-1990	2"	1	4	20	48	79	90
	RA-1645	1"	3	11	27	70	88	98
	RA-1645	2"	1	5	27	50	73	84
	Acrylocoat	1"	0	9	23	56	71	89
	Acrylocoat	2"	1	3	20	48	70	85
	Nu-Film 17	1"	0	0	1	2	3	4
	Nu-Film 17	2"	1	2	7	14	18	19
	Chevron	1"	0	0	1	2	2	2
Dipel 4L	-	-	0	0	7	29	49	71
	-	-	1	1	8	33	73	87
	-	1"	0	0	0	1	2	2
	-	2"	0	0	0	0	0	0
	RA-1990	1"	1	4	11	33	40	52
	RA-1990	2"	1	6	12	22	27	33
	RA-1645	1"	0	0	5	11	33	43
	RA-1645	2"	0	2	6	9	18	20
	Acrylocoat	1"	0	0	2	8	15	18
	Acrylocoat	2"	0	0	2	3	4	5
	Nu-Film 17	1"	0	0	1	1	1	1
	Nu-Film 17	2"	0	0	0	0	0	0
	Chevron	1"	0	0	0	0	0	0
Control	-	-	0	0	0	0	0	0
	-	-	0	0	0	0	0	0
	-	1"	0	0	0	0	0	0

Table 12. Weathering tests with Dipel 4L and Thuricide 32B with various stickers. Materials were applied at 8 BIU/a. At least 2 hrs lapsed between treatment and weathering of plants.

Material	Sticker	Rain	PERCENT LARVAL MORTALITY					
			24 Hr	48 Hr	72 Hr	96 Hr	120 Hr	144 Hr
Dipel 4L	1% RA-1645	1"	0	9	16	38	72	79
	1% RA-1645	2"	0	3	4	17	53	71
	1% RA-1645	3"	0	5	8	13	26	54
	3% RA-1645	2"	2	8	22	48	82	95
	3% RA-1645	3"	2	2	8	27	77	95
	1% Acrylocoat	1"	0	0	1	1	16	53
	1% Acrylocoat	2"	0	0	0	0	8	54
	1% Acrylocoat	3"	0	0	0	1	15	54
	3% Acrylocoat	2"	0	0	4	10	24	59
	3% Acrylocoat	3"	0	2	4	5	22	44
Thuricide	1% RA-1645	1"	0	5	23	63	96	100
	1% RA-1645	2"	0	11	28	62	90	100
	1% RA-1645	3"	5	10	27	54	86	100
	3% RA-1645	2"	0	14	33	88	100	
	3% RA-1645	3"	0	6	16	57	90	98
	1% Acrylocoat	1"	0	1	13	36	58	94
	1% Acrylocoat	2"	0	1	7	20	51	88
	2% Acrylocoat	2"	0	0	11	34	54	84
	3% Acrylocoat	2"	2	19	49	81	87	95
	3% Acrylocoat	3"	1	5	30	67	79	95
Control	-	-	0	0	0	0	0	0
	-	-	-	-	-	1	2	31

Table 13. Laboratory tests with Dipel 4L with and without stickers. Materials were applied at 8 BIU/a.

3% Sticker	Drying Time (Min.)	Rain	PERCENT LARVAL MORTALITY 24 Hr	48 Hr	72 Hr	96 Hr	120 Hr	144 Hr
BT only	-	-	4	16	42	79	94	97
RA-1645	-	-	1	3	28	53	74	84
RA-1645	15	1"	0	0	5	18	42	63
RA-1645	30	1"	0	2	4	15	49	73
RA-1645	60	1"	1	0	3	24	48	75
RA-1645	120	1"	1	2	8	21	48	80
Control	-	-	0	0	0	1	3	25
Bt only	-	-	-	16	27	45	76	97
Acrylocoat	-	-	-	13	27	41	78	95
Acrylocoat	15	1"	-	8	9	11	17	64
Acrylocoat	30	1"	-	0	2	10	28	72
Acrylocoat	60	1"	-	5	7	8	28	70
Acrylocoat	120	1"	-	13	17	29	42	59
Control	-	-	-	0	1	1	4	19
Control	-	1"	-	0	1	1	3	19
Bt only	-	-	-	16	26	36	67	89
Rhoplex B-15	-	-	-	24	32	47	69	86
Rhoplex B-15	15	1"	-	4	8	17	27	60
Rhoplex B-15	30	1"	-	3	5	9	16	36
Rhoplex B-15	60	1"	-	3	4	9	30	74
Rhoplex B-15	120	1"	-	2	3	11	21	74
Control	-	-	-	0	0	1	3	3
Control	-	1"	-	0	0	0	0	3

Table 14. Laboratory tests with Thuricide 16B and 32B at various rates. Plants were dried at least 1 hr before exposure to rain.

Material	Dosage (BIU)	Rate (qt)	Rain	Sticker	PERCENT LARVAL MORTALITY 24 Hr	48 Hr	72 Hr	96 Hr	120 Hr
Thuricide 16B	8	4	-	-	10	25	49	79	97
	8	4	1"	.2% C-13	0	2	9	42	77
	8	2	-	-	0	3	16	29	51
	8	2	1"	.2% C-13	0	2	7	24	47
	8	2	1"	.2% C-13	0	0	11	41	81
	4	1	-	-	2	6	14	33	71
	4	1	1"	-	0	0	1	8	36
	4	1	1"	.2% C-13	0	0	0	5	10
Thuricide 32B	8	4	-	-	1	10	36	71	95
	8	4	1"	.5% C-13	0	8	44	89	99
	8	2	-	-	6	19	31	58	89
	8	2	1"	-	0	5	14	48	79
	8	2	1"	.2% C-13	0	6	9	58	84
	4	1	-	-	5	13	22	55	68
	4	1	1"	-	-	1	10	28	52
	4	1	1"	.2% C-13	0	1	4	27	55
Control	-		-	-	0	0	0	0	0
Control	-		1"	-	0	0	0	0	0

Table 15. Laboratory tests with 8 BIU Thuricide 16B against gypsy moth larvae.

Rate (gal/a)	Rain	Sticker	PERCENT LARVAL MORTALITY 4 Days	8 Days	10 Days
2 1/	-	-	36	98	100
2	2"	-	0	18	22
1 1/	-	-	35	90	100
1	2"	-	0	0	5
1	-	3% RA-1645	21	83	95
1	2"	3% RA-1645	2	8	11
1	-	3% NuFilm	40	87	96
1	2"	3% NuFilm	0	1	1
1	-	3% Bond	36	85	92
1	2"	3% Bond	0	5	9
1 2/	2"	5% NuFilm	0	9	9
1	2"	5% Bond	16	74	86
1	2"	5% CIB	0	0	2
1	2"	5% Target NL	0	14	21
1	2"	5% Rhoplex	2	15	20
1	2"	5% Chevron	0	7	7
1	2"	5% Mobait	0	1	1
1	2"	5% Plyac	0	0	0
1 3/	2"	3% NuFilm	0	0	0
1	2"	-	0	0	0
1	2"	3% Plyac	0	10	27
1	2"	3% Mobait	0	1	1
1	2"	3% Chevron	0	2	4
1	2"	3% Rhoplex	0	5	13
1	2"	3% Target NL	1	11	17
1	2"	3% CIB	0	0	8
1	2"	3% Bond	1	2	5
.66		-	37	92	100
.66	2"	-	0	1	1
.5 4/	-	-	15	87	100
.5	2"	-	0	3	4
.5	2"	3% Bond	0	0	0
.5	2"	3% Plyac	0	0	0
.5	2"	3% Chevron	0	4	12
.5	2"	3% NuFilm	0	1	13
.5	2"	3% Rhoplex	0	2	4
.5	2"	3% Mobait	1	2	3
Control	-	-	0	0	0

1/ Plants treated with rain immediately following treatment with Thuricide.
2/ Plants treated with rain 2.5 hrs after treatment with Thuricide.
3/ Plants treated with rain 24 hrs after treatment with Thuricide.
4/ Plants treated with rain 3 hrs after treatment with Thuricide.

Table 16. Tests results with 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 17. Laboratory test of Dipel 4L at 1.0 BIU/acre and Pro Tec.

Formulation	PERCENT MORTALITY			
	2 Day	5 Day	6 Day	7 Day
No ProTec	1	13	37	57
Pro Tec A 10:1	1	7	10	25
Pro Tec A 5:1	0	12	26	38
Pro Tec B 10:1	1	23	34	48
Pro Tec B 5:1	0	22	41	55
Check	0	0	0	5

Table 18. Laboratory weathering test with Dipel 4L at 1.0 BIU/acre and ProTec.

Formulation	Inches Rain	PERCENT MORTALITY		
		2 Day	4 Day	7 Day
No Pro Tec	-	0	0	3
No Pro Tec	0.5	0	0	0
No Pro Tec	1.0	0	0	1
No Pro Tec	2.0	0	0	0
Pro Tec A 10:1	-	1	2	15
Pro Tec A 10:1	0.5	0	3	6
Pro Tec A 10:1	1.0	0	4	9
Pro Tec A 10:1	2.0	1	2	4
Pro Tec A 5:1	-	0	1	4
Pro Tec A 5:1	0.5	0	3	8
Pro Tec A 5:1	1.0	1	7	10
Pro Tec A 5:1	2.0	0	2	8
Pro Tec B 10:1	-	0	2	4
Pro Tec B 10:1	0.5	0	0	1
Pro Tec B 10:1	1.0	0	1	2
Pro Tec B 10:1	2.0	0	2	4
Pro Tec B 5:1	-	0	3	11
Pro Tec B 5:1	0.5	0	4	8
Pro Tec B 5:1	1.0	0	0	4
Pro Tec B 5:1	2.0	0	0	3
Check	-	0	0	0

Table 29. Weathering test with Thuricide 16B, 32B and Dipel 4L at 8 BIU/acre.

Material	Sticker	Inches Rain	PERCENT MORTALITY 2 Day	4 Day	6 Day
Thuricide 16B	-	-	9	60	96
Thuricide 16B	-	1.0	0	4	17
Thuricide 16B	3% Acrylocoat	1.0	0	8	24
Thuricide 16B	3% RA-1990	1.0	0	12	27
Thuricide 32B	-	-	8	50	93
Thuricide 32B	-	1.0	3	20	49
Thuricide 32B	3% Acrylocoat	1.0	2	73	99
Thuricide 32B	3% RA-1990	1.0	10	66	93
Dipel 4L	-	-	14	60	90
Dipel 4L	-	1.0	0	0	0
Dipel 4L	3% Acrylocoat	1.0	1	9	27
Dipel 4L	3% RA-1990	1.0	3	25	59
Check	-	1.0	0	0	0

Table 30. Weathering tests with Bacillus thuringiensis formulations using 8 BIU/acre and droplets of 200 microns (mmd).

Material	Sticker	Inches Rain	PERCENT MORTALITY 2 Day	4 Day	6 Day
Thuricide 16B	-	-	27	65	95
Thuricide 16B	-	1.0	0	2	3
Thuricide 16B	3% Acrylocoat	-	16	57	92
Thuricide 16B	3% Acrylocoat	1.0	1	12	56
Thuricide 16B	RA-1645	-	15	49	89
Thuricide 16B	RA-1645	1.0	0	3	22
Thuricide 16B	RA-1990	-	20	65	99
Thuricide 16B	RA-1990	1.0	0	3	20
Dipel 4L	-	-	21	53	79
Dipel 4L	-	1.0	0	2	6
Dipel 4L	3% Acrylocoat	-	11	53	90
Dipel 4L	3% Acrylocoat	1.0	7	30	67
Dipel 4L	3% RA-1645	-	11	43	82
Dipel 4L	3% RA-1645	1.0	1	16	42
Dipel 4L	3% RA-1990	-	20	70	94
Dipel 4L	3% RA-1990	1.0	3	33	64
Check	-	1.0	0	0	0

Table 31. Weathering test with Bacillus thuringiensis formulations using 8 BIU/acre and droplets of 75 microns (mmd).

Material	Sticker	Inches Rain	PERCENT MORTALITY 2 Day	4 Day	6 Day
Thuricide 16B	-	-	12	71	90
Thuricide 16B	-	1.5	0	4	7
Thuricide 16B	3% Acrylocoat	-	12	68	97
Thuricide 16B	3% Acrylocoat	1.5	0	11	23
Thuricide 16B	3% RA-1990	-	19	67	92
Thuricide 16B	3% RA-1990	1.5	0	10	19
Dipel 4L	-	-	10	56	95
Dipel 4L	-	1.5	0	1	2
Dipel 4L	3% Acrylocoat	-	17	69	87
Dipel 4L	3% Acrylocoat	1.5	0	1	3
Dipel 4L	3% RA-1990	-	5	54	98
Dipel 4L	3% RA-1990	1.5	6	44	90
Check	-	-	0	0	0
Check	-	1.5	0	0	0

Table 32. Weathering tests with Dipel 4L and Thuricide 16B. Treatments were dried 2 hrs before weathering.

Material	Dosage (BIU)	Inches Rain	PERCENT MORTALITY 3 Day	4 Day	6 Day
Thuricide 16B	8	-	19	67	89
Thuricide 16B	8	1.0	0	1	2
Thuricide 16B	8	2.0	0	0	2
Thuricide 16B	4	-	28	65	93
Thuricide 16B	4	1.0	0	1	2
Thuricide 16B	4	2.0	0	0	2
Thuricide 16B	2	-	20	55	92
Thuricide 16B	2	1.0	0	1	3
Thuricide 16B	2	2.0	0	0	0
Dipel 4L	8	-	20	54	78
Dipel 4L	8	1.0	1	1	3
Dipel 4L	8	2.0	1	1	2
Dipel 4L	4	-	15	28	58
Dipel 4L	4	1.0	1	1	2
Dipel 4L	4	2.0	0	1	1
Dipel 4L	2	-	2	17	25
Dipel 4L	2	1.0	0	1	1
Dipel 4L	2	2.0	0	0	1
Check		2.0	0	0	0

Table 33. Weathering tests with Dipel 4L and Thuricide 16B at 8 BIU/acre.

Material	Sticker	Inches Rain	PERCENT MORTLITY 2 Day	4 Day	5 Day
Dipel 4L	-	-	26	86	97
Dipel 4L	-	1.0	1	6	14
Dipel 4L	3% RA-1645	-	19	61	92
Dipel 4L	3% RA-1645	1.0	6	35	66
Dipel 4L	3% RA-1990	-	3	36	54
Dipel 4L	3% RA-1990	1.0	9	43	76
Dipel 4L	3% Acrylocoat	-	22	75	99
Dipel 4L	3% Acrylocoat	1.0	0	33	33
Dipel 4L	3% Vaporgard	-	5	44	75
Dipel 4L	3% Vaporgard	1.0	0	0	5
Thuricide 16B	-	-	19	73	91
Thuricide 16B	-	1.0	0	8	14
Thuricide 16B	3% RA-1645	-	18	77	94
Thuricide 16B	3% RA-1645	1.0	1	6	38
Thuricide 16B	3% RA-1990	-	12	70	92
Thuricide 16B	3% RA-1990	1.0	0	4	18
Thuricide 16B	3% Acrylocoat	-	19	78	96
Thuricide 16B	3% Acrylocoat	1.0	1	6	28
Thuricide 16B	3% Vaporgard	-	47	94	100
Thuricide 16B	3% Vaporgard	1.0	1	8	30
Check	-	-	0	0	0

Table 34. Weathering tests with 4 Bacillus thuringiensis formulations at 8 BIU/acre.

Formulation	Sticker	Inches Rain	PERCENT MORTALITY 2 Day	4 Day	6 Day
Thuricide 16B	-	-	9	60	96
Thuricide 16B	-	1.0	0	4	17
Thuricide 16B	3% Acrylocoat	1.0	0	8	24
Thuricide 16B	3% RA-1990	1.0	0	12	27
Thuricide 32B	-	-	8	50	93
Thuricide 32B	-	1.0	3	20	49
Thuricide 32B	3% Acrylocoat	1.0	2	73	99
Thuricide 32B	3% RA-1990	1.0	10	66	93
Thuricide 32BX	-	-	8	42	91
Thuricide 32BX	-	1.0	0	3	22
Thuricide 32BX	3% Acrylocoat	1.0	8	53	94
Thuricide 32BX	3% RA-1990	1.0	6	44	80
Dipel 4L	-	-	14	60	90
Dipel 4L	-	1.0	0	0	0
Dipel 4L	3% Acrylocoat	1.0	1	9	27
Dipel 4L	3% RA-1990	1.0	3	25	59
Check	-	-	0	0	0
Check	-	1.0	0	0	0

Table 19. Laboratory weathering test with Dipel 4L at 4 BIU/acre and Pro Tec.

Formulation	Inches Rain	PERCENT MORTALITY 1 Day	2 Day	5 Day
Pro Tec A 5:1	-	0	0	6
Pro Tec A 5:1	1.0	0	0	8
Pro Tec A 10:1	-	0	2	12
Pro Tec A 10:1	1.0	0	1	4
Pro Tec B 5:1	-	0	0	15
Pro Tec B 5:1	1.0	0	0	4
Pro Tec B 10:1	-	0	1	4
Pro Tec B 10:1	1.0	0	0	3
Check	-	0	0	0

Table 20. Laboratory weathering test with Dipel 4L at 8 BIU/acre and Pro Tec.

Formulation	Inches Rain	PERCENT MORTALITY 2 Day	3 Day	4 Day	7 Day
No Pro Tec	-	23	44	53	96
No Pro Tec	1.0	1	3	4	6
Pro Tec A 5:1	-	27	43	54	98
Pro Tec A 5:1	1.0	2	4	10	49
Pro Tec A 10:1	-	16	30	47	98
Pro Tec A 10:1	1.0	2	2	2	16
Pro Tec B 5:1	-	20	37	50	93
Pro Tec B 5:1	1.0	2	2	4	19
Pro Tec B 10:1	-	11	28	36	87
Pro Tec B 10:1	1.0	4	4	6	26
Check	-	0	0	0	0

Table 21. Laboratory weathering test with Dipel 4L at 8 BIU/acre and Pro Tec.

Formulation	Inches Rain	PERCENT MORTALITY 2 Day	3 Day	6 Day
No Pro Tec	-	21	42	93
No Pro Tec	0.5	0	0	2
Pro Tec A 5:1	-	21	42	84
Pro Tec A 5:1	0.5	6	7	39
Pro Tec A 10:1	-	26	35	90
Pro Tec A 10:1	0.5	8	10	44
Pro Tec B 5:1	-	15	27	87
Pro Tec B 5:1	0.5	0	0	20
Pro Tec B 10:1	-	16	29	91
Pro Tec B 10:1	0.5	1	2	12
Check	-	0	0	0

Table 22. Laboratory weathering test with Dipel 4L at 8 BIU/acre and Pro Tec.

Formulation	Inches Rain	PERCENT MORTALITY 2 Day	4 Day	5 Day	7 Day
No Pro Tec	-	13	40	79	96
No Pro Tec	1.0	2	5	9	16
Pro Tec A 40:1	-	16	47	63	88
Pro Tec A 40:1	1.0	4	9	17	41
Pro Tec B 40:1	1.0	4	8	12	29
Check	-	0	0	0	0

Table 23. Laboratory weathering test with Thuricide 16B at 8 BIU/acre and Pro Tec.

Formulation	Inches Rain	PERCENT MORTALITY 2 Day	4 Day	5 Day	7 Day
No Pro Tec	-	8	46	70	88
No Pro Tec	1.0	0	2	4	6
Pro Tec A 5:1	-	5	36	60	87
Pro Tec A 5:1	1.0	2	30	56	91
Pro Tec B 5:1	-	7	40	71	95
Pro Tec B 5:1	1.0	0	36	57	88
Check	-	0	0	0	0

Table 24. Laboratory weathering test with Dipel 4L at 8 BIU/acre and Pro Tec.

Formulation	Inches Rain	PERCENT MORTALITY 2 Day	5 Day	7 Day
No Pro Tec	-	13	93	95
No Pro Tec	1.0	0	14	20
Pro Tec A 1:1	1.0	3	57	69
Pro Tec B 1:1	1.0	2	31	44
Check	-	0	0	0

Table 25. Laboratory weathering test with Thuricide 32 BX at 8.0 BIU/acre and Pro Tec.

Formulation	Inches Rain	PERCENT MORTALITY 2 Day	5 Day	7 Day
No Pro Tec	-	5	75	95
No Pro TEc	1.0	0	23	63
Pro Tec A 5:1	1.0	1	38	81
Pro Tec A 10:1	1.0	2	36	81
Pro Tec A 1:1	1.0	8	75	98
Pro Tec B 5:1	1.0	0	13	76
Pro Tec B 10:1	1.0	4	36	55
Pro Tec B 1:1	1.0	2	66	78
Check	-	0	0	0

Table 26. Weathering test with Bacillus thuringiensis (Bugtime) at 8 BIU/acre.

Inches Rain	Sticker	PERCENT MORTALITY 2 Day	4 Day	6 Day
-	-	2	12	50
1.0	-	0	0	0
2.0	-	0	1	1
3.0	-	1	2	3
1.0	3% RA-1990	2	3	10
1.0	3% Acrylocoat	0	0	0
Check	-	0	0	0

Table 27. Weathering test with Thuricide 32B at 8 BIU/acre.

Inches Rain	Sticker	PERCENT MORTALITY 2 Day	4 Day	6 Day
-	-	23	76	100
1.0	-	1	9	18
2.0	-	0	4	6
3.0	-	0	7	14
1.0	3% RA-1990	17	72	97
1.0	3% Acrylocoat	10	50	97
Check	-	0	0	0

Table 28. Weathering test with Dipel 4L at 8 BIU/acre.

Inches Rain	Sticker	PERCENT MORTALITY 2 Day	4 Day	6 Day
-	-	17	50	95
1	-	0	0	0
2	-	0	2	4
3	-	0	0	0
1	3% Acrylocoat	0	0	8
1	3% RA-1990	0	0	0
Check		0	0	0

Table 35. Simulated ground applications with Thuricide 16B.

Dosage	Rate	Application Method	PERCENT MORTALITY 3 Day	7 Day
2 BIU	400 gal	Dip	5	49
4 BIU	400 gal	Dip	11	55
8 BIU	400 gal	Dip	10	78
16 BIU	400 gal	Dip	28	96
32 BIU	400 gal	Dip	29	96
48 BIU	400 gal	Dip	28	97
64 BIU	400 gal	Dip	37	100
2 BIU	400 gal	Brushed	1	54
4 BIU	400 gal	Brushed	3	51
8 BIU	400 gal	Brushed	2	65
16 BIU	400 gal	Brushed	6	8
32 BIU	400 gal	Brushed	10	93
48 BIU	400 gal	Brushed	17	85
64 BIU	400 gal	Brushed	13	89
32 BIU	200 gal	Dip	34	100
16 BIU	200 gal	Dip	42	100
8 BIU	200 gal	Dip	30	99
32 BIU	200 gal	Brushed	11	99
16 BIU	200 gal	Brushed	23	96
8 BIU	200 gal	Brushed	14	86
Check		-	0	0

Table 36. Simulated ground applications with Dipel 4L.

Dosage	Rate	Application Method	PERCENT MORTALITY 3 Day	7 Day
64 BIU	200 gal	Brushed	8	80
32 BIU	200 gal	Brushed	4	79
16 BIU	200 gal	Brushed	1	77
8 BIU	200 gal	Brushed	0	74
4 BIU	200 gal	Brushed	2	47
64 BIU	200 gal	Dip	32	99
32 BIU	200 gal	Dip	17	93
16 BIU	200 gal	Dip	6	52
8 BIU	200 gal	Dip	4	43
4 BIU	200 gal	Dip	4	45
Check		-	0	26

Table 37. Weathering tests with 8 BIU Dipel 4L.

Sticker	Rain	PERCENT MORTALITY 24 Hrs	48 Hrs	72 Hrs	144 Hrs
-	-	2	15	61	100
-	1"	1	4	30	83
Acrylocoat	-	2	6	56	100
Acrylocoat	1"	1	13	48	100
Check	-	0	0	0	0

Although substantial progress has been made in improving Bacillus thuringiensis formulations, further improvements leading to greater consistency of field applications and lower cost are needed. New stickers such as Acrylocoat, RA-1990, TS 100, TS 85 and TS 30 may prove to make the material more efficacious, possibly eliminating the need for multiple applications.

Various formulations of Carbaryl have been developed for gypsy moth control. Some are used strictly for aerial application while others for both aerial and ground treatments. In general, most applications are effective in controlling the pest. However, some treatments with mist blowers and hydraulic equipment have not been successful and a second application is required to control migrating larvae. This lack of activity may partially be due to the poor weathering characteristics of the formulation being used.

Tests were conducted according to the standard oak seedling techique. Second instar, laboratory reared, gypsy moth larvae were used as test insects. All dosages were replicted 5 times with 20 larvae/replicate. Test chambers were held at 27°C and 55% RH. Twenty-four hour mortality readings were made on each test dosage and check.

Rain for weathering was produced in the laboratory using a Delavan raindrop nozzle.

Table 38. Laboratory tests with Sevimol 4 applied at 1.0 lbs AI/ac.

Ant Sticker/100 gal	Inches Rain	Percent Mortality after 5 days
-	-	98
-	1	38
-	3	18
Acrylocoat 3 gal	3	99
Acrylocoat 1 gal	3	86
Acrylocoat 2 qt	3	95
Acrylocoat 1 qt	3	44
Acrylocoat 1 pt	3	7
Acrylocoat 8 oz	3	6
Acrylocoat 4 oz	3	20 *
RA-1990 3 gal	3	96
RA-1990 1 gal	3	90
RA-1990 2 qt	3	82
RA-1990 1 qt	3	26
RA-1990 1 pt	3	9
RA-1990 8 oz	3	1
RA-1990 4 oz	3	5 *
Chevron 1 gal	3	31 *
Chevron 2 qt	3	22 *
Chevron 1 qt	3	27
Chevron 1 pt	3	14 *
Chevron 8 oz	3	28 *
Chevron 4 oz	3	24 *
Nu-Film 17 1 gal	3	44
Nu-Film 17 2 qt	3	49
Nu-Film 17 1 qt	3	28 *
Nu-Film 17 1 pt	3	4 *
Nu-Film 17 8 oz	3	21 *
Nu-Film 17 4 oz	3	19 *
Check		0

* Mortality possibly due to starvation - plants were completely defoliated.

Table 39. Laboratory tests with Sevin 4 flowable (Union Carbide) applied at 1.0 lbs AI/ac.

Amt Sticker/100 gal	Inches Rain	Percent Mortality after 5 days
-	-	98
-	3	3
Acrylocoat 1 qt	3	100
Acrylocoat 1 pt	3	13 *
Acrylocoat 8 oz	3	4 *
Acrylocoat 4 oz	3	3 *
RA-1990 1 qt	3	17 *
RA-1990 1 pt	3	85
RA-1990 8 oz	3	39
RA-1990 4 oz	3	7 *
Chevron 1 qt	3	39 *
Chevron 1 pt	3	26 *
Chevron 8 oz	3	28 *
Chevron 4 oz	3	6 *
Nu-Film 17 1 qt	3	40
Nu-Film 17 1 pt	3	14 *
Nu-Film 17 8 oz	3	22
Nu-Film 17 4 oz	3	7 *
Check		0

* Mortality probably due to starvation - plants were completely defoliated.

Table 40. Laboratory tests with Sevin 5 Aqua (Miller) applied at 1.0 lbs AI/ac.

Wet Sticker/100 gal	Inches Rain	Percent Mortality after 5 days
-	-	99
-	1	20
-	3	16
Acrylocoat 3 gal	3	93
Acrylocoat 1 qt	3	99
Acrylocoat 1 pt	3	81
Acrylocoat 8 oz	3	91
Acrylocoat 4 oz	3	56
RA-1990 3 gal	3	96
RA-1990 1 qt	3	99
RA-1990 1 pt	3	100
RA-1990 8 oz	3	100
RA-1990 4 oz	3	95
Chevron 1 qt	3	77
Chevron 1 pt	3	43
Chevron 8 oz	3	41
Chevron 4 oz	3	39 *
Nu-Film 1 qt	3	93
Nu-Film 1 pt	3	84
Nu-Film 8 oz	3	60
Nu-Film 4 oz	3	10 *
Check		0

Table 41. Laboratory tests with Sevin 4 flowable (Agway) applied at 1.0 lbs AI/ac.

Amt Sticker/100 gal	Inches Rain	Percent Mortality after 5 days
-	-	100
-	1	97
Acrylocoat 1 gal	1	98
Acrylocoat 2 qt	1	96
RA-1990 1 gal	1	94
RA-1990 1 qt	1	99
Rhoplex AC33 1 gal	1	94
Rhoplex AC33 1 qt	1	98
Chevron 1 gal	1	97
Chevron 2 qt	1	97
Nu-Film 1 gal	1	96
Nu-Film 2 qt	1	96
Check		0

Table 42. Laboratory tests with Sevin UCSF-4 (Union Carbide) diluted with kerosene and applied at 1.0 lbs AI/ac.

Amt Sticker/100 gal	Inches Rain	Percent Mortality after 5 days
-	-	98
-	1	98
-	3	98
Acrylocoat 3 gal	3	100
RA-1990 3 gal	3	100
Check		0

Table 43. Laboratory tests with Sevin XLR.

Formulation	Sticker	Inches Rain	Percent Mortality after 5 days
1 X 50 mix - 2 gal/ac	-	-	54
1 X 50 mix - 5 gal/ac	-	-	99
1 x 100 mix - 2 gal/ac	-	-	30
1 X 100 mix - 2 gal/ac	-	1	1
1 X 100 mix - 5 gal/ac	-	-	80
1 X 100 mix - 10 gal/ac	-	-	90
1 X 100 mix - 10 gal/ac	-	1	0
1 X 200 mix - 2 gal/ac	-	-	4
1 X 200 mix - 2 gal/ac	-	1	0
1 X 200 mix - 5 gal/ac	-	-	26
1 X 100 mix - 2 gal/ac	3% Acrylocoat	1	0
1 X 100 mix - 10 gal/ac	3% Acrylocoat	1	50
1 x 100 mix - 2 gal/ac	3% Acrylocoat	1	0
1 X 100 mix - 2 gal/ac	3% RA-1990	1	0
1 X 100 mix - 10 gal/ac	3% RA-1990	1	3
1 X 100 mix - 2 gal/ac	3% RA-1990	1	0
1 X 100 mix - 10 gal/ac	3% 1956	1	4
1 X 100 mix - 10 gal/ac	3% Chevron	1	1
1 X 100 mix - 10 gal/ac	3% Nu-Film 17	1	2
1 X 100 mix - 10 gal/ac	3% Rhoplex B15	1	22
Check		0	

Table 44. Weathering tests with Sevin formulations applied at 1.0 lbs per acre.

Formulation	Sticker	Inches Rain	PERCENT MORTALITY 2 Day	4 Day	5 Day
4F (Agway)	3% Chevron	3	48	87	96
4F (Agway)	3% Nu-Film 17	3	74	96	99
Sevimol 4	3% Chevron	3	50	83	91
Sevimol 4	3% Nu-Film 17	3	22	61	74
5 Aqua (Miller)	3% Chevron	3	37	76	95
5 Aqua (Miller)	3% Nu-Film 17	3	38	83	92
Check	-	-	0	0	0
Check	-	3	0	0	0

Table 45. Weathering tests with Sevin 4F (Agway) applied at 1.0 lbs per acre.

Sticker	Inches Rain	PERCENT MORTALITY 2 Day	4 Day	5 Day
2% Rhoplex AC33	-	30	89	97
2% Rhoplex AC33	1.0	38	91	94
2% Chevron	-	41	94	97
2% Chevron	1.0	38	89	96
2% Nu-Film 17	-	45	98	99
2% Nu-Film 17	1.0	58	98	99
2% RA-1990	-	28	86	92
2% RA-1990	1.0	28	84	95
2% Acrylocoat	-	27	83	95
2% Acrylocoat	1.0	21	90	97
-	1.0	43	92	96
Check	-	0	0	1
Check	1.0	0	0	1

All formulations except Sevin 4 Flowable (Agway) and Sevin UCSF-4 were found to require sticker to retain activity with 3" rainfall. Although the Agway formulation of Sevin 4 Flowable was not weathered with more than 1.0 inches of rainfall, tests at that rate indicate no sticker needs to be added to the formulation. As expected, additional sticker was not needed with Sevin UCSF-4 as it is an oil based formulation. Sevin XLR displays good deposit retention when not diluted below a 1 X 11 mix. However, if used for hydraulic spraying at high dilution, a sticker would be required.

Although Sevimol 4, Sevin 5 Aqua and Sevin 4 Flowable (Union Carbide) did not weather well we were able to improve their performance by adding sticker. Of the stickers tested Acrylocoat (Rohm Haas) and RA-1990 (Monsanto) proved to be the most effective. It must be noted that NuFilm-17 may have performed better than it did if it was exposed to sunlight for a short period of time.

Based on this work it is recommmended that Acrylocoat be used with the formulations of Sevin needing additional sticker. (Sevin 5 Aqua - 8.0 ounces per 100 gallons, Sevimol 4 - 64 ounces per 100 gallons, Sevin 4 Flowable - 32 ounces per 100 gallons).

RA-1990 gave excellent results in most tests. This material was as reliable as Acrylocoat, however, at this time we are not sure if it is exempt from tolerance.

Over the past several years PPQ has used carbaryl as a regulatory treatment for campsites infested with gypsy moth. Sevin 80S is used with ground equipment and Sevin 4-oil with aircraft. In general, results have been favorable. Recently there has been much interest in a formulation in an aqueous medium. The formulation is readily dispersable in water and is reported to be less toxic to bees than the other mixes. It is also reported to have excellent residual characteristics.

A series of laboratory tests have been conducted at the Otis Methods Development Center to evalute various performance parameters of this new formulation.

Table 46. Laboratory tests with Sevin XLR and Sevin 4-oil at various application volumes.

Material	Application Volume (1.0 lb AI/A)	PERCENT MORTALITY 24 Hr	48 Hr	120 Hr	144 Hr
4-oil	32 oz/A	8	42	97	98
4-oil	40 oz/A	12	49	98	100
XLR	32 oz/A	9	47	93	96
XLR	40 oz/A	9	48	96	98
4-oil	48 oz/A	15	50	98	98
4-oil	64 oz/A	18	59	100	
XLR	48 oz/A	7	45	94	97
XLR	64 oz/A	4	59	98	99
4-oil	96 oz/A	15	47	96	98
4-oil	128 oz/A	30	58	97	98
XLR	96 oz/A	9	56	98	98
XLR	128 oz/A	12	65	99	100
Check		0	0	0	10

Table 47. Laboratory tests with Sevin XLR and Sevin 4-oil at various application rates.

Material	Application Rate (40 oz/A)	PERCENT MORTALITY 24 Hr	48 Hr	72 Hr	144 Hr
4-oil	1.0 lb AI/A	7	44	87	100
4-oil	0.75 lb AI/A	9	52	89	100
XLR	1.0 lb AI/A	8	36	82	100
XLR	0.75 lb AI/A	8	25	80	99
4-oil	0.5 lb AI/A	5	50	86	100
4-oil	0.25 lb AI/A	5	41	82	100
XLR	0.5 lb AI/A	6	25	70	99
XLR	0.25 lb AI/A	7	26	65	97
4-oil	0.125 lb AI/A	9	30	79	100
4-oil	0.06 lb AI/A	9	33	69	100
XLR	0.125 lb AI/A	4	20	50	68
XLR	0.06 lb AI/A	2	15	39	97
4-oil	0.03 lb AI/A	0	10	25	32
4-oil	0.01 lb AI/A	2	6	9	13
XLR	0.03 lb AI/A	3	13	36	98
XLR	0.01 lb AI/A	2	5	10	11
Check		0	0	0	0

Table 48. Weathering tests with Sevin XLR and Sevin 4-oil.

Material	Application Volume (1 lb AI/A)	Rain	PERCENT MORTALITY 24 Hr	96 Hr	120 Hr	144 Hr
4-oil	32 oz/A	1"	29	93	96	98
XLR	32 oz/A	1"	26	77	83	91
4-oil	32 oz/A	3"	24	92	94	97
XLR	32 oz/A	3"	19	85	87	92
4-oil	40 oz/A	1"	59	96	97	98
XLR	40 oz/A	1"	28	95	97	98
4-oil	40 oz/A	3"	43	96	97	98
XLR	40 oz/A	3"	19	78	91	98
4-oil	48 oz/A	1"	74	99	99	100
XLR	48 oz/A	1"	27	88	96	99
4-oil	48 oz/A	3"	74	97	97	99
XLR	48 oz/A	3"	28	87	96	99
4-oil	64 oz/A	1"	76	96	97	99
XLR	64 oz/A	1"	42	91	97	100
4-oil	64 oz/A	3"	72	90	98	99
XLR	64 oz/A	3"	35	91	95	98
4-oil	96 oz/A	1"	60	96	98	99
XLR	96 oz/A	1"	23	95	97	99
4-oil	96 oz/A	3"	60	95	97	99
XLR	96 oz/A	3"	19	97	100	
4-oil	128 oz/A	1"	86	96	100	
XLR	128 oz/A	1"	13	93	99	99
4-oil	128 oz/A	3"	60	97	98	99
XLR	128 oz/A	3"	18	99	100	
Check		-	0	0	0	0
Check		3"	0	0	0	0

Table 49. Weathering tests with Sevin XLR and Sevin 4-oil.

Material	Application Volume (40 oz/A)	Rain	PERCENT MORTALITY 24 Hr	48 Hr	72 Hr	144 Hr
4-oil	1.0 lb AI/A	1"	11	30	81	100
XLR	1.0 lb AI/A	1"	10	56	86	100
4-oil	1.0 lb AI/A	3"	15	52		100
XLR	1.0 lb AI/A	3"	16	28		98
4-oil	0.75 lb AI/A	1"	16	34	86	100
XLR	0.75 lb AI/A	1"	5	57	87	99
4-oil	0.75 lb AI/A	3"	14	40		100
XLR	0.75 lb AI/A	3"	18	48		99
4-oil	0.50 lb AI/A	1"	9	33	74	100
XLR	0.50 lb AI/A	1"	8	49	90	100
4-oil	0.50 lb AI/A	3"	13	44		99
XLR	0.50 lb AI/A	3"	14	33		98
4-oil	0.25 lb AI/A	1"	6	23	79	100
XLR	0.25 lb AI/A	1"	3	19	69	99
4-oil	0.25 lb AI/A	3"	6	29		99
XLR	0.25 lb AI/A	3"	8	11		93
4-oil	0.12 lb AI/A	1"	1	9	46	94
XLR	0.12 lb AI/A	1"	3	19	57	96
4-oil	0.12 lb AI/A	3"	5	16		69
XLR	0.12 lb AI/A	3"	14	18		83
4-oil	0.06 lb AI/A	1"	2	11	35	82
XLR	0.06 lb AI/A	1"	0	13	27	75
4-oil	0.06 lb AI/A	3"	2	9		54
XLR	0.06 lb AI/A	3"	1	7		31
4-oil	0.03 lb AI/A	1"	1	6	16	26
XLR	0.03 lb AI/A	1"	0	8	12	18
4-oil	0.03 lb AI/A	3"	2	4		4
XLR	0.03 lb AI/A	3"	1	2		18
4-oil	0.01 lb AI/A	1"	1	4	4	12
XLR	0.01 lb AI/A	1"	0	0	0	0
4-oil	0.01 lb AI/A	3"	1	4		4
XLR	0.01 lb AI/A	3"	0	1		0
Check	-	-	0	0	0	0
Check	-	-	0	0	0	0

Table 50. Ultraviolet light tests with Sevin 4-oil and Sevin XLR.

Material	Application Volume (1.0 lb/A)	UV (Hr)	PERCENT MORTALITY 24 Hr	48 Hr	72 Hr	96 Hr
4-oil	32 oz/A	-	14	59	97	99
XLR	32 oz/A	-	10	42	79	89
4-oil	32 oz/A	2	20	67	99	100
XLR	32 oz/A	2	3	37	75	92
4-oil	40 oz/A	-	35	89	100	
XLR	40 oz/A	-	1	49	88	90
4-oil	40 oz/A	2	22	79	100	
XLR	40 oz/A	2	4	42	72	94
4-oil	40 oz/A	-	16	49	91	99
XLR	40 oz/A	-	6	34	76	97
4-oil	40 oz/A	6	17	55	96	99
XLR	40 oz/A	6	7	36	80	98
4-oil	48 oz/A	-	28	88	99	100
XLR	48 oz/A	-	7	63	83	97
4-oil	48 oz/A	2	35	86	100	
XLR	48 oz/A	2	9	56	79	97
4-oil	64 oz/A	-	21	88	100	
XLR	64 oz/A	-	8	62	92	99
4-oil	64 oz/A	2	34	80	99	99
XLR	64 oz/A	2	3	64	100	
4-oil	64 oz/A	-	19	58	97	100
XLR	64 oz/A	-	9	31	77	99
4-oil	64 oz/A	6	17	55	96	99
XLR	64 oz/A	6	18	44	88	99
4-oil	96 oz/A	-	19	60	94	96
XLR	96 oz/A	-	16	84	98	98
4-oil	96 oz/A	2	18	69	93	94
XLR	96 oz/A	2	13	84	100	
4-oil	128 oz/A	-	26	70	91	92
XLR	128 oz/A	-	17	86	100	
4-oil	128 oz/A	2	25	71	99	99
XLR	128 oz/A	2	17	80	99	100
Check		-	0	0	0	1
Check		2	0	0	0	0

Table 51. Ultraviolet light tests with Sevin 4-oil and Sevin XLR using various dosages.

Material	Application Volume (40 oz/A)	UV (Hr)	PERCENT MORTALITY 24 Hr	48 Hr	144 Hr
4-oil	1.0 lb AI/A	-	19	37	100
XLR	1.0 lb AI/A	-	9	33	95
4-oil	1.0 lb AI/A	2	11	50	99
XLR	1.0 lb AI/A	2	17	38	99
4-oil	0.75 lb AI/A	-	19	48	99
XLR	0.75 lb AI/A	-	14	42	100
4-oil	0.75 lb AI/A	2	17	42	98
XLR	0.75 lb AI/A	2	9	47	96
4-oil	0.5 lb AI/A	-	15	38	98
XLR	0.5 lb AI/A	-	8	48	99
4-oil	0.5 lb AI/A	2	15	41	99
XLR	0.5 lb AI/A	2	9	40	94
4-oil	0.25 lb AI/A	-	18	33	99
XLR	0.25 lb AI/A	-	6	41	99
4-oil	0.25 lb AI/A	2	24	29	99
XLR	0.25 lb AI/A	2	6	36	99
4-oil	0.12 lb AI/A	-	17	32	100
XLR	0.12 lb AI/A	-	11	42	100
4-oil	0.12 lb AI/A	2	14	19	96
XLR	0.12 lb AI/A	2	8	40	95
4-oil	0.06 lb AI/A	-	17	31	95
XLR	0.06 lb AI/A	-	6	36	87
4-oil	0.06 lb AI/A	2	19	25	86
XLR	0.06 lb AI/A	2	8	18	85
4-oil	0.03 lb AI/A	-	9	15	76
XLR	0.03 lb AI/A	-	8	16	61
4-oil	0.03 lb AI/A	2	9	12	58
XLR	0.03 lb AI/A	2	3	13	64
4-oil	0.01 lb AI/A	-	0	1	17
XLR	0.01 lb AI/A	-	2	12	40
4-oil	0.01 lb AI/A	2	0	3	10
XLR	0.01 lb AI/A	2	3	9	43
Check		-	0	0	0
Check		2	0	0	0

Table 52. Comparison of Sevin 4-oil and Sevin XLR formulations applied to oak seedlings. No aging of the material.

Material	Application Volume (1 lb/A)	Rainfall	PERCENT LARVAL MORTALITY 24 Hr	48 Hr	72 Hr	144 Hr
Sevin 4-oil + Kerosene	64 oz/A	0	31	58	90	100
		1"	28	66	94	98
Sevin XLR + H_2O	128 oz/A	0	38	75	90	100
		1"	37	64	84	99
Control	-	0	0	0	0	0

Table 53. Efficacy of Sevin XLR formulation aged for 6 weeks. Material applied at 1 lb/A to oak seedlings.

Material	No. weeks of Aging	PERCENT LARVAL MORTALITY 24 Hr	48 Hr	72 Hr	96 Hr	120 Hr
Sevin XLR + H_2O	0	46	82			99
	1	16	57	81	96	
	2	5	42		97	
	3	33	-	99	-	100
	4	32	67	88	-	99
	5	15	43	-	-	100
	6	-	47	88	95	99

Table 54. Comparison of Sevin 4-oil and Sevin XLR treated oak seedling aged for 3 weeks.

Material	Application Rate (1 lb/A)	Age of Treatment	PERCENT LARVAL MORTALITY 24 hr	48 Hr	72 Hr	96 Hr	144 Hr
Sevin 4-oil + Kerosene	64 oz/A	0	31	58	90		100
		1	39	80	98	100	
		2	22	58	99		
		3	22	69		100	
Sevin XLR + H_2O	128 oz/A	0	38	75	90		100
		1	41	78	99	100	
		2	15	69	96		
		3	29	62		99	

The flow rate of undiluted XLR formulation was tested in a laboratory spray system equipped with flat fan nozzles. A flat fan nozzle (No. 8006) should deliver 0.42 gallons or 53.76 ounces per minute at 20 psi. Undiluted material was sprayed through of No. 8006 and 8002 nozzles for a period of 10 minutes and no problems developed. There was no buildup of material on the nozzle *tips and the material flowed through 50 mesh screens with little effort.*

Viscosity of three Sevin XLR mixes was as follows:

Undiluted	1,050 cps
Sevin 32 oz. + water 8 oz.	290 cps
Sevin 32 oz. + Water 32 oz.	60 cps

Based on the data in this report, it appears that Sevin XLR compares favorably with Sevin 4-oil; weathering characteristics may be slightly superior to the oil formulation. Ultraviolet tests show both formulations to be resistant to sunlight.

Tests with various nozzle tips indicate Sevin XLR may be used undiluted for gypsy moth control. Dilution, if necessary, can be achieved with small amounts of water, thus eliminating the use of costly kerosene as with Sevin 4-oil. Clean-up would also be much less of a problem with the XLR formulation.

The use of Sevin XLR is acceptable for gypsy moth control. However, definitive field work should be conducted with this new formulation undiluted, and diluted to 40 and 64 oz/A.

In the past, some formulations of pesticides have caused damage to automotive paint finishes. To avoid such costly mistakes, new formulations are tested on automotive paint finishes in the laboratory to determine what effect, if any, they may have on paint finishes.

In general all automobiles made in the past few years were painted with acrylic enamel or acrylic lacquer. General Motors cars are painted with acrylic lacquer and Chrysler Corporation cars with acrylic enamel.

Recently, tests have been conducted using Union Carbides new formulation of Carbaryl, Sevin XLR. Using a laboratory insecticide spray chamber, acrylic lacquer plates were sprayed with 3 formulations of Sevin XLR. One drop of each formulation and 1 drop of undiluted product were placed on each plate by hand. Drops were 0.5 cm in diameter. The various deposits were allowed to dry for 72 hours. Each plate was then rubbed vigorously with cheese cloth for approximately 30 seconds. No water or liquid was used to rub the plates.

After rubbing the plates with cheese cloth, each was examined under a light and then microscopically.

Table 55. Effects of spray tower application of Sevin XLR on auto paint finishes.

Paint Type	Paint Color	Formulation 40 oz. 1/	64 oz. 1/	128 oz. 1/
Acrylic lacquer	Yellow	N	N	N
	Maroon Metallic	N	N	N
	Mahogany	N	N	N
	White	N	N	N
	Brt Blue Metallic	N	N	N
	Silver Metallic	N	N	N
	Dk Blue Metallic	N	N	N
Dispersion Lacquer	Bright Yellow	N	N	N
	Black	N	N	N
	White	N	N	N
	Gold Metallic	N	N	N
	Norfolk Gray	N	N	N
	Spectra Red	N	N	N
	Gray Metallic	N	N	N
	Beige	N	N	N
	Med Waxberry Metallic	N	N	N
	Dk Maple Metallic	N	N	N
	Charcoal Metallic	N	N	N
	Dk Brown Metallic	N	N	N
	Orange Metallic	N	N	N
	Dk Sandstone Metallic	N	N	N
	Med Sandstone Met.	N	N	N
	Citrus Orange Met.	N	N	N
	Pastel Sandstone	N	N	N
	Mulbry Grayfire Mist	N	N	N
	Saxony Red	N	N	N
	Silver Metallic	N	N	N
	Lt Maple Metallic	N	N	N

N - No spot
1 - Spot faint (Barely visible)
2 - Spot definite
3 - Deep spot (almost to metal)
4 - Spot to the metal

1/ 40 oz. - 32 oz. XLR + 8 oz. water
64 oz. - 32 oz. XLR + 32 oz. water
128 oz. - 32 oz. XLR + 96 oz. water

Table 56. Effects of 0.5 cm diameter spots of Sevin XLR on auto paint finishes.

Paint Type	Paint Color	Formulation 32 oz.[1/]	40 oz.[1/]	64 oz.[1/]	128 oz.[1/]
Acrylic Lacquer	Yellow	N	N	N	N
	Maroon Metallic	N	N	N	N
	Mahogany	N	N	N	N
	White	N	N	N	N
	Bright blue metallic	N	N	N	N
	Silver metallic	N	N	N	N
	Dk blue metallic	N	N	N	N
Dispersion Lacquer	Bright Yellow	N	N	N	N
	Black	1	1	1	1
	White	N	N	N	N
	Gold Metallic	1	1	1	1
	Norfolk Gray	1	1	1	1
	Spectra red	1	1	1	1
	Gray metallic	N	N	N	N
	Beige	1	N	N	N
	Med Waxberry Metallic	1	N	N	N
	Dark Maple Metallic	1	N	N	N
	Charcoal Metallic	N	N	N	N
	Dark Brown Metallic	N	N	N	N
	Orange Metallic	1	1	1	1
	Dk Sandstone Metallic	N	N	N	N
	Med Sandstne Met.	N	N	N	N
	Citrus Orange Met.	N	N	N	N
	Pastel Sandstone	N	N	N	N
	Mulbry Grayfire Mist	N	N	N	N
	Saxony Red	1	1	1	1
	Silver Metallic	N	N	N	N
	Light Maple Metallic	N	N	N	N

N - No spot
1 - Spot faint (barely visible)
2 - Spot definite
3 - Deep spot (almost to metal)
4 - Spot to the metal

1/ 32 oz. - undiluted XLR
40 oz. - 32 oz. XLR + 8 oz. water
64 oz. - 32 oz. XLR + 32 oz. water
128 oz. - 32 oz. XLR + 96 oz. water

All material cleaned off acrylic lacquer plates after dry rubbing with cheese cloth. No spots or damage to plates was observed under bright lights and a microscope.

Some dispersion lacquer plates still had spots that were barely visible after medium rubbing. However, after they were lightly washed with water, spots were no longer visible.

Based on this test data we can say that any accidental contamination of automobiles will not result in damage to acrylic or dispersion lacquer finishes. The formulation now must be tested on enamel and vinyl surfaces.

Gypsy moth eggs collected from Long Island were hatched, reared to 2nd instar and tested against Sevimol, Sevin 80S, Sevin XLR and Sevin 4-oil. All formulations gave excellent mortality in our oak seedling bioassays. Predictably there was no indication of resistance. Egg hatch was near 85 percent.

Table 57. Bioassay of four Sevin formulations against gypsy moths collected on Long Island.

Formulation	Dosage AI/acre	Percent Mortality 24 Hr	48 Hr	72 Hr	168 Hr
Sevimol	1.0 lb.	32	76	96	100
	0.5 lb.	44	58	86	98
	0.25 lb.	28	58	82	100
	0.125 lb.	26	48	66	98
Sevin 4-oil	1.0 lb.	58	90	98	100
	0.5 lb.	48	80	90	98
	0.25 lb.	40	74	92	100
	0.125 lb.	52	72	92	98
Sevin XLR	1.0 lb.	58	74	86	100
	0.5 lb.	30	64	72	92
	0.25 lb.	32	52	74	90
	0.125 lb.	52	72	80	98
Sevin 80S	1.0 lb.	68	92	100	
	0.5 lb..	36	76	82	100
	0.25 lb.	42	50	66	92
	0.125 lb.	26	52	80	94
Check		0	0	0	6

Table 58. Efficacy tests with Sevin formulations at .125 lb/a with and without P4 buffer.

Formulation	0.1% Buffer	PERCENT MORTALITY 2 Day	4 Day	6 Day
Sevimol 4	No	12	63	75
Sevimol 4	Yes	12	53	73
4F (Stauffer)	No	13	62	79
4F (Stauffer)	Yes	16	74	93
4F (Agway)	No	20	80	98
4F (Agway)	Yes	17	74	91
5 Aqua (Miller)	No	16	75	88
5 Aqua (Miller)	Yes	21	66	78
Check	-	0	0	0

A series of tests have recently been conducted with Methoxychlor and Malathion formulations supplied by Lowden Tree Specialists. Results show Malathion to be effective as a contact insecticide against 2nd instar gypsy moth larvae. Tests with Methoxychlor continue to demonstrate its limited effectiveness against gypsy moth larvae when applied in a laboratory spray chamber simulating an aerial application. However, when seedlings were dipped in a solution containing 1.5 quarts of 32.4 percent Methoxychlor per 100 gallons water, results were good.

Table 59. Results of tests with Methoxychlor against laboratory reared 2nd instar gypsy moth larvae.

Formulation	Application	Percent Mortality after 5 days
3 pt/100 gal H_2O	DIP	90
3 pt/100 gal H_2O	Painted on top surface	33
3 pt/100 gal H_2O	Sprayed at 1.0/gal/ac	1
Applied at 2.7 lbs AI/gal/ac	Spray	70
Applied at 1.33 lbs AI/gal/ac	Spray	69
Check		0

Table 60. Laboratory results with Malathion against laboratory reared 2nd instar gypsy moth larvae.

Formulation	Application	Percent Mortality After 5 Days
1.0 pt/100 gal H_2O	DIP	100
1.0 pt/100 gal H_2O	Painted on top surface	98
1.0 pt/100 gal H_2O	Sprayed at 1.0 gal/ac	1
Applied at 6.6 lbs AI/gal/ac	Spray	100
Applied at 3.3 lbs AI/gal/ac	Spray	100
Applied at 1.6 lbs AI/gal/ac	Spray	99
Check		0

Table 61. Laboratory results using Malathion and Methoxychlor against laboratory reared 2nd instar gypsy moth larvae.

Type Application	Percent Mortality After 5 Days
Plants dipped	96
Brushed onto plants	84
Plants sprayed at 1.0 gal/ac	0
Plants sprayed at 5.0 gal/ac	0
Plants sprayed at 10.0 gal/ac	3 *
Check	0

* Mortality due to starvation - plants completely eaten.

The formulation used contained 1 pint of 80 percent Malathion and 1.5 quarts of 32 percent Methoxychlor per 100 gallons of water.

Orthene was registered in the mid-1970's as a 75S formulation to be applied at 0.66 - 1.0 lb/acre in 0.5 gallons of water. It has a low toxicity to mammals, birds, fish and soil microorganisms, is degraded to innocuous products and is not bioconcentrated.

Orthene has had little use in the PPQ gypsy moth program. Recently, there has been renewed interest in the product. Although Orthene 98 is not yet registered for gypsy moth use, a series of laboratory tests have been conducted with the formulation. Original tests were conducted during May 1979.

Table 62. Mortality of second instar gypsy moth larvae challenged with Orthene 75S and Orthene 98.

Dosage (lb AI/ac)	Rain	Percent Mortality after 3 days 75S	98%
1.0	–	100	100
	1"	59	79
	2"	44	42
	3"	54	43
0.5	–	99	100
	1"	59	26
	2"	41	19
	3"	32	2
0.25	–	99	97
	1"	25	11
	2"	17	21
	3"	28	1
0.125	–	99	95
	1"	13	3
	2"	4	2
	3"	4	3
Control		0	0

Table 63. Mortality of 2nd instar gypsy moth larvae challenged with Orthene 75S and Orthene 98 with the addition of selected stickers (1gallon/acre application rate).

Dosage (lb AI/ac)	Sticker 3%	Rain	Percent Mortality after 4 days 75S	98%
0.5	NuFilm 17	2"	95	98
	Chevron	2"	47	11
	Rhoplex	2"	39	20
	Plyac	2"	28	69
0.25	NuFilm 17	2"	34	87
	Chevron	2"	40	9
	Rhoplex	2"	5	9
	Plyac	2"	20	50
Control			0	0

The data indicate little difference in activity between the two formulations. However, when exposed to rainfall, the active material of both formulations was lost. Consequently, tests were conducted with a number of stickers; NuFilm 17 gave the best results. This was anomolous because in the original work done with Orthene (prior to registration) the material demonstrated fair weathering characteristecs and we felt the addition of a sticker may not be needed. However, tests conducted during 1979 and the latest series indicate that both formulations wash off. Furthermore, we found that the addition of most stickers did not improve the residual quality of the formulations appreciably.

During January 1981 more testing was conducted with the Orthene formulations.

Table 64. Sticker tests with Orthene 75S and Orthene 98% (1.0 lb AI/acre).

Sticker (3%)	Rain	Percent Mortality after 3 days 75S	98%
-	-	97	99
-	1"	12	3
-	2"	22	8
-	3"	27	9
RA-1990	-	100	100
	1"	6	3
	2"	2	8
	3"	15	1
Chevron	-	100	100
	1"	17	16
	2"	12	12
	3"	0	0
NuFilm 17	-	100	100
	1"	4	6
	2"	5	8
	3"	0	6
Acrylocoat	-	100	100
	1"	6	14
	2"	1	9
	3"	0	0
Check	-	0	0
Check	1"	0	0
Check	2"	0	0
Check	3"	0	0

Results were very poor with all sticker tests. However, when sticker was added to the formulation and no rain was applied, good mortality occurred. We felt we may be adding too much emulsifier (at 3% sticker) to the formulation; we therefore tested at 1.5 and 0.5 percent.

Table 65. Sticker tests with Orthene 75S and Orthene 98%.

Sticker	Concentration	Rain	Percent Mortality after 3 days 75S	98%
	-	-	96	99
	-	1"	30	24
RA-1990	1.5	1"	10	17
	0.5	1"	10	11
Chevron	1.5	1"	27	15
	0.5	1"	20	14
Acrylocoat	1.5	1"	18	11
	0.5	1"	30	9
NuFilm	1.5	1"	27	12
	0.5	1"	22	27
Check		-	0	0
		1"	1	0

Reducing the amount of sticker used did not improve the weathering quality of the material. The present label for Orthen 75S states nothing about adding sticker to the formulation. Tests reported here show that the material washes off foliage rapidly; additional work with the formulation to improve weathering is warranted.

Table 66. Laboratory test results with RH-0994 against 2nd instar gypsy moth larvae.

Pounds active ingredient per 1.0 gal/acre	Percent Mortality after 4 days
1.0	100
0.5	100
0.25	98
0.125	99
0.062	95
0.0312	97
0.0156	48
Check	0

Table 67. Weathering tests with RH-0994 at 0.06 lbs AI/acre (1 gal/acre).

Sticker	Inches Rain	Percent Mortality 2 Days	Percent Mortality 5 Days
-	-	71	99
-	1.0	0	1
-	-	20	100
-	2.0	0	1
-	-	11	95
1% Acrylocoat	-	10	89
1% Acrylocoat	1.0	11	76
1% Chevron	-	13	96
1% Chevron	1.0	0	1
1% NuFilm 17	-	12	96
1% NuFilm 17	1.0	0	9
1% RA-1990	-	8	97
1% RA-1990	1.0	7	98
-	-	32	100
-	1.0	0	0
0.5% Acrylocoat	1.0	14	99
0.5% RA-1990	1.0	18	99
0.5% Chevron	1.0	0	1
0.5% NuFilm 17	1.0	0	2
-	-	22	100
-	2.0	0	0
0.5% Acrylocoat	2.0	11	98
0.5% RA-1990	2.0	12	100
0.5% Chevron	2.0	0	0
0.5% NuFilm 17	2.0	0	0
-	-	41	98
-	2.0	0	0
1.0% Acrylocoat	2.0	0	0
1.0% RA-1990	2.0	18	95
1.0% Chevron	2.0	1	10
1.0$ NuFilm 17	2.0	0	11
-	-	12	99
-	1.0	0	0
2% Acrylocoat	1.0	10	97
2% RA-1990	1.0	9	100
2% Chevron	1.0	0	58
2% NuFilm 17	1.0	4	55
-	-	52	99
-	1.0	1	2

Table 67. (Cont'd)

Sticker	Inches Rain	Percent Mortality 2 Days	Percent Mortality 5 Days
0.5% NuFilm 17	1.0	0	4
0.5% NuFilm 17	1.0[1]	1	2
0.5% NuFilm 17	1.0[2]	1	13
2.0% NuFilm 17	1.0	3	11
2.0% NuFilm 17	1.0[1]	1	44
2.0% NuFilm 17	1.0[2]	4	64
0.125% Acrylocoat	1.0	8	89
0.25% Acrylocoat	1.0	16	97
1.0% TS-30	1.0	21	96
1.0% TS-85	1.0	34	100
-	-	50	100
-	1.0	0	1
0.03% RA-1990	1.0	4	65
0.06% RA-1990	1.0	9	83
0.125% RA-1990	1.0	9	85
0.25% RA-1990	1.0	17	93
0.03% Acrylocoat	1.0	1	53
0.06% Acrylocoat	1.0	3	39
0.125% Acrylocoat	2.0	16	87
0.25% Acrylocoat	2.0	23	95
1.0% TS-30	2.0	40	97
1.0% TS-85	2.0	43	95
-	-	39	100
-	3.0	0	13
0.125% RA-1990	3.0	0	43
0.25% RA-1990	3.0	4	94
0.125% Acrylocoat	3.0	2	57
0.25% Acrylocoat	3.0	9	94
1.0% TS-30	3.0	15	91
1.0% TS-85	3.0	25	91
Check	-	0	0
Check	3.0	0	0

[1] Treatment exposed to natural sunlight for 30 minutes.

[2] Treatment exposed to natural sunlight for 60 minutes.

Table 68. Weathering tests with RH RA-0994.

Dosage (lb/a)	Sticker	Inches Rain	Percent Mortality 2 Day	Percent Mortality 5 Day
0.03	-	-	56	93
0.03	-	1.0	1	1
0.03	-	-	14	83
0.03	-	2.0	0	0
0.03	-	-	7	83
0.03	1.0% Acrylocoat	-	3	87
0.03	1.0% Acrylocoat	1.0	1	81
0.03	1.0% Chevron	-	5	81
0.03	1.0% Chevron	1.0	0	0
0.03	1.0% NuFilm 17	-	8	96
0.03	1.0% NuFilm 17	1.0	0	2
0.03	1.0% RA-1990	-	3	69
0.03	1.0% RA-1990	1.0	1	64
0.01	-	-	17	64
0.01	-	1.0	0	0
0.01	-	-	4	75
0.01	-	2.0	0	1
Check	-	-	0	0
Check	-	2.0	0	0

Table 69. Ultraviolet light test with NC-6897.

Dosage (lbs/acre)	Minutes UV Lights	Percent Mortality 2 Days	4 Days	5 Days
0.5	-	93	100	
0.5	15	81	100	
0.5	30	91	100	
0.5	60	90	98	99
0.5	120	79	99	100
0.125	-	93	100	
0.125	15	67	98	99
0.125	30	63	92	99
0.125	60	67	96	97
0.125	120	83	99	99
Check	-	0	0	0
Check	120	0	0	0

Table 70. Weathering tests with NC-6897.

Dosage (lbs/acre)	Inches Rain	PERCENT MORTALITY 2 Days	4 Days	7 Days
1.0	1.0	27	38	51
0.5	1.0	3	10	11
0.25	1.0	0	2	2
0.125	1.0	0	0	3
0.062	1.0	0	0	0
0.031	1.0	0	0	0
1.0	3.0	11	11	11
0.5	3.0	13	19	25
0.25	3.0	0	3	3
0.12	3.0	0	0	0
0.06	3.0	0	1	1
0.03	3.0	0	0	0
Check		0	0	0
Check	3.0	0	0	0

Table 71. Laboratory tests with Advantage 25 WP.

Dosage lbs AI/ac	PERCENT MORTALITY 2 Days	4 Days	5 Days	7 Days
1.0	100			
0.5	84	98	100	
0.25	62	96	96	100
0.125	53	84	89	99
0.062	28	74	89	97
0.031	35	52	68	82
0.015	16	29	34	35
0.007	5	10	11	13
Check	2	2	2	2

Table 72. Laboratory tests with Bacto-Zyme.

Dilution	PERCENT MORTALITY 2 Days	4 Days	7 Days
Undiluted	23	34	47
1:1	17	25	27
1:5	12	20	23
1:10	27	27	31
1:20	11	25	29
1:30	19	30	54
1:40	19	26	44
1:50	30	35	54
Check	8	11	16

Table 73. Laboratory tests with BAY FCR-1272.

Dosage lbs AI/ac	PERCENT MORTALITY 24 Hr	48 Hr
1.67	100	
0.83	100	
0.41	100	
0.20	100	
0.10	100	
0.05	100	
Check	0	0

Table 74. Laboratory tests with Phosphoric Acid to lower PH of H_2O.

Grams of Acid Added per 100 gal	Pounds of Acid Added per 100 gal	PH Reading
0	0	7.0
378.5	0.83	5.6
946.3	2.09	4.8
2,838.9	6.26	4.6
3,785.3	8.34	4.5
5,677.9	12.52	4.3
7,570.6	16.69	4.2
9,463.3	20.86	4.2
18,926.6	41.73	4.1
37,853.3	83.47	4.0
75,706.6	166.73	3.8
113,559.9	250.40	3.6

Nursery stock treated with carbaryl and DDT was bioassayed to test for residue. Test insects used were laboratory reared gypsy moth neonates.

Two shipments of various stock were received and tested. Stock was placed in plastic petri dishes and neonates were allowed to move freely over treated material for given periods of time. They were then removed and placed on artificial diet where mortality readings were made every 24 hours.

All nursery stock tested was treated on April 15, 1981. The first shipment to be bioassayed had 11 types of foliage and the second one had 5 types. In each test foliage types were tested individually. There was no important difference in activity between various types of foliage.

The bioassay results indicate little residue. Very limited mortality occurred even after an exposure of 6 hours.

Table 75. Bioassay of nursery stock treated with Carbaryl and DDT.

Exposure Time	Bioassay Date	Percent Mortality 6 Days after exposure
1 Min.	4/22	0.9
3 Min.	4/22	1.8
5 Min.	4/22	1.8
1 Hr.	4/22	6.8
1 Hr	4/28	1.0
6 Hr	4/28	8.0
Check		0.0

Carbaryl and DDT treatment made 4/15/81.

During early March 1981, John Belding collected a number of gypsy moth egg masses (above the snow line) from 4 locations in Northern Vermont. They were sent to the Otis Methods Development Center for hatch tests. The egg masses were placed individually in plastic petri dishes and held for 6 weeks in an environmental chamber. Each petri dish was provisioned with moisture; holding temperature was 26°C with relative humidity of 60 percent.

Table 76. Hatch from Northern Vermont egg masses.

Location	No. Egg Masses	No. Egg Masses with Hatch
Essex Center	101	2
Middlebury	102	0
Newport	42	4
Stowe	54	0

Table 77. Temperatures recorded at Newport, Burlington and Stowe, VT during December, 1980 and January, 1981.

Date	Newport Max.	Newport Min.	Burlington Max.	Burlington Min.	Stowe Max.	Stowe Min.
12/1	43	30	47	31	28	20
12/2	42	28	52	31	32	24
12/3	41	7	44	15	32	0
12/4	15	4	19	12	10	-10
12/5	16	5	18	9	18	- 6
12/6	24	8	28	12	28	6
12/7	30	7	33	12	36	25
12/8	44	22	50	33	42	31
12/9	31	24	38	23	42	10
12/10	31	14	32	19	18	10
12/11	29	- 4	19	2	18	-13
12/12	22	- 6	28	8	10	- 9
12/13	29	8	33	11	21	5
12/14	18	- 8	20	- 1	7	- 4
12/15	5	-17	18	- 6	2	-22
12/16	18	5	17	3	17	1
12/17	7	-10	10	- 4	18	- 6
12/18	27	-14	25	10	15	- 5
121/19	19	-10	28	- 6	16	-10
12/20	2	-22	4	-15	- 5	-18
12/21	6	-16	10	-15	- 5	-13
12/22	13	-21	18	-12	8	-10
12/23	28	12	27	18	11	4
12/24	30	- 8	31	- 5	20	11
12/25	- 3	-28	- 5	- 25	-19	-38
12/26	10	-28	14	-26	3	-31
12/27	23	- 4	21	- 5	22	0
12/28	34	- 9	34	- 5	28	3
12/29	40	30	37	30	32	27
12/30	30	1	30	- 2	30	- 9
12/31	6	-11	7	-10	6	-15
1/1	13	-18	16	-10	24	- 4
1/2	23	-15	22	-11	12	- 2
1/3	- 9	-25	- 8	-21	- 1	-32
1/4	-19	-31	-13	-27	-18	-35
1/5	4	-20	3	-13	- 1	-32
1/6	20	-14	23	- 7	12	- 7
1/7	30	- 3	29	3	15	5
1/8	1	- 6	10	- 4	5	-20

Table 77. (Cont'd)

Date	Newport Max.	Newport Min.	Burlington Max.	Burlington Min.	Stowe Max.	Stowe Min.
1/9	15	-12	14	- 7	9	- 9
1/10	10	-11	19	- 3	9	- 9
1/11	- 5	-17	2	-10	- 7	-27
1/12	7	-20	5	-12	- 1	-22
1/13	3	-26	1	-18	5	-12
1/14	10	-22	11	-17	2	-10
1/15	6	-17	15	- 8	16	- 2
1/16	12	-15	12	- 4	15	6
1/17	11	- 7	13	- 3	13	- 5
1/18	21	-14	20	-10	4	- 9
1/19	32	15	35	20	20	4
1/20	29	- 8	34	2	25	15
1/21	17	-16	23	- 6	20	0
1/22	29	16	20	22	21	9
1/23	31	22	31	21	15	10
1/24	27	11	20	17	15	5
1/25	28	2	33	1	13	1
1/26	42	23	43	33	32	10
1/27	41	25	42	29	30	21
1/28	26	0	29	12	21	0
1/29	16	- 5	17	11	10	-10
1/30	13	- 8	16	0	- 5	-13
1/31	25	-13	21	- 5	12	- 8

All temperatures Fahrenheit

No hatch occurred from egg masses collected at 2 locations and very limited hatch from those at 2 other locations. Based upon these low hatch rates, it is obvious that excessive winter kill occurred at the locations where study egg masses were collected. This mortality was presumably due to extremely cold conditions. Its hard to tell how wide-spread this winter kill may be. In central Massachusetts where temperatures were also moderately low, hatch was near 85 percent. Based on this test it appears that a large number of gypsy moth egg masses in the northern New England states will not hatch this spring due to low winter tempertures and other factors.

Project Number: GM 0.1.2
Project Title: Field Studies with SIR-8514, Bacillus Thuringiensis and Gypchek (1980)
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: W.H. McLane and J.A. Finney

Field tests were conducted in Massachusetts and Rhode Island to study the insect growth regulator, SIR-8514, establish effectiveness of a new formulation of Gypchek and test new sticker materials with BT formulations. Tests in New Jersy during 1979 resulted in 78% reduction in egg mass density when SIR-8514 was applied at 0.03 lbs. AI/acre. A Gypchek formulation containing Pro Tec has proven to be effective in the laboratory and, to some degree, in the field. Laboratory screening has demonstrated the need for a sticking material to be added to BT formulations when used on oak foliage.

Twelve 50 acre experimental plots used were established in Rhode Island to test 3 dosages of SIR-8514 (Table 1) on State land at Washington State Forest and Pulaski Memorial State Forest in the towns of Glocester and Burrillville. Little defoliation had occurred in and around test plots during 1979. Egg masses were large and ranged in numbers from 117 to 735 per acre. Approval for this work was obtained from the Rhode Island Department of Enviromental Management and Mobay Chemical Company acquired an Experimental Use Permit from the United States Environmental Protection Agency.

In Massachusetts, 50 plots of 50 acres each were established on State lands (Table 1). Plots were located in Northfield, Warwick, Erving and Wendell State Forests. Although little defoliation had occurred in and around the test plots during 1979, this infestation was much heavier than that found in Rhode Island plots. Egg mass size was medium to large and densities ranged from 33 to 3,596 per acre. Approval for this work was obtained from the Department of Environmental Management, State Pesticide Board and Department of Environmental Affairs. An Experimental Use Permit was also obtained for the high dosage of Gypchek.

Table 1. Materials, rates and dosages tested on 50 acre plots during 1980. The SIR-8514 plots were in Rhode Island and the remainder in Massachusetts.

Material	Sticker	AI / Acre	Gallons/ Acre	Number Applications	No. Plots
SIR-8514	-	0.015	0.5	1	3
SIR-8514	-	0.031	0.5	1	3
SIR-8514	-	0.062	0.5	1	3
Check	-	-	-	-	3
Dipel 4L	-	8.0 BU	1.0	2	4
Dipel 4L	1645	8.0 BU	1.0	2	4
Dipel 4L	Acrylocoat	8.0 BU	1.0	2	4
Thuricide 32B	-	8.0 BU	1.0	2	4
Thuricide 32B	1645	8.0 BU	1.0	2	4
Thuricide 32B	Acrylocoat	8.0 BU	1.0	2	4
Thuricide 32B	-	8.0 BU	1.0	2	2
Gypcheck	-	$25X10^6$	2.0	2	4
Gypcheck	-	$6.25X10^7$	2.0	2	4
Gypcheck	-	$12.5X10^7$	2.0	2	4
Gypcheck	-	$25X10^7$	2.0	2	4
Check	-	-	-	-	8

Sticker was used at the rate of 3% of total volume.

Plots were established in such a manner that at least one side was always bordering on a roadway or power line. Back corners were determined by compass lines. All plots were square in shape. Within the central 10 acres of each plot, a rectanglar grid of 20 equidistant prism points was constructed for the purpose of egg mass, larval and pupal sampling procedures.

Compass lines to each corner were marked with orange surveyor tape at 20 foot intervals. Each corner tree was marked with double orange tape and a tag identifying the plot and corner aspect. Survey lines leading into 10 acre study areas were marked in blue tape and prism point lines were marked with white tape. Each prism point was identified by a tag and double white tape. At each prism point 5 egg masses were collected above the snow line and 5 below. Eggs were incubated in the laboratory to establish percentage of hatch (Table 2). Pre spray egg mass counts were also made using the fixed and variable prism point method (Table 2).

Six egg masses from each virus plot and 4 check plats were sent to Dr. DuBois, U.S. Forest Service, Hamden, CT, to determine virus load. Results of this test were never received.

Table 2. Results of egg mass hatchability tests with eggs collected from 1980 spray plots.

Treatment	Percent Hatch	E/M Per Acre	Larvae Per 50 Acre Spray Plot (x1000)
SIR-8514 0.015	75	735	8,596
SIR-8514 0.031	80	152	2,705
SIR-8514 0.062	78	117	2,111
Check	82	285	6,059
Dipel 4L	70	1,342	23,735
Dipel 4L + 1645	85	1,613	21,691
Dipel 4L + Acrylocoat	92	1,700	609
Thuricide 32B	87	1,721	27,070
Thuricide 32B + 1645	86	1,420	21,860
Thuricide 32B + Acrylocoat	93	694	15,264
Thuricide 32B/1qt	86	355	6,813
Thuricide 16B	89	1,293	24,115
Check	86	852	14,836
Gypchek $25X10^7$	87	842	16,917
Gypchek $12.5X10^7$	87	772	13,232
Check	90	563	9,611

All totals are based on the average of samples collected from 4 plots.

Mixing of formulations and aircraft operations for the projects were based at Orange airport, Orange Massachusetts and Danielson Airport, Danielson, Conneticut.

The application schedule was designed to allow the first application of BT and Gypchek to go on in Western Massachusetts when the majority of larvae were in first and early second instar. At that time foliage development was approximately 40 percent. The second application of BT and Gypchek was to be applied 7 to 10 days following the first (Table 3). Between the 1st & 2nd applications of BT and Gypchek all SIR-8514 plots were treated in Rhode Island (Table 3). Foliage and larvae development in Rhode Island was 60 percent and 2nd instar respectively.

Table 3. Date of treatment for 1980 spray plots.

Treatment	Date 1st App.	Date 2nd App.
SIR-8514 0.016	5/27	
SIR-8514 0.031	5/27	
SIR-8514 0.062	5/28	
Dipel 4L	5/23	5/30
Dipel 4L + 1645	5/23	5/31
Dipel 4L + Acrylocoat	5/25	6/06
Thuricide 32B	5/24	5/31
Thuricide 32B + 1645	5/24	6/01
Thuricide 32B + Acrylocoat	5/25	6/06
Thuricide	5/28	6/06
Thuricide 16B	5/24	6/01
Gypchek 25 X 10^7	6/06	
Gypchek 12.5 X 10^7	6/07	

In order to better compare formulations, at least one replicate of each formulation was treated on a given day.

All plots were sprayed with a Cessna Ag-Truck equpped with a conventional spray system. The aircraft yielded a 60 foot swath at 125 mph, 20 feet above tree top level. Flat fan nozzles were used to disperse the material at 40 PSI. In order to coordinate the spray operation, radio communications were maintained between pilot, airport and field crew. For aircraft navigational aids, helium-inflated weather balloons were suspended 10 - 20 feet above the forest canopy at each plot corner.

Spray materials were first mixed in a nurse tank before being loaded into the spray aircraft. The nurse tank was thoroughly cleaned between formulation changes. When stickers were used, the sticking agent was added to the mix last. All virus tested was first blended on site and then added to the final formulation 15 minutes prior to loading aircraft. All virus was stored at cool temperatures until thee time of use.

Following application, 10 minute larval counts were made in plots treated with Gypchek and check plots (Table 4). Two parallel lines were established between prism point lines in each plot. The same two observers made all post spray counts. A 10 minute count was made on each of the two lines and then a 5 minute count on each line was combined to give a total of three 10 minute counts per plot. This survey was continued for 26 days following treatment.

Because of mixing problems associated with the use of Pro Tec, we had to cancel most of the scheduled Gypchek treatments. As a result of production errors at United States Trade International, we were unable to use the first shipment of Pro Tec received. By the time we received a new production lot, larvae were in the late 3rd and 4th instar stage. As a result we applied only one application of Gypchek and that only at 25×10^7 and 12.5×10^7 per acre. We expected little if any control on the treated plots.

Table 4. Ten minute larval counts in Gypchek plots and checks.

Days Following Treatment	Average Number Larvae Counted		
	25×10^7	12.5×10^7	Check
6	211	333	98
12	91	125	88
16	122	189	74
20	104	178	102
26	112	214	70

At peak defoliation time (July 1-5), experienced personnel made defoliation estimates at each prism point in all plots (Table 5). Aerial photographs were taken of all spray plots in Massachusetts and Rhode Island.

Table 5. Average post spray defoliation in 1980 spray plots in Massachusetts and Rhode Island.

Treatment	State	Percent Defoliation at Peak
Dipel 4L	MA	10
Dipel 4L + 1645	MA	11
Dipel 4L + Acrylocoat	MA	9
Thuricide 32B	MA	22
Thuricide 32B + 1645	MA	16
Thuricide 32B + Acrylocoat	MA	10
Thuricide 16B	MA	10
Thuricide 32B	MA	17
Check (BT)	MA	25
Gypchek 25 X 10^7	MA	57
Gypchek 12.5 X 10^7	MA	72
Check (Gypchek)	MA	41
SIR-8514 (0.06 lbs/a)	RI	14
SIR-8514 (0.03 lbs/a)	RI	16
SIR-8514 (0.01 lbs/a)	RI	12
Check	RI	43

In the Rhode Island and Massachusetts treatment and check areas we also utilized burlap bands to collect data on larval populations. At each prism point, 10 oak trees were numbered, tagged and banded with 12 inch wide burlap bands. Weekly burlap readings commenced after spraying and were made until adult flight (Tables 6 and 7). At time of fall egg mass counts, burlaps were also checked for new egg masses.

Table 6. Larval counts in Rhode Island SIR-8514 plots.

Dosage	Number Live Larvae Under Burlaps			
	15 days[1]	20 days	25 days	30 days
0.06 lbs.	181[2]	31	8	0
0.03 lbs.	491	137	38	14
0.01 lbs.	318	157	37	4
Check	242	257	129	289

1 Number of days after treatment.
2 Average number of larvae found under 200 burlaps.

Table 7. Larval counts in Massachusetts plots treated with BT.

Treatment	Number Live Larvae Under Burlaps		
	9 days[1]	24 days	51 days
Dipel 4L	454[2]	269	543
Dipel 4L + 1645	303	165	1,003
Dipel 4L + Acrylocoat	319	162	642
Thuricide 32B	376	242	1,113
Thuricide 32B + 1645	521	232	779
Thuricide 32B + Acrylocoat	214	370	997
Thuricide 16B	364	111	318
Thuricide 32B	55	390	2,388
Check	236	578	1,937

1/ Number of days following first application.
2/ Average number of larvae found under 200 burlaps

After each aerial treatment was completed 100 tender northern red oak seedlings were placed on an open area near the runway and sprayed from the air with the same tank mix that was used to treat the 50 acre plots. The plants were returned to Otis where they were stored outside and exposed to natural elements. Five plants from each treatment were bioassayed at various time intervals (Table 8).

Table 8. Bioassay results of oak seedlings treated by aircraft at Orange airport. All mortality readings were made 6 days after introduction of larvae.

Treatment	Bioassay I			Bioassay II			Bioassay III		
	D	R	M	D	R	M	D	R	M
Dipel 4L	4	0	92	6	0.25	87	8	0.85	25
Dipel 4L + 1645	4	0	100	6	0.25	83	8	0.85	51
Dipel 4L + Acrylocoat	5	1.0	92	7	1.0	76	10	2.15	80
Thuricide 32B	4	0	48	6	0.25	47	8	0.85	10
Thuricide 32B + 1645	4	0	100	6	0.25	94	8	0.85	73
Thuricide 32B + Acrylocoat	5	1.0	97	7	1.0	99	10	2.15	75
Thuricide 16B	4	0	100	6	0.25	98	8	0.85	27
Check	4		0	6	0.25	0	8	0.85	2

D - Days following treatment
R - Number of inches of natural rain on plants
M - Percent mortality of 2nd instar test larvae

Little rainfall occurred over the treatment areas following the first application of BT. The first measurable rain was recorded one week following the first application. However, rain was recorded nearly every day during and following the 2nd application of BT and the first and only treatment of Gypchek (Table 9). No rainfall was recorded at the Rhode Island test site.

Table 9. Measureable rainfall recorded following BT and Gypchek treatments in Massachusetts.

Date	Rainfall	Date	Rainfall
5/23	0.0	6/3	0.46
5/24	0.0	6/4	0.20
5/25	0.0	6/5	0.06
5/26	0.0	6/6	0.0
5/27	0.0	6/7	0.37
5/28	0.0	6/8	0.14
5/29	0.0	6/9	0.0
5/30	0.0	6/10	0.25
6/1	0.0	6/11	0.0
6/2	0.01	6/12	0.0

During late October, after most foliage had dropped, post spray egg mass counts were made in all test plots following the procedure described for pretreatment counts. Results are tabulated in Table 10.

Table 10. Egg Mass density change in 1980 spray plots and checks

Treatment	Preseason egg masses/acre	Postseason egg masses/acre	Percent Change
Dipel 4L	1,342	1,003	-25 *
Dipel 4L + 1645	1,613	897	-44 *
Dipel 4L + Acrylocoat	34	282	+729 X
Thuricide 32B	1,721	2,399	+39 *
Thuricide 32B +1645	1,420	1,510	+6 *
Thuricide 32B +Acrylocoat	694	1,640	+136 #
Thuricide 32B/1qt.	355	1,513	+326 #
Thuricide 16B	1,293	810	-37 *
Check *	1,215	6,356	+423
Check #	528	5,449	+932
Check X	43	3,362	+7,719
Gypchek $25X10^7$	842	4,322	+413
Gypchek $12.5X10^7$	1,362	3,571	+162
Check	563	3,140	+458
SIR 8514/0.06 lbs.	117	0.5	-99.5
SIR 8514/0.03 lbs.	153	114[1]	-25
SIR 8514/0.01 lbs.	735	60	-91.8
Check	285	3,426	+1,102

1/ Nearly all egg masses found on five points indicating that a spray skip occurred during application.

Final results of BT tests were somewhat discouraging in that, generally, egg mass densities increased over the season. Comparing the various formulations without sticker added, Thuricide 16B and Dipel 4L resulted in similar foliage protection, and final egg mass counts. Dipel 4L was an easier material to handle and mix. On cold mornings, Thuricide 16B is very viscous and hard to pump. Best results were achieved with Dipel 4L and RA-1645 sticker. Treatments using Acrylocoat gave poor results in terms of egg mass reduction. Thuricide 32B gave poor results in all plots treated, however, with the addition of RA-1645 some reduction in egg masses was noted. Although egg mass reduction was poor, nearly all treatments f BT gave fair to good foliage protection.

Thuricide 32B applied at one quart per acre gave only fair foliage protection. All Dipel treatments and some Thuricide sprays showed a decrease in larval population 24 days following the first application. However, as the season progressed, the number of live larvae under burlaps increased.

When oak seedlings were sprayed by aircraft and bioassayed, both stickers were effective, regardless of the formulation used. Based on this test one can say that satisfactory foliage protection can be achieved with the use of 2 applications of 8 BIU Thuricide 16B or Dipel 4L, without additional sticker. When RA-1645 and acrylocoat were used additional egg mass reduction occurred. RA-1645 was the most effective of the stickers tested.

The poor results with BT may be due in part to droplet size. We had hoped to produce a 75-100 micron dia droplets by using flat fan nozzles directed 90° to the aircraft slipstream. However, with the formulations used we found that this technique gave us a droplet in the range of 25-50 microns. By morning the boom to the point nozzles were actually pointing 10° aft of the slip stream we were able to increase size of droplets. However, the over all size was still very small. The end result may have been some movement of material from the target area.

Our application of Gypchek gave very poor results. No noticable control took place in treated plots. This was likely due to the advanced development of the larvae when treatment occured and the fact that only one application was applied. The special blending needed for this mix made it hard to work with. Because of the larger particle sizes, the virus did not pass through 50 mesh screens well. Therefore, screens were removed and some clogging of nozzle openings took place. Much work has to be conducted to improve this active ingredient and produce a workable, effective formulation. Virus should not be field tested again until a sound formulation is developed and throughly tested in the laboratory. Our experience with Pro Tec, rules out its future use until better quality control of the product can be assured.

Test results with SIR-8514 this year corroborated previous years laboratory and field data. This material is the most effective compound tested against gypsy moth in the past 5 years. As an insect growth regulator it ranks in effectiveness with Dimilin. Excellent egg mass reduction was achieved at dosages as low as 0.01 pounds per acre. The 25W is an easy formulation to work with and can be mixed in a nurse tank or in the aircraft. SIR-8514 is now at the stage of development that would call for acreage average treatment in 1981.

Project Number: GM 1.1.1
Project Title: Field Studies with SIR-8514 and Bacillus thuringiensis (1981)
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

In cooperation with the Pennsylvania Department of Environmental Resources, 3 one-thousand acre spray plots were treated with the insect growth regulator BAY SIR-8514. Three 1,000 acre untreated plots were used as checks. In central Massachusetts, 21 fifty acre woodland plots were treated with Bacillus thuringiensis (BT). Six plots without treatment were used as checks.

BAY SIR-8514, a Mobay product, has been tested in the field for the past two years. In 1979 New Jersey tests, SIR-8514 gave 78% egg mass reduction at 0.03 lbs AI/acre. 1980 applications in Rhode Island also gave excellent gypsy moth egg mass reduction (Table 1).

Table 1. Egg mass density change in 1980 SIR-8514 spray plots and checks.

Dosage/Rate	Preseason Egg masses/acre	Postseason Egg masses/acre	Percent Change
0.06 lbs. AI/ac	117	0.5	- 99.5
0.03 lbs. AI/ac	153	114 1/	- 25
0.01 lbs. AI/ac	735	60	- 91.8
Check	285	3,426	+ 1,102

1/ Nearly all egg masses were found on 5 points in one plot, indicating a spray skip. Eliminating those points from the calculations yields 96% reduction.

Our objective for 1981 work was to demonstrate the effectiveness of SIR-8514 against gypsy moth larvae under operational conditions (pilot study).

With the cooperation of the Pennsylvania Department of Environmental Resources we were able to locate suitable treatment areas near New Germantown, Pennsylvania, in Tuscarora State Forest, Perry County. Plots were established in such a manner to allow all 4 corners to be on or near roads or pipelines. Treatment areas were rectangular in shape.

Egg mass counts were made in late March using a grid systemof 50 prism points per plot. Pre spray egg mass counts were made in late March. Egg masses were counted on all prism trees and on a fixed radius plot at each of 50 points (Table 2). A DBH reading was recorded for each tree included in the survey. Lines and prism points were not marked for use during post spray counts.

Table 2. Pre-spray egg mass counts in 1981, SIR-8514 treatment and check plots.

Plot No.	Dosage	Egg Masses/Acre
1	0.06 lbs. AI/ac	1,632
2	0.06 lbs. AI/ac	1,521
3	0.06 lbs. AI/ac	843
C1	-	2,252
C2	-	213
C3	-	279

During April 1981, the Environmental Protection Agency granted Mobay Chemical Corporation an experimental use permit (3125-EUP-176) for application of SIR-8514 to the Pennsylvania plots. Clearance to conduct the program was acquired from various state agencies. A certificate of waiver for low flying aircraft was then obtained from the Pennsylvania Aeronautics Commission and Federal Aeronautics Admininstration.

Treatments were applied over a period of 4 days, May 15-18. At the time of treatment insects were generally 50% 1st instar and 50% 2nd instar. Foliage expansion was somewhat less than we had hoped for, 20-30 percent. Although a small amount of rain fell shortly after treating part of plot #2, little rain occurred during or following treatment. Application was made at 0.5 gallons per acre.

Mixing and aircraft operations were conducted out of Chambersburg Airport, Chambersburg, Pennsylvania. The material was mixed in a nurse tank before being transferred to the aircraft. The 25 percent wettable powder mixed with ease and handled very well. No sticker was added. The formulation could be mixed in the aircraft to eliminate the need for a nurse tank. A USDA, Cessna-Ag-Truck was used to apply the formulation.

The aircraft was equipped with 18 flat fan nozzles, number 8004. The spray boom was 90° to the aircraft slipstream and a pressure of 40 PSI was used. Application was at 120 mph in 60 foot swaths, 20 feet above the foliage. Plot corners were marked with kytoons and radios were used to communicate between ground crews and aircraft.

Defoliation surveys were made at 25 locations in each plot by experienced personnel at peak defoliation time (Table 3).

Table 3. Post spray defoliation in Pennsylvania SIR-8514 plots.

Plot Number	Percent Defoliation [1/]
1	18.25
2	11.75
3	10.5
C1	99
C2	60.5
C3	34.5

1/ Average of 25 readings per plot.

Aerial photographs were also taken of the study areas; these also showed that SIR-8514 gave excellent foliage protection.

The next phase of this will be during the fall when an extensive postspray egg mass survey will be conducted. Results of this survey will be summarized in a final report.

In central Massachusetts, Thuricide 16B and Thuricide 32BX, Bacillus thuringiensis formulations from Sandoz, Inc., were applied by aircraft (Table 4).

Table 4. Central Massachusetts BT treatments, 1981.

Material	Dosage/Rate [1]	Number Applications	Number Plots
Thuricide 16B	4.0 BIU/ac	1	3
"	"	2	3
Thuricide 16B	8.0 BIU/ac	1	3
"	"	2	3
Thuricide 16B	12.0 BIU/ac	1	3
"	"	2	3
Thuricide 32BX	8.0 BIU/ac	2	3
Check	-	-	6

[1] All treatments were applied at 1 gal/acre. Three percent Acrylocoat sticker was used with each formulation.

Our objective in this test was to determine the most suitable application formulas for this registered material. Recommended applications of BT presently call for 2 treatments, 7 to 10 days apart, using 8.0 BIU/acre. This schedule is relatively expensive compared to chemical treatments; the cost is further increased by the need for 2 applications. Costs could be reduced if efective control would be obtained with a single application or a double application of less than the presently recommended amount of active ingredient. Our objective in testing Thuricide 32BX was to establish its efficacy in the field and evaluate its mixing and handling characteristics.

With cooperation from the Massachusetts Department of Environmental Management, we located suitable study areas at Brimfield State Forest, Brimfield, Massachusetts. The area selected had limited defoliation during 1980 and egg mass density was 822 to 5,438 per acre. Egg masses were of large size and all indications pointed to a healthy, building population.

Using a compass, topographical maps, rope and surveyor tape, 27 fifty acre plots were established. Boundary lines were surveyed and marked in orange tape and each corner tree was marked with double orange tape and a tag identifying corner and plot number. Minimum distance between plots was 400 feet. This was less than desired, however, a limited amount of State land required us to place plots close together. Plots were located in such a manner as too have a maximum number of corners on or near roadways.

Within the center 10 acres of each plot 20 prism points were established, 5 points on 4 parallel lines. No markers were used to identify lines or prism points within individual plots. In March prespray egg mass counts were made on each prism tree and within each fixed radius plot (Table 5). Egg masses were collected at each plot and returned to the laboratory of hatchability tests (Table 5).

Table 5. Pre-spray egg mass counts in 1981 Bacillus thuringiensis treatment and check plots using Thuricide 16B.

Dosage	Applications	Egg Masses/acre 1/	Percent Hatch 1/
4.0 BIU/acre	1	2,812	84
"	2	3,087	74
8.0 BIU/acre	1	2,678	84
"	2	2,193	90
8.0 BIU/acre 2/	2	2,852	91
12.0 BIU/acre	1	2,135	86
"	2	1,481	87
Check	-	2,966	87

1/ Average of 3 study plots. Check is average of 6 plots.

2/ Formulation used was Thuricide 32 BX.

At our request, Sandoz applied for an Experimental Use Permit for 2 applications of Thuricide 16B at 12.0 BIU/acre. A permit was granted in April (11273-EUP-24). We submitted an Environmental Notification Form with the Massachusetts Department of Environmental Affairs and clearance was granted in early April. The Massachusetts Department of Environmental Management also gave clearance for the project. A certificate of waiver for low flying aircraft was also obtained from the Massachusettts Aeronautics Commission and Federal Aeronautics Administration. Home owners within 0.5 miles of any spray plot were notified in person and advised about the spraying. People around the treatment area were also notified by 2 radio stations and 5 newspapers. The selectmen and health officials of each town in or near the treatment plots were notified well in advance of treatments by letter and in person. State and local police were also informed of the treatment dates.

Actual application of material started on May 23 and ended June 3. Plots that were to receive 2 applications were treated first and then plots that were to receive only 1 application were treated. The first round of applications was completed on May 26. Second applications were started on June 2 and were completed on June 3. At the time of the first application, foliage was extended nearly 40 percent and larvae were 66 percent 2nd instar, 20 percent 3rd instar and 14 percent 1st instar. At the time of the 2nd application there were 58 percent 3rd instar, 28 percent 4th instar and 14 percent 2nd instar. Foliage was extended to nearly 80 percent. Very little rain occurred during application of material or during the days following the applications.

Mixing and aircraft operations were conducted out of Palmer Airport, Palmer, Massachusetts. No mixing problems were encountered with Thuricide 16B or Thuricide 32BX. We did find problems in using Acrylocoat in the formulations as a sticking agent. When adding Acrylocoat to the Thuricide and water in the nurse tank, we observed a small number of lumps in the material. After using the first 5 gallon pail of sticker we then screened the remaining pails as they were added to the mix. Although we did not experience any clogging of nozzles or screens in the aircraft system we did experience some buildup of sticker on screens after 3-4 loads were dispersed. However, when we tried to apply 12.0 BIU/acre we had complete clogging of 50 mesh nozzle screens after only 3 swaths of 0.25 miles each. Apparently this formulation was so thick that the sticker did not mix well. The material was applied with no further problems after the nozzle screens were removed. Problems with Acrylocoat may be solved by using an in line screen on the nurse rig and/or the premixing the sticker with water before adding to the mixing tank.

A USDA, Cessna-Ag Truck was used to apply the material. The aircraft was equipped with 8008 flat fan nozzles. The spray boom was 90° to the slipstream and a boom pressure of 40 PSI was used. Application was at 120 mph in 60 foot swaths, 20 feet above the foliage. To aid aircraft guidance, yellow balloons were used at each plot corner. Radios were used to communicate between ground crews and aircraft.

Postspray defoliation surveys were made in all spray plots and checks at the time of peak defoliation (Table 6). Defoliation estimates were made at each prism point by experienced personnel. Final estimates were based on all trees within the experimental plot.

Table 6. Post spray defoliation in Thuricide spray plots.

Dosage	No. Applications	Percent Defoliation 1/
4.0 BIU	1	25
"	2	41
8.0 BIU	1	42
"	2	38
12.0 BIU	1	8
"	2	5
8.0 BIU 2/	2	29
Check	-	70

1/ Average of 3 replications

2/ Thuricide 32 BX

The next phase of the experiment will be conducted in October when post spray egg mass counts are made. Results of the posttreatment egg mass survey will be summarized in a final report.

The encouraging part of the program was the consistently good foliage protection acquired using single and double applications of 12.0 BIU per acre. All replicates had foliage protection comparable to that expected of checmical sprays.

Project Number: GM 1.1.2
Project Title: Fumigation Tests with Carboxide and Methyl Bromide Against Gypsy Moth Egg Masses
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: E. C. Paszek, W. S. Wood, C. P. Schwalbe, J. F. Fons

Introduction:

Methyl bromide causes damage to certain food stuffs, rubber, leather, plastic and various other products and is not suitable for fumigating recreational vehicles. Ethylene oxide does not cause the adverse effects associated with methyl bromide and was tested as a possible agent for the fumigation of sensitive articles infested with gypsy moth egg masses.

Four series of fumigations were done in the fall of 1980 and the spring of 1981 with an ethylene oxide-carbon dioxide 1:9 mixture (Carboxide) on laboratory reared and field collected egg masses. A total of 155 treatments were made, 145 with carboxide and 11 with methyl bromide. Previous data [1/] from 14 fumigation treatments conducted in 1969 indicated that carboxide gave 100% kill of laboratory reared egg masses which had completed diapause. Eggs were exposed for 8, 16 or 24 hours to dosages of 10, 15, 20 or 25 lb/1000 ft^3 at 5, 10 or 22°C. In another series, 11 treatments of freshly collected wild egg masses that had not completed diapause at the time of treatment were exposed for 16 or 24 hours to dosage rates of 10, 15, 20 or 25 lb/1000 ft^3 at temperatures of 7, 12 or 22°C. Hatch occurred only in one of the 2 treatments that were exposed for 24 hours to 20 lb/1000 ft^3 carboxide at 22°C.

1/ Preliminary tests using Vikane, Carboxide and Vertifume against eggs of the gypsy moth, Porthetria dispar (L.), MDL Quarterly Informative Memorandum 1969-4, Project: Insect-Gypsy Moth, PP 1-6, Wesley A. Merriam.

Procedure:

Ten intact masses were randomly selected from specific lots of laboratory reared or field collected egg masses that were stored at 5°C. The masses were placed in kraft paper bags, 1.5 lb size. The opened end of the bag was folded over and stapled to prevent spillage.

Before treatment the samples were held at treatment temperature for 16-24 hours. The treatments were performed in 250, 540, or 1416 liter fumigation chambers, utilizing either a 10% ethylene oxide-90% carbon dioxide mixture or methyl bromide.

The fumigant was introduced into the chamber under a 20 inch vacuum and the chamber was immediately returned to normal atmospheric pressure (NAP). The chamber fans were operated for the first 15 minutes of treatment to assure complete distribution. Thermal conductivity concentrations were taken with a Gow-Mac Gasmaster at the beginning and end of the fumigation. An Ascarite reading to determine EO concentration was taken at the end of each EO-CO_2 treatment. The treatment exposure was calculated from completion of gas introduction. At the conclusion of each treatment, the fumigant was evacuated by drawing a 20 inch vacuum at least three times. The samples were maintained at treatment temperature for 12 to 24 hours. Control, untreated egg masses were stored next to the fumigation chambers at the same temperature. Control masses were placed inside the chamber and treated in the same manner as fumigated egg masses by drawing a 20 inch vacuum 3 times and returning the chamber to NAP. To determine hatchability, each egg mass was placed in a 50 x 9 mm plastic petri dish with a tight lid and incubated in a chamber at 25°C at 90% RH. The incubating egg masses were checked for hatch and when hatching commenced, the larvae were removed daily by aspiration.

Results:

The first series of fumigations was with laboratory reared eggs that had completed diapause (Table 1). Egg masses were exposed for 8, 16 and 24 hours at temperatures of 22, 10 or 5°C at a dosage of 20 lb/1000 ft^3. Complete kill was achieved in both Carboxide and methyl bromide treatments.

In the second series of tests (Table 2), samples of laboratory reared egg masses in 4 stages of development and field collected egg masses in diapause were fumigated Deccember 8-11, 1980.

Table 1. Gypsy moth egg masses[1] fumigated with 10% ethylene oxide, 90% carbon dioxide mixture or with methyl bromide.

Time Exposed	Temp. °C	Dosage lb/1000 ft^3	No. Larvae[2/]	% Kill
		Carboxide		
0	22	0	641	0
24	22	20	0	100
0	10	0	587	0
8	10	20	0	100
16	10	20	0	100
24	10	20	0	100
0	5	0	600	0
8	5	20	0	100
16	5	20	0	100
24	5	20	0	100
		Methyl Bromide		
0	22	0	641	0
2.3	22	1.5	0	100
2.66	22	2	0	100
3.55	22	3	0	100

1/ Laboratory reared NJF_{21} eggs, oviposited 4/25/80, refrigerated 5/23/80 to 11/18/80, fumigated 11/18-20/80, incubated 11/20/80.

2/ Average hatch from 10 egg masses

Table 2. Gypsy moth egg masses fumigated with 10% Ethylene Oxide - 90% Carbon Dioxide mixture.

			Field Collected in Diapause				Laboratory Reared in 4 Stages of Development							
			From Incipient Infestation[1/]		From Area Defoliated[1/]		180 Days In Diapause[2/]		120 Days In Diapause[3/]		30 Days In Diapause[4/]		10 Days After Oviposition[5/]	
Time Exposed	Temp. °C	Dosage lb/1000 ft^3	No. Larvae[6/]	% Kill	No. Larvae	% Kill	No. Larvae	% Kill	No. Larvae	% Kill	No. Larvae	% Kill	No. Larvae	% Kill
0	22	0	383	0	157	0	630	0	589	0	488	0	221	0
2	22	5	320	16.5	96	38.9	33	94.8	7	98.9	51	89.5	0.5[7/]	99.8
4	22	5	107	72.1	24	84.8	0	100	0	100	0	100	0.1	99.9
6.5	21	10	0	100	0	100	0	100	0	100	0	100	0	100
8	22	5	0	100	0	100	0	100	0	100	0	100	0	100
0	10	0	388	0	131	0	517	0	565	0	582	0	357	0
2	10	5	319	17.8	121	7.7	329	36.4	318	43.8	174	70.2	94[7/]	73.7
4	10	5	338	12.9	141	0	36	93.1	34	94	33	94.3	0	100
8	10	5	0	100	0.4	99.7	0	100	0	100	0	100	0	100
0	5	0	403	0	138	0	603	0	545	0	514	0	324	0
2	5	10	345	14.4	104	24.7	467	22.6	454	16.7	15	97.1	0.5[7/]	99.8
4	5	10	2	99.6	7	95	0	100	0	100	0	100	0	100
8	5	10	0	100	0.1	99.9	0	100	0	100	0	100	0	100
8	10	10	0	100	0	100	0	100	0	100	0	100	0	100
8	10	20	0	100	0	100	0	100	0	100	0	100	0	100
8	5	20	0	100	0	100	0	100	0	100	0	100	0	100

1/ Collected 11/22/80, Sandwich, MA, fumigated 12/8-11/80, incubated 2/4/81.
2/ NJF_{21} fumigated 12/8-11/80, incubated 12/15/80.
3/ NJF_{21} fumigated 12/8-11/80, incubated 2/9/81.
4/ NJF_{21} fumigated 12/8-11/80, incubated 5/1/81.
5/ NJF_{21} fumigated 12/8-11/80, sampled for embryonation 1/27/81, all fumigated masses partially embryonated.
6/ Average hatch from 10 egg masses.
7/ Partial hatch occurred in masses under refrigeration.

Laboratory reared egg masses (NJF21)

1) Unembryonated, treated 10 days after oviposition.
2) Embryonated, in diapause 30 days at treatment.
3) Embryonated, in diapause 120 days at treatment.
4) Completed diapause, 180 days at treatment.

Field collected wild egg masses, Sandwich, MA.

5) Small masses, in diapause from an area of total defoliation.
6) Large masses, in diapause from an incipient infestation.

Complete kill was achieved in all 6 sets of the following treatments:

Hours exposed	Temp °C	Dosage lb/1000 ft^3
8	22	5
6.5	21	10
8	10	10
8	10	20
8	5	20

99.7% kill and 99.9% kill occured in the following 2 single treatments of field collected masses from a totally defoliated area in Sandwich, MA. The remaining 5 samples in each of these 2 sets had 100% Kill.

8	10	5
8	5	10

The treatments at the short time exposures of 2 and 4 hours in the dosage range of 5 to 10 lb/1000 ft^3 temperature ranges of 22, 10 and 5°C had a low % kill. The fumigation treatments at 5 lb/1000 ft^3 for 8 hours at 22°C, 5 lb/1000 ft^3 for 8 hours at 10°C, and 10 lb/1000 ft^3 for 8 hours at 5°C are the lowest combination of dosage rates, hours exposed and optimum temperatures that are required to get a high % kill.

The third series of fumigations was conducted on 3/19/81 (Table 4, third column). Egg masses were collected in Sandwich 3/2/81 near the site of the previous collections made on 11/22/80. These masses had completed diapause. The results of 5 lb/1000 ft^3 for 8 hours at 22°C was 100% kill. The following treatments were inconclusive: 10 lb/1000 ft^3 for 8 hour at 10°C treated in duplicate had 100% kill in one replicate and only 27.7% in the other replicate. The treatment 15 lb/1000 ft^3 at 8 hours for 5°C was done in triplicate. The first sample 100% kill, the second had 15.1% kill and in the third sample there was no mortality.

Egg masses treated at 7.5 lb/1000 ft^3 for 8 hours at 10°C showed no mortality. Because of this erratic mortality pattern these treatments were repeated in the next series of fumigations.

Table 3. Gypsy moth egg masses[1/] fumigated with 10% Ethylene Dioxide, 90% Carbon Dioxide Mixtures.

Time Exposed	Temp °C	Dosage lb/1000 ft^3	No Larvae[2/]	% Kill
0	22	0	525	0
4	22	5	0.1	99.9
8	22	5	0	100
8	22	5	0	100
0	10	0	465	0
4	10	5	467	0
8	10	5	402	13.6
8	10	7.5	463	0
8	10	7.5	513	0
8	10	10	383	17.7
0	5	0	565	0
4	5	10	181	68
8	5	10	0	100
8	5	10	465	27.7
8	5	15	0	100
8	5	15	610	0
8	5	15	480	15.1

1/ Large masses collected 3/2/81, Sandwich, MA, fumigated 3/7-13/81, incubated 3/19/81.

2/ Average hatch from 10 egg masses.

The fourth series of fumigations was done 4/2 - 5/21/81 on egg masses collected in Sandwich, MA 3/2/81 and stored at 5°C until treatment (Table 4, column 4); egg masses collected in Sandwich 4/2/81 near the same location of those collected on 3/2/81 (Table 4, column 5); and on egg masses collected at Harriman State Park, NY (Table 4, column 6).

100% kill was achieved at 5 lb/1000 ft^3 for 8 hours at 22°C in the triplicate samples of eggs collected in Sandwich 4/2/81 and at Harriman, NY, and the duplicate samples of egg masses collected in Sandwich 3/2/81. Complete kill was achieved in all single replicates of the 3 sets of egg masses treated with 5 lb/1000 ft^3 for 8 hours at 10° and in duplicate samples of the 3 sets treated at 7.5 lb/1000 ft^3 for 8 hours at 10°C. There was also 100% kill in the 3 samples fumigated at the rate of 12.5 lb/1000 ft^3 for 8 hours at 10°C. Treatment with 10 lb/1000 ft^3 for 4 hours at 5°C had 100% kill in the 2 samples of egg masses from Sandwich, MA and 99.9% kill in the sample from Harriman State Park, NY. The treatments 10 lb/1000 ft^3 for 8 hours at 5°C were done in duplicate in all of the 3 sets. The 2 sets collected in Sandwich on 3/2/81 and 4/2/81 had 100% kill while the set from Harriman, NY had 100% mortality in one sample and 98.9% in the second sample. In egg masses fumigated at 15 lb/1000 ft^3 for 8 hours at 5°C, 4 replicates from Sandwich collected 3/2/81 and 4/2/81 and 3 replicates from Harriman, NY had 100% mortality.

Eight methyl bromide treatments, were made on wild egg masses that completed diapause and were collected in Sandwich, MA and Harriman State Park, NY (Table 5). The 3 treatments at 2.5 lb/1000 ft^3 for 3 hours at 10°C had 100% kill. Two treatments at the lower dosage at the rate of 1.5 lb/1000 ft^3 for 2.5 hours at 22°C had 99.6 and 99.8% kill. Three treatments at the higher dosage rate of 3 lb/1000 ft^3 for 3 hours at 5°C had 97.9, 99.8 and 98.9% kill.

Conclusion:

Four series of fumigations were conducted in the fall of 1980 and the spring of 1981 with the fumigant ethylene oxide-carbon dioxide 1:9 mixture (Carboxide). A total of 156 treatments were made on field collected and laboratory reared egg masses that had completed diapause, were in several stages of development, and on egg masses treated shortly after oviposition before completion of embryonation. Data (Table 6) indicate that a dosage of 5 lb/1000 ft^3 for 8 hours is an adequate treatment at 22°C. At 10°C the dosage would have to be increased to 12.5 lb/1000 ft^3 for an 8 hour treatment. At 5°C the dosage rate would have to be increased 20 lb/1000 ft^3 for an 8 hour treatment. Because of hatch occuring in two higher dosage replicates treated in March 1981 additional treatments will be made in three new series of fumigations in 1981-82. A search of the data showed no apparent reason why that hatch occured. The first of the new series of fumigations was made on non-embryonated egg masses that were fumigated in August 1981. The second and third series will be fumigated in the fall of 1981 and the spring of 1982.

Three methyl bromide treatments at 2.5 lb/1000 ft^3 for 3 hours at 10°C had 100% kill while the remaining 5 treatments at a lower and higher dosage rates did not have 100% mortality (Table 5). Treatments similiar to those using the rate schedule set up in the manual for methyl bromide fumigation should be done in the new series of fumigations in 1981-82.

Table 4. Gypsy moth egg masses fumigated with 10% Ethylene Oxide - 90% Carbon Dioxide mixture.

			Field Collected on Specified Dates from the Following Areas:											
			Sandwich, MA[1/] 11/22/80		Sandwich, MA[2/] 11/22/80		Sandwich, MA[3/] 3/2/81		Sandwich, MA[4/] 3/2/81		Sandwich, MA[4/] 4/2/81		Harriman, NY[4/] 4/2/81	
Time Exposed	Temp. °C	Dosage lb/100 ft^3	No.[5/] Larvae	% Kill	No. Larvae	% Kill	No. Larvae	% Kill	No. Larvae	% Kill	No. Larvae	% Kill	No. Larvae	% Kill
0	22	0	383	0	157	0	525	0	321	0	423	0	396	0
2	22	5	329	16.5	96	38.9								
4	22	5	107	72.1	24	84.8	0.1	99.9			0	100	0	100
6.5	21	10	0	100	0	100								
8	22	5	0	100	0	100	0	100	0	100	0	100	0	100
8	22	5					0	100	0	100	0	100	0	100
8	22	5									0	100	0	100
0	10	0	388	0	131	0	465	0	326	0	344	0	289	0
2	10	5	319	17.8	121	7.7								
4	10	5	338	12.9	141	0	467	0	0	100	0.05	99.9	47.5	83.3
8	10	5	0	100	0.4	99.7	402	13.6	0	100	0	100	0	100
8	10	7.5					463	0	0	100	0	100	0	100
8	10	7.5					513	0	0	100	0	100	0	100
8	10	10	0	100	0	100	383	17.7						
8	10	12.5							0	100	0	100	0	100
8	10	20	0	100	0	100								
0	5	0	403	0	138	0	565	0	365	0	623	0	429	0
2	5	10	345	14.4	104	24.7								
4	5	10	2	99.6	7	95	181	68	0	100	0	100	0.8	99.9
8	5	10	0	100	0.1	99.9	0	100	0	100	0	100	0	100
8	5	10					465	27.7	0	100	0	100	0.4	99.9
8	5	15					0	100	0	100	0	100		
8	5	15					610	0	0	100	0	100	0	100
8	5	15					480	15.1	0	100	0	100	0	100
8	5	15							0	100	0	100	0	100
8	5	20	0	100	0	100								

1/ Large masses from Incipient infestation, fumigated 12/8 -11/80. Incubated 12/4/81
2/ Small masses from defoliated area, fumigated 12/8-11/80, Incubated 2/4/81
3/ Large Masses from Incipient infestation, fumigated 3/7-13/81, Incubated 2/4/81
4/ Large Masses fumigated 4/3-5/21/81, Incubated 6/8/81
5/ Average hatch from 10 egg masses

Table 5. Gypsy Moth Egg Masses Fumigated with Methyl Bromide

Laboratory Reared Masses [1/]

Time Exposed	Temp °C	Dosage lb/1000ft^3	No. [2/] Larvae	% Kill
0	22	0	641	0
2.3	22	1.5	0	100
2.66	22	2	0	100
3.55	22	3	0	100

Field Collected Masses [3/] from the Following Areas

Time Exposed	Temp °C	Dosage lb/1000ft^3	Sandwich, MA 3/2/81 No Larvae [2/]	Sandwich, MA 3/2/81 % Kill	Sandwich, MA 4/2/81 No Larvae [2/]	Sandwich, MA 4/2/81 % Kill	Harriman, NY 4/2/81 No Larvae [2/]	Harriman, NY 4/2/81 % Kill
0	22	0	321	0	423	0	396	0
2.5	22	1.5			1.1	99.6	0.5	99.8
0	10	0	326	0	344	0	289	0
3	10	2.5	0	100	0	100	0	100
0	5	0	365	0	623	0	429	0
3	5	3	7.5	97.9	1.2	99.8	4.4	98.9

1/ NJF[21], oviposited 4/25/80, 180 day diapause completed 11/18/80
Fumigated 11/18-20/80, Incubated 11/20/80
2/ Average hatch from 10 egg masses
3/ Fumigated 5/7/81, Incubated 6/8/81

Table 6. 21 Carboxide Control/treatments where 95% or more kill was observed

Temp. °C	Time Exposed	Dosage lb/1000 ft^3	No. Reps	% Control
22	24	20	1	100
22	8	5	16	100
22	6.5	10	6	100
22	4	5	9	78
22	2	5	6	34
10	24	20	1	100
10	16	20	1	100
10	8	20	7	100
10	8	12.5	3	100
10	8	10	7	86
10	8	7.5	8	76
10	8	5	10	90
10	4	5	10	30
10	2	5	6	0
5	24	20	1	100
5	16	20	1	100
5	8	20	7	100
5	8	15	14	86
5	8	10	14	93
5	4	10	10	90
5	2	10	6	34

Project Number: GM 7.2.4
Project Title: Development of Laboratory Evaluation Techniques for Gypsy Moth Male Comparison
Project Period: October 1, 1980 - September 30, 1981
Project Type: Final
Project Leader: D. R. Lance

This project has involved the development of a sustained-flight wind tunnel as a tool for monitoring the pheromone-mediated behavior of adult male gypsy moths. The project was divided into 2 sections, one of which dealt with the effects of rearing and flight temperatures, and another which compared the pheromone-mediated behavior of wild and laboratory-reared males. This report constitutes only a summary and final analysis of data. For details of results and techniques, refer to GM 7.2.4A and GM 7.2.4B in the October, 1979 - September, 1980 annual report.

Section A. The effect of temperature on the pheromone-mediated behavior of laboratory-reared male gypsy moths.

Final tests were run and data were analyzed using ANOVA and chi-square tests. Tables 1 and 2 show the results of the tests in which the flight tunnel temperature was 23°C (these tests were not complete when the previous report was written). From this study, the following general conclusions may be drawn:

1. As flight tunnel temperature was increased, slightly higher percentages of the males responded to pheromone. The mean time for the males to respond or Response Latency Period (RLP) did not vary greatly with temperature. At cooler temperatures, the males wing-fanned (often while walking) for longer periods, Duration of Preflight Fanning, (the DPF) prior to taking flight.

2. Logarithmic increases in pheromone concentration resulted in linear increases in the percentage of males which responded to pheromone. At 50 ng per wick, almost 100% of the males initiated wing-fanning. RLP's increased with pheromone concentrations. At higher pheromone concentrations, males may perceive females to be nearby, and thus may spend more time searching the immediate vicinity prior to taking flight.

3. Neither the percentage of males which responded to pheromone nor the RLP's were affected greatly by the temperature at which males were held as pupae. However, in the morning, males from cooler pupal treatments appeared to be slightly more responsive than were the males from warmer treatments.

4. Males from cooler pupal treatments were more prone to appetitive flight than were males from the warmer treatments.

5. Though the males from pupae held at 30° responded to pheromone in proportions similar to males from the other treatments, a significantly lower proportion of responding 30° males took flight during the 5 minute test period. Also, compared to males from the other pupal treatments, the 30° males that did fly had consistently longer DPF's and were less capable of sustaining flight in a pheromone plume.

6. Cool pupal-holding temperatures (i.e. with a mean of ca. 20°C) should result in highly competitive sterile males in terms of periodicity of sexual behavior, tendency toward appetitive flight, and ability to locate sources of pheromone.

Section B. Comparing the pheromone-mediated behavior of wild and laboratory-reared males.

The Scheffe procedure was used to separate the mean values for the RLPs and the DPFs for 4 wild strains and 1 laboratory-reared strain (NJS-F20) of male gypsy moths. These moths were tested in a flight tunnel where they were exposed to 1 of 4 concentrations of (+) disparlure (0, 2, 10, or 50 ng). Within each pheromone concentration, there were no significant differences in RLP's among the 5 strains. There also were no significant differences among DPF's, except when a 50 ng wick was used. At 50 ng, the laboratory strain had a significantly longer mean DPF (65.8 sec.) than did one of the wild strains (New Lisbon, NY, with 31.5 sec.). Neither of these strains had a mean DPF which was significantly different from that of any of the other 3 wild strains.

In general, the pheromone-mediated behavior of the NJS males was similar to that of wild males. Some differences arose in DPF's and in ability to sustain flight in a pheromone plume; however, by holding NJS males at cool temperatures (e.g. 20°C) as pupae, it may be possible to minimize even these differences.

Table 1. Response-latency period (RLP) and duration of preflight fanning (DPF), in seconds, for male gypsy moths which were exposed to (+) disparlure in a flight tunnel at 23°C.

	Mean seconds to respond 1/					
	ng (+) disparlure on wick					
	2		10		50	
Pupal Holding Temperature	RLP	DPF	RLP	DPF	RLP	DPF
FT 2/	66.8a	51.9a	29.5a	63.6a	14.3a	82.4a
20 C	98.9a	83.3a	76.1b	68.4ab	14.0a	92.9a
25 C	70.3a	108.8a	50.2ab	81.1ab	14.5a	86.5a
30 C	101.2a	126.1a	64.1ab	140.0b	22.1a	119.3a

1/ Within a column, means which are not followed by the same letter are significantly different at P<0.05, as determined by the Scheffe procedure.

2/ A fluctuating temperature with a daily high of 25°C and a low of 12°C.

Table 2. Responses of males gypsy moths to various concentrations of (+) disparlure in a flight tunnel at 23°C.

Pupal holding temperature	Percentage of males which wing-fanned ng (+) disparlure				Percentage of wing-fanning males which took flight 1/	Percentage of males which sustained flight in a 50 ng plume
	0	2 1/	10 1/	50		
FT	9.5	22.2a	82.5a	100.0	94.3a	9.6%
20°C	6.3	32.1a	79.4a	98.4	90.9a	13.1%
25°C	1.6	26.2a	79.4a	100.0	90.4a	6.7%
30°C	0.0	28.6a	66.7a	98.4	60.2b	2.0%

1/ Within a column, percentages which are not followed by the same letter are significantly different at P<0.05, as determined by chi-square analysis.

Project Number: Gm 8.2.2
Project Title: Radiological Sterilization of Male Gypsy Moth
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leader: Victor C. Mastro

Introduction

The objective of investigations previously reported in this section was to describe the radiation dose required to completely sterilize male gypsy moths. In the course of these studies it was discovered that when males were exposed to some doses which were less than sterilizing and mated with untreated females, the resulting progeny were sterile. Albeit this type of mating does not result in a normal complement of progeny, potentially, several hundred sterile progeny could be produced. These progeny would theoretically provide a population suppression effect in the year following release much greater than the released of a greater number of totally sterile males. Because the sterile progeny would develop in the field, factors which influence development and behavior would be the same as those influencing the feral population. If viable, potential savings in rearing costs and field operations would permit use of the technique in populations of higher densities or over larger areas than presently can be supported by the Otis Methods Development Center's rearing facility.

To determine if the basic principle of induced inherited sterility (F_1 sterility) was sound, a small test was initiated using one level of radiation to treat several ages of male pupae. The results of this test, presented in the following report, demonstrate the principle of induced inherited sterility is workable, however, a second larger test has been initiated to define the dose x age combination which will result in a maximum number of progeny which are totally sterile. The larger study included irradiation of 6 age gropus of male pupae with 7 different levels of radiation or a total of 42 treatments. When completed, results of this study will define the best pupal age and dose to use for production of males for release.

Methods and Materials

Preliminary Test

All male insects for these tests were collected as prepupa (spins), from New Jersey standard strain (NJSS) rearing stock so that exact pupal ages could be determined. Male pupae were irradiated (8 krads) in four different age classes: 4-5, 6-7, 8-9, and 10-11 days after pupation. Twenty males from each treatment group were individually mated to untreated females in 8 oz squat cups. Mating cups were lined with paper toweling which served as an ovipositional substrate. All adults, when paired, were one day old (i.e. the day after eclosion). In addition, twenty untreated males were mated with untreated females to serve as a control.

After oviposition was complete, egg masses were held for 28 days at 25°C, 50-60% RH for embryonation, and then transferred to 6°C for 150 days to satisfy diapause requirements. When the chilling period was complete, egg masses were individually placed in plastic 5 cm dia. petri dishes and held at 25°C, 80% RH for hatch. When hatch was complete all unembryonated eggs, larvae, and embryonated eggs which did not hatch were counted. Eggs from masses which did not contain any embryonated eggs were counted but excluded from analysis of the data.

To compare development and survival of F_1 progeny, 1500 newly hatched larvae from each treatment group were infested onto standard B-4 diet. Larvae were selected so that approximately equal numbers were taken from each egg mass in a treatment group. From past experience, survival rates in the control group were known to be high and, therefore, only 750 larvae were infested from this treatment. Data were collected from all treatments to compare the length of larval development, larval survival, pupal survival and the proportion of abnormalities in the adult stage. Once development was complete, one-day-old F_1 adults were inbred and outcrossed to determine degree of sterility. The following mating types were made for each of the 4 treatments (i.e. age groups when irradiated).

1) F_1 male x untreated female
2) F_1 female x untreated male
3) F_1 male x F_1 female

Resulting F_2 egg masses were embryonated, chilled, and held for hatch in exactly the same manner and under the same conditions as F_1 egg masses. Again at the conclusion of hatch all larvae, embryonated eggs which did not hatch and unembryonated eggs were counted.

Dose-Age Optimization Test

A second test has been initiated for the purpose of defining the optimum age for irradiation and the optimum dose. This test, which is currently in progress included the following male pupal radiation treatments:

Pupal age (days) at treatment time 1/	Radiation Doses (krads) 2	4	6	8	10	12	15	Control
0 - 1	X	X	X	X	X	X	X	X
2 - 3	X	X	X	X	X	X	X	X
4 - 5	X	X	X	X	X	X	X	X
6 - 7	X	X	X	X	X	X	X	X
8 - 9	X	X	X	X	X	X	X	X
10 - 11	X	X	X	X	X	X	X	X

1/ 0 days = the day of pupation.

All insects used in the dose x age optimization test were reared under standard rearing conditions (SRC), on B-4 diet, at 8 insects infested per cup. As in the previous test, males were collected from diet cups as prepupae (spins) so that exact pupation dates were known. Pupae were held under SRC, irradiated at the proper age, and returned to SRC for eclosion. Male and female pupae were held in separate cups which were checked daily for newly eclosed insects. Adults were removed from pupal cups and held until the following day when they were paired in mating cups. Ten pairs were mated for each treatment group for each replicate and the test was replicated five times (i.e. 50 matings were made for each treatment).

Resulting egg masses were held for embryonation under SRC for 28 days, then individually packaged in labeled glassine envelopes and held at 6°C for an additional 150 days. At the conclusion of the cold treatment, egg masses were sampled (plugged), using a procedure developed in preliminary studies. Three samples (plugs) from each egg mass were placed in a petri dish and held at 25°C, 80+% RH for hatch. After hatch was completed all larvae, unhatched embryonated eggs and unembryonated eggs were counted. The portion of each egg mass remaining after sampling was cleaned (dehaired) and an estimate of the total number of eggs made using a volumetric technique which was also developed in preliminary studies. The technique involves dehairing the egg mass, manually counting 50 eggs, determining the volume displaced by 50 eggs in a .5 ml graduated pipette and then calculating the total number of eggs from the total volume displaced by all remaining eggs.

From each treatment, 520 newly hatched larvae (140/replicate) were infested onto B-4 diet at 8 larvae per container and held under standard rearing conditions. Beginning 23 days after infestation, and at 2-day intervals thereafter, rearing cups were checked for pupation. All pupae were removed from rearing containers and placed in labeled containers. Checks of rearing containers continued for 30 days (15 observations) after the initial check. At the time of the last observation, (i.e. 53 days after infestation) any remaining insects were counted, classified for stage of development and discarded. Pupal containers were checked daily for adult eclosion. Adults were removed from containers and classified as normal or abnormal. Abnormal adults were further classified as to types of abnormality (e.g. failure to eclose, crumpled wings, etc).

Both adult males and females were one day old when paired in mating containers. The following types of matings were made for all treatments:

1) F_1 males (progeny of (IR) ♂ x N ♀) x untreated females
2) Untreated males x F_1 females (progeny of ♂(IR) x N♀)
3) F_1 males x F_1 females

F_2 egg masses, now in diapause, will be handled according to the same procedures described for F_1 egg masses. Any neonates hatching from egg masses will be reared under standard procedure and appropriate back crosses with normal adults will be made to determine the degree of sterility in the F_2 generation.

Results

Preliminary Test

Fecundity of mating pairs appears to be affected by the age at which the male parent was irradiated. Pairs, which included a male treated in the youngest age group (4-5 days old), were less likely to mate successfully (i.e. result in an egg mass which contained embryonated eggs); and for those pairs which did mate successfully produced smaller numbers of eggs. Although the mean number of eggs produced was not significantly different from the control group it was significantly smaller than the other three treatments.

Table 1. Proportions of successful matings and mean numbers of eggs produced when untreated females were mated to males which had been irradiated (8 krads) in 4 age groups.

	P_1 Male Treatment				
	Group 1 IR 4-5 days old	Group 2 IR 6-7 days old	Group 3 IR 8-9 days old	Group 4 IR 10-11 days old	Control
No. of successful matings/No. of pairs	16/20	20/20	20/20	20/20	20/20
Mean no. of eggs/ mass	569.5a	731.8b	819.9b	865.3b	719.3ab

1/ Horizontally, mean proportions followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

The proportion of eggs in masses which were embryonated also varied with male treatment age. The smallest proportions of embryonated eggs resulted when male parents were either treated in the youngest or oldest age groups (Table 2).

Table 2. Mean proportions of embryonated eggs produced by untreated females mated to males irradiated (8 krads) in 4 age groups.

P_1 Male Treatment				
Group 1 IR 4-5 days old	Group 2 IR 6-7 days old	Group 3 IR 8-9 days old	Group 4 IR 10-11 days old	Control
74.06a 1/	86.65b	95.12b	73.20a	96.14b

1/ Horizontally, mean proportions followed by the same letter are not significantly different at the 5% level of significance according to Duncan's Multiple Range Test. For analysis, proportions were transformed to the arcsine of the square root. Actual percentages are presented above.

The percentage of embryonated eggs which hatched was nearly the same for all treatment groups (Table 3).

Table 3. Mean proportions of embryonated eggs which hatched when untreated females were mated to males irradiated (8 krads) in 4 age groups.

P_1 Male Treatment				
Group 1 IR 4-5 days old	Group 2 IR 6-7 days old	Group 3 IR 8-9 days old	Group 4 IR 10-11 days old	Control
44.24a 1/	45.24a	55.42a	47.52a	93.63b

1/ Horizontally, mean proportions followed by the same letter are not significantly different at the 5% level of significance according to Duncan's Multiple Range Test. For analysis, proportions were transformed to the arcsine of the square root. Actual percentages are presented above.

The results presented on Tables 2 and 3 suggest that the age at which males are irradiated affects sperm transfer or mobility, but that sperm that are able to fertilize eggs are equally damaged regardless of adult parental treatment age. In other words matings involving males, irradiated in the youngest age classes, produced egg masses which had a lower proportion of embryonated eggs but nearly equal proportions of embryonated eggs hatch in all treatments.

Table 4 presents the proportions of total eggs which hatched for the various treatment groups. These proportions are useful for computing the number of F_1 progeny one would expect from each treatment.

Table 4. Mean proportions of the total eggs which hatched when untreated females were mated to males irradiated (8 krad) in 4 age groups.

Group 1 IR 4-5 days old	Group 2 IR 6-7 days old	Group 3 IR 8-9 days old	Group 4 IR 10-11 days old	Control
37.25 a [1/]	43.68 a	53.05 a	35.78 a	90.34 b

1/ Horizontally, mean proportions followed by the same letter are not significally different according to Duncan's multiple range test at the 5% level of significance. For analysis, proportions were transformed to the arcsine of the square root. Actual percentages are presented.

For example, from Table 1, one would expect an average of 596.5 eggs to result from mating a male treated at 4-5 days of age with a normal female. From Table 4, an average of 37.25% of the eggs would be expected to hatch, producing 212 F_1 progeny.

Survival and development of F_1 progeny are also important considerations in selecting the optimum dose age combination. Table 5 presents developmental data for all treatment groups. Survival is nearly the same for all treatments through the pupal and to the adult stages. Of note, however, are the skewed sex ratios resulting from all treatments where the male parent was irradiated. This ratio varies from ca 1.5:1 to ca 2.2 male:1 female ratio. These ratios would result in an even greater impact in the F_1 generation than if the ratio was a normal 1M:1F ratio as seen in the control group. Disproportionate effects of irradiation on female F_2 progeny are further described by the data in Table 6. Large numbers of adult female progeny were abnormal (incomplete ecdysis, crumpled wings, wings without scales, etc). F_1 adult males also displayed a higher level of abnormalities than the control group but the largest proportion of abnormal males was found in the oldest treatment group and this was only five percent.

Larval development appears to be longer for progeny of irradiated males (Table 7). Differences between progeny of normal matings and progeny from matings which had an irradiated male parent were small and well within the range of normal development times. Larval development under natural conditions is slower than development in the laboratory. It is not known how natural conditions will affect F_1 larval development or survival. These areas must be studied before the result of partially sterile males release can be predicted with certainty but the development and suvival data presented here indicate that progeny of irradiated males are comparable to normal insects.

Table 5. Survivorship of F-1 progeny resulting from irradiated (8 krads) males mated with untreated females.

	TREATMENT PARENTAL GENERATION					
	IR(4-5)MxNF[1/]	IR(6-7)MXNF	IR(8-9)MXNF	IR(10-11)MXNF	Total all IR	NM XNF(Control)
Total No. Infested	1500	1500	1500	1500	6000	750
No. of pupae harvested	864	735	730	788	3117	440
Percent of total surviving to pupal stage	57.6	49.0	48.7	52.5	52.0	58.7
Male:female pupal ratio	1.63:1	1.43:1	2.20:1	1.94:1	1.77:1	0.97:1
No. of pupae observed for eclosion	795	721	690	732	2938	423
No. of adults eclosing	748	661	628	672	2709	416
$\frac{\text{No. adult}}{\text{No. pupae}} \times 100$	94.09	91.68	91.01	91.80	92.20	98.34

1/ NF = untreated female, NM = untreated male

Table 6. Development of progeny from irradiated (8 krads) males mated with untreated females.

	TREATMENT PARENTAL GENERATION																	
	IR(4-5) M x NF			IR(6-7) M x NF			IR(8-9) M x NF			IR(10-11) M x NF			Total all IR Treatments			NM x NF Control		
	♂	♀	Σ	♂	♀	Σ	♂	♀	Σ	♂	♀	Σ	♂	♀	Σ	♂	♀	Σ
No. of adults	483	265	748	394	267	661	450	178	628	456	216	672	1783	929	2709	193	223	416
No. of adults abnormal	6	27	33	8	47	55	19	38	57	23	59	82	56	171	227	0	10	10
No. of adults with incomplete ecdysis	3	17	20	3	23	26	3	18	21	0	12	12	9	70	79	1	4	5
Total of abnormal adults & those with incomplete ecdysis	9	44	53	11	70	81	22	56	78	23	71	94	65	241	306	1	14	15
Percentage of adults with incomplete ecdysis	1.9	16.6	7.1	2.8	26.2	12.3	4.9	31.5	12.5	5.0	32.9	14.0	3.6	25.9	11.3	0.5	6.3	3.6

Table 7. Rate of development of progeny from irradiated (8 krads) males mated with untreated females.

	TREATMENT PARENTAL GENERATION																	
	IR(4-5) M x NF			IR(6-7) M x NF			IR(8-9) M x NF			IR(10-11) M x NF			Total all IR Treatments			NM x NF Control		
	♂	♀	Σ	♂	♀	Σ	♂	♀	Σ	♂	♀	Σ	♂	♀	Σ	♂	♀	Σ
$\bar{x}$ total development time in days	46.6	46.4	46.5	47.2	47.1	47.2	47.1	46.4	46.9	47.9	47.5	47.8	47.2	46.9	47.1	45.3	44.7	44.9
$\bar{x}$ larval development time in days	33.0	34.5	33.6	33.7	35.4	34.4	34.2	35.2	34.5	31.9	36.1	35.3	34.0	35.2	34.4	31.8	32.9	32.4
$\bar{x}$ pupal development time in days	13.6	11.9	12.8	13.6	11.7	12.8	12.9	11.3	12.4	13.0	11.4	12.5	13.3	11.6	12.7	13.5	11.8	12.6

Results of inbreeding and outcrossing F_1 adults are presented in Tables 8-11. Smaller egg masses result when mating F_1 males with normal female. Although analysis indicates no difference between treatments (i.e. treatment age of P_1 male) there is a significant difference between mating types. F_1 females crossed with normal males produced egg masses which contained the greatest numbers of eggs.

Table 8. Mean total eggs per mass (F_2 egg masses) produced when F_1 progeny of irradiated males were inbred and outcrossed.

Pupal treatment age of P_1 male parent	Mating Type					
	F_1 M X N F	No.	N M x F_1 F	No.	F_1 M x F_1 F	No.
4-5 days old	748.2	10	776.0	10	537.5	8
6-7 days old	634.8	9	887.4	10	706.1	9
8-9 days old	561.3	10	889.6	9	531.1	8
10-11 days old	627.0	6	846.9	10	586.8	6

		DF	F	SIG
ANOVA	Main effect	5	4.349	.00134
	Treat	3	.502	.68159
	Mating	2	10.055	.00011

Embryonation of F_2 egg masses is affected by both mating type and the parentage (age group of the P_1 male when treated) (Table 9). Generally, when the F_1 male was a parent the proportion of embryonated eggs decreased with increasing pupal irradiation age. Poor sperm transfer or mobility could decrease embryonation. Both of these reasons could also explain the small proportion of pairs producing embryonated egg masses in the oldest treatment group (Table 8, 10-11 day old group). Both mating types involving F_1 males in this group produced only 6 embryonated egg masses out of 10 pairs. Because pairs were not observed at mating time it is not possible to determine if this resulted from a lack of mating or because of mating without sperm transfer.

Table 9. Mean proportions of total eggs (F_2 egg masses) which were embryonated when F_1 progeny of irradiated males were inbred and outcrossed.

Pupal treatment age of P_1 male parents	F_1 M X N F	No.	N M X F_1 F	No.	F_1 M X F_1 F	No.
4-5 days old	85.83	10	76.86	10	8.29	8
6-7 days old	53.99	9	90.50	10	38.59	9
8-9 days old	38.11	10	87.97	9	12.04	8
10-11 days old	12.54	6	77.91	10	4.95	6

Source	DF	MF	F	SIG
Main	5	2.9733	35.245	.0000
Treat	3	0.67792	8.036	.0008
Mating	2	6.63658	78.669	.0000

The smallest mean proportion of embryonated eggs which hatched were from the 8-9 day old male treatment group, regardless of mating type (Table 10). Fertility of mating pairs from the oldest treatment group (10-11 days old) appears to be greater. However, because of the small number of embryonated eggs in this group, the number of eggs hatching was very small (Table 11). Progeny of males from the two oldest treatment groups are very sterile, producing egg masses that averaged less than 2% hatch, independent of mating type (outcrossing or inbreeding). However, the two youngest age groups produced progeny which were partially fertile. This anomoly may be partly explained by poor sperm transfer of F_1 progeny from the older age groups. However, embryonation in the third treatment group averaged ca 40%, yet hatch was very low, ca 3%. Whatever the mechanism of sterility, these data indicate that age at treatment is as important as dose. Results of the presently incomplete dose-age optimization test will provide the optimum age(s) and dose combination(s) for inducing sterility in the F_1 generation. These results will be reported in the next annual report.

Table 10. Mean proportions of embryonated eggs which hatched from F_2 egg masses produced when progeny of P_1 irradiated males were inbred and outcrossed.

Pupal treatment age of P_1 male parents	Mating Type F_1 M x N F	No.	N M x F_1 F	No.	F_1 M x F_1 F	No.
4-5 days old	47.07	10	9.73	10	0.74	8
6-7 days old	33.85	9	19.20	10	1.27	9
8-9 days old	2.53	10	1.79	9	0.00	8
10-11 days old	23.89	6	2.52	10	16.67	6

		DF	F	SIG
ANOVA	Main effects	5	5.970	.00008
	Treat	3	3.853	.01198
	Mating	2	9.439	.00019

Table 11. Mean proportions of total F_2 eggs per mass which hatched when progeny of P_1 irradiated males were inbred and outcrossed.

Pupal treatment age of P_1 male parents	Mating Type F_1 M x N F	No.	N M x F_1 F	No.	F_1 M x F_1 F	No.
4-5 days old	44.59	10	8.21	10	0.08	8
6-7 days old	25.58	9	18.6	10	0.32	9
8-9 days old	1.09	10	1.45	9	0.00	8
10-11 days old	0.85	6	1.88	10	0.04	6

		DF	F	SIG
ANOVA	Main effects	5	8.336	.000
	Treat	3	6.939	.00029
	Mating	2	10.227	.00010

Project Number: GM 8.2.6
Project Title: Comparison of 6 Large Capacity Gypsy Moth Traps
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: E. C. Paszek and C. P. Schwalbe

The objective of this study was to compare the commercially produced Baker and Hercon traps with the standard 1/2 gallon USDA MC trap and the 1 gallon size USDA MC trap. Studies conducted in 1980 indicated that the 1 gallon size MC trap with 2 pieces of 3.2" (DDVP) dog collar was the most efficient trap in capturing moths. Traps (as described in the following table) were tested in sparse population. There were 5 replicates of each trap. Results are summarized below:

Trap Type and Size	Lure Type	Killing Agent (DDVP)	Total No. Moths Captured in 5 Traps
USDA - MC 1 gal	500 ug (+) (Hercon)	6.4" dog collar	874
USDA - MC 1/2 gal	500 ug (+) (Hercon)	3.2" dog collar	677
Baker - 1.2 qt	0.8 % (+) (Baker)	3.2" Bag-A-Bug Insect Strip	403
Baker - 1.2 qt	500 ug(+) (Hercon)	3.2" dog collar	389
Hercon - 1/2 gal	500 ug(+) (Hercon)	3.2" dog collar	362
Hercon - 1/2 gal	13% (±) (Hercon)	1X4" Hercon Insect tape	87

The gallon size MC trap was the most efficient trap in capturing moths. The 2 Baker Bag-A-Bug traps were both baited with (+) lure but captured a reduced number of moths. Evidently the design of the Baker Bag-A-Bug is deficient in some regard. The Hercon trap most closely resembles the USDA 1/2 gallon trap but captured fewer moths than any other design. This was partially attributed to the inferior quality of the paper from which the trap was constructed. After exposure to rain, it's hood sags, causing it to have an improper alignment with the body of the trap. In this study the hood was stapled to the trap to prevent slippage, however, sagging did occur.

The Hercon trap baited with the racemic 1x1" lure tape captured very few insects. This study indicates that the USDA 1 gallon and 1/2 gallon size large capacity traps are the most efficient traps in capturing moths.

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, 1980 - September 30, 1981
Report Type: Final
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. A total of 36 plots were ultimately established and the preseason egg mass counts for each set of treatment and control plots are summarized in Table 1.

Table 1. Preseason estimates of egg mass densities in mating disruption test plots. PA, 1980

Treatment	Egg masses/ha		
	Low	Medium	High
Hercon	2.6	45.6	204.0
Conrel	3.6	29.3	200.0
Check	3.6	43.7	236.8

After eggs hatched in the spring, 4 burlap bands were placed in each subplot (64 bands/plot). These were examined periodically and observed insects (larvae and pupae) were taken as further measure of population density within the plots. The average number of female pupae/band at peak pupation in each set of treatment plots is summarized in Table 2 and the results indicate that real differences in population densities existed.

Table 2. Average number of female pupae/burlap band at peak pupation in mating disruption test plots. PA, 1980.

Treatment	Female pupae/burlap band		
	Low	Medium	High
Hercon	.8	17.8	23.4
Conrel	.5	15.8	23.5
Check	1.3	16.3	25.9

The treatment of plots commenced shortly after first observed pupation. The Conrel fibers were applied July 2-4, 1980 and Hercon flakes were applied July 4, 1980.

Treatment effectiveness was monitored by male moth captures in 1/2 gal traps baited with (+) disparlure. There were 8 traps in each plot; traps were examined every other day and the number of moths recorded. These results are tabulated in Table 3.

Table 3. Total number of male moths captured in 8 disparlure-baited traps in each test plot. PA, 1980.

Treatment	Number of males captured		
	Low	Medium	High
Hercon	129 (94) 1/	885 (86)	2063 (70)
Conrel	45 (98)	332 (95)	1587 (77)
Check	1986	6371	6926

1/ Values in parentheses represent percent reduction from the corresponding check.

Capture of male moths in pheromone-baited traps was clearly reduced by disparlure treatment. The Conrel formulation appeared to cause the greatest reduction and the effect was population density dependent. Note, however, that catch was similar in the medium and high density check plots, indicating that adult male population densities were similar. No attempt was made to quantify adult female densities (other than through pupal counts summarized in Table 2).

Mating incidence of female moths was also monitored by placing 8 laboratory-reared moths in each plot. These insects were replaced every other day and any deposited eggs were examined for embryonation ca 6 weeks later. These data are summaried in Table 4.

Table 4. Incidence of mating of laboratory-reared female moths in test plots. PA, 1980

Treatment	Percent of females mated		
	Low	Medium	High
Hercon	16.4 (71)[1]	28.3 (66)	35.2 (55)
Conrel	10.2 (82)	35.7 (56)	45.3 (38)
Check	57.1	82.0	73.4

1/ Values in parentheses represent percent reduction from the corresponding check.

Female moths in the treated plots had a lower incidence of mating than those in check plots. Parenthesized values represent percent reduction in mating from the check. Mating was reduced anywhere from 38-82%, depending upon treatment and population density. It is noteworthy that treatments were more effective in suppressing mating at lower population densities than at higher densities. As before, differences between treatment and control are statistically significant.

After adult flight had ceased, each plot was surveyed to estimate post season egg mass densities. These results are summarized in Table 5.

Table 5. Postseason estimates of egg mass densities in mating disruption test plots. PA, 1980

Treatment	Egg masses/ha		
	Low	Medium	High
Hercon	50.4	4864	7427
Conrel	19.2	1851	7042
Check	134.4	4782	8842

At low population densities, egg mass counts in both treatments were lower than those in the check plots. Also, the medium density plots treated with the Conrel formulation had lower post-season counts than the checks. No apparent differences were observed in the higher population plots. Because of variation among plots, these differences were not found to be statistically significant, but outwardly, it appears that greatest effects were found in low population plots.

All postseason egg mass counts, regardless of treatment, were higher than those preseason (much higher, in fact, than we would predict using trap data as a measure of efficacy). Incidence of female mating more closely correlates with population growth, but this relationship is still imperfect. While it is clear that populations increased, the increase seems to have been dampened by disparlure treatment, especially at sparse population densities. This dampening of population increase may have significance in slowing an outbreak. The large increases observed (at least) in the check plots are definite indication that these populations were surviving as larvae and reproducing as adults very efficiently. In view of the fact that the mating disruption method is non-lethal to the parasite-supporting larval and pupal stages of the gypsy moth, parasite reproduction in the year of treatment is not affected. Thus, parasite abundance at the beginnning of the following season should be the same as if no treatment was made. Maintaining parasite levels and simultaneously suppressing gypsy moth mating could result in better synchronization or balance of parasite and host densities the following generation. Integration of parasite conservation with mating disruption may be useful in lengthening the period of time between outbreaks or, perhaps even changing population trends from increasing to decreasing densities. However, such tactics are likely to be successful only at the lower population densities at which mating disruption appears to be effective.

Project Number: GM 0.2.7
Project Title: Sterile Male Trial Berrien County Michigan
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leader: V. C. Mastro

Introduction

Preliminary results of releasing sterile males in Berrien County during the 1980 field season were summarized in the last annual report (October 1, 1979 - September 30, 1980 pp 132-140). The following is intended to finalize the reporting of 1980 data (egg mass evaluations, etc.) and report the preliminary results of the 1981 release.

Methods and Materials

Sterile Male Production - 1981

Rearing procedures described in the last annual report were used again in 1981. These procedures provided pupae which, when shipped to the release site, were internally marked, and at least 8 days old when exposed to 15 krads of gamma radiation. Females, for monitoring purposes, were reared in the same manner except that dye was not included in the larval diet and they were not exposed to any gamma radiation.

Plot Design

With the following exception, plot design and layout in 1981 duplicated the 1980 effort. Density of burlap banding in the core area was increased from a single tree burlaped per point (47.5 m^2 grid) in 1980 to all trees (an average of 6) burlaped within a 5m radius of the point. Banding density within the core area, which was ca 18 ha and contained 93 points, was increased to provide a better definition of larval distribution and also to provide feral females for mating evaluations. Density of banding in the remainder of the plot (eg. around the core area) remained the same as in 1980.

Sterile Male Release - 1981

Beginning on July 15 ca 9000 irradiated male pupae daily were placed in the pilot study area. Releases continued until August 20 which resulted in a total release of ca 333,000 males. A weighted release system was again used to distribute the pupae. Because the 1981 larval survey failed to provide much information on feral insect distribution, the same release pattern used in 1980 was repeated in 1981 with the following adjustments: 1) Within the core area, clusters of rotation points (eg. 4 release sites spaced 47.5 m apart on a grid) were provisioned with 2 containers per site for a total of 8 release containers. This strategy, which was used throughout the flight period, provided for an 8 day eclosion period before reprovisioning each release container. 2) Outside the core area where an 8 day rotation was also maintained, release sites were spaced 90 m apart.

Wire mesh cylinders were used for release at those sites where more than 50 males were placed. These containers were similar to those used in 1980. However, to discourage avian predators the wire mesh size was reduced from 1"w X 1"h (used in 1980) to 1"w X 1/2"h. Also, wrapping the lower portion of the container with fine mesh screening (1/8"), a practice initiated mid-season 1980, was adopted for use in the 1981 season.

A maximum of 450 males were placed in the wire mesh cylinders during the 1981 season. Testing at the Otis laboratory indicated that when 600 male pupae were placed in a container, as was done at some release sites in 1980, eclosion was retarded. Where 50 males or less were released at a site the modified quart milk-carton container developed in 1980 was employed.

Evaluation 1981

A trap density of 32/sq mi, as in 1980, was maintained through the 1981 field season. Delta traps, used for survey in areas surrounding the release area in 1980, were replaced with high capacity milk carton traps. Males captured in milk carton traps can be more reliably identified than those captured in delta traps. Traps within, and immediately adjacent to the sterile male release area (e.g. 28 traps) were checked daily. The remaining traps were checked weekly. All captured males were identified as either wild-type or sterile released males (i.e. internally marked red).

Male mating success was again monitored daily by placing twenty laboratory-reared one day old virgin females in the infested core area. In 1980, females were placed in the morning and collected the following morning. However, in 1981, because of increased predation pressure, females were collected during the evening of the same day they were placed. Virtually all females were mated when collected. Egg masses oviposited by placed females have been held for embroynation and will be evaluated when 150 days of cold treatment is complete.

Control matings, as in 1980, were done at the Otis laboratory. In addition to the 25 IR male X untreated female and 25 untreated male X untreated female crosses done daily, IR male X feral female and feral male X feral female crosses were done when wild-type insects were available. Both untreated and irradiated males were randomly selected from sterile male production lots.

At the conclusion of the field season a thorough search of the core area was carried out in an attempt to locate egg masses deposited by feral females. In 1980, this search resulted in the location of 49 egg masses, two of which were egg masses produced in 1979 (hatched egg masses). The search in 1981 succeeded in locating only one feral egg mass presumably because of the decrease in the density of native insects.

Results

Evaluation of 1980 Egg Masses

Egg masses produced by females which were placed for monitoring mating success in the study area were returned to the Otis laboratory for evaluation. Egg masses were packaged individually in glassine envelopes and held at 25° ± 1°C, 80-90% RH for 28 days to complete embryonation. To satisfy diapause requirments the egg masses were held at 6°C for an additional 150 days. A total of 305 egg masses were recovered from monitor females. In addition, the 47 egg masses collected from feral females at the end of the field season were evaluated. Because the oviposition dates for feral egg masses were unknown the 28 day embryonation period was timed from the probable oviposition date (i.e. peak feral flight). Holding and handling of these wild-type egg masses was otherwise the same as for masses produced by monitor females.

To provide base line data on the degree of sterility that would be expected from 1) irradiated male X untreated female and 2) untreated male X untreated female crosses, 25 males were randomly selected daily from production lots before and after irradiation treatment. Appropriate crosses were made and resulting egg masses were handled and held in the same manner as monitor female egg masses. A total of 637 egg masses from each type of mating were available at the end of the season for evaluation.

The mean number of eggs resulting from both mating types was nearly identical (Table 1) indicating that irradiated males appear to induce the same ovipositional response as normal males.

Table 1. Mean number of eggs per mass produced by females used in control matings.

Mating Type 1/	No. of Matings	$\bar{x}$ No. of eggs/mass	S. D.	F
IR X N	637	774.89	221.57	.025
N X N	637	772.93	222.26	

1/ IR and N denote irradiated (15 Krads) and untreated insects respectively.

Normal females when mated to irradiated males produced a smaller proportion of embryonated eggs (Table 2). This reduction indicates that sperm transfer is not as complete as normal matings or a portion of the sperm are in some manner defective.

Table 2. Mean proportions of total eggs which were embroynated when females were crossed with irradiated (15K) males and untreated males.

Mating Type 1/	No. of Matings	$\bar{X}$ % of total eggs which were embroynated	S. D.	F 2/
IR X N	637	76.55	21.90	196.09
N X N	637	88.84	19.84	

1/ IR and N denote irradiated (15 Krads) and untreated insects respectively.
2/ Analysis using transformed values (arcsine $\sqrt{\text{proportion}}$)

Proportions of embroynated eggs which hatched provides a clear separation of the two mating types (Table 3). This is the most reliable method for determining the parentage of an egg mass.

Table 3. Mean proportions of embroynated eggs which hatched from egg masses produced by females used in control matings.

Mating Type 1/	No. of Matings	$\bar{X}$ % of embroynated eggs which hatched	S. D.	F 2/
X N	637	18.04	16.55	7019.273
N X N	637	88.51	13.30	

1/ IR and N denote irradiated (15 Krads) and untreated insects respectively.
2/ Anaylsis using transformed values (arcsine $\sqrt{\text{proportion}}$)

To categorize egg masses (produced by monitor females and feral females) as being either the result of mating with a irradiated or a feral male the following test statistic was employed:

where: $\chi^2 = (\bar{X} - X)\sigma_{\bar{X}}^2$

X = the observed proportion (No. of neonates/No. of embryonated eggs)

$\bar{X}$ = the mean proportion derirved from control matings

$\sigma_{\bar{X}}^2$ = the variance of the control population mean

Chi square was tested at the 95% confidence level.

Of the 305 egg masses collected from monitor females: 261 (86%) were the result of sterile male X untreated female crosses and 44 (14%) were from untreated male X untreated female crosses. This is a ratio of 5.9:1 sterile to fertile matings.

Results of evaluating the 47 feral egg masses indicated that 38 matings were sterile and 9 matings were fertile; ca. 81% sterile matings or a 4.2:1 sterile:fertile mating ratio.

Larval Evaluation.

Weekly checks of burlap bands began during the week of June 1-7. The first larvae were located during the week of June 15-21. The greatest number of larvae (11) were found at 6 sites during the week of June 22. Final checks of banding sites were made during the week of July 13-20, at which time only 2 larvae were located. In all, 14 larvae were identified between June 15 and July 13. Results of the 1980 banding survey identified at total of 110 larvae. The greatest number found during a single weekly check (July 13-17) was 102. Therefore, even with increased effort, eg. greater banding density, ca 87% fewer larvae were located in 1981. As in 1980, as larval development progressed, fewer were found during subsequent weekly check. Loss of larvae under bands can be partially attributed to predation by Calosoma spp and vertebrates.

Results from the evaluations of monitor female and feral female egg masses agree with the apparent reduction in larval density. The sites at which larvae were found in 1981 all were within the same area where larvae were found in 1980. Apparently burlap banding trees on a grid pattern resulted in a fairly accurate description of the distribution of the infestation. The pattern that emerged from male captures in both years of study is also well correlated with larval distribution.

Adult Evaluation

Trapping in 1981 resulted in a total of 50 feral males captured. This is an ca 82% reduction from the number of feral males trapped in 1980 (274 feral males). The flight period during 1981 was nearly the same as in 1980 (Table 4); the first feral male was captured 3 days earlier in 1981. There was a 27 day feral male flight period in 1980 and a 25 day flight period in 1981. Peak male flight in 1980 occurred between 7/31 - 8/12. In 1981 the largest number of males captured on any one day occured 7/26. A total of six males were captured on this date, five within the confirmed infested area, or inner trap zone (ITZ) and one male was captured in a trap located immediately adjacent to the zone. However, the majority of males were captured between 8/1 - 8/10 and this appears to be the time of peak male flight.

Overflooding ratios in 1980 were low at peak feral male flight and on several occasions the ratios were below 10S:1F (Table 4). The ratios during peak native flight (7/31 - 8/12) in 1980 averaged 14.4S:1F. In 1981 during the period of peak feral capture (8/1 - 8/10) overflooding ratios averaged 128.6S:1F. The lowest overflooding ratio recorded in 1981 was on 7/22 the first day of feral male capture (ie. 35S:1F).

Discussion

The initial objective of this sterile male pilot study was to demonstrate the interaction of sterile males with fertile females in a natural gypsy moth infestation. The results reported here demonstrate that interaction and additionally demonstrate a marked effect on the density of a native population. The measured effect on the native population is nearly the same regardless of the evaluation technique used:

1)	Monitor female egg mass evaluation	86% sterile matings
2)	Feral female egg mass evaluation	81% sterile matings
3)	Larval evaluation (burlap bands)	87% reduction
4)	Adult evaluation (trapping)	82% reduction
5)	Native egg mass finds	98% reduction

Clearly, the native population has deceased in density in the pilot study area over the 1980-1981 field season. Reduction is concluded in light of the past history of the population where males captures increased before intervention by approximately 3 fold from 1979 (90 males) to 1980 (274 males). Other populations in Michigan and indeed all throughout the area infested with gyspy moths were increasing in density during 1980 and 1981. Results of releasing males again in 1981 should demonstrate a further reduction in the native insect density and provide more evidence that sterile male release is an efficacious treatment for population suppression and/or elimination.

To demonstrate that the technique is a tool for elimination of sparse isolated populations we propose to release sterile males again in the 1982 field season. The native population density in 1982 should be much lower than in 1981 given the high overflooding ratios achieved (128S:1F) during peak flight. This overflooding ratio should result in an ca. 88% reduction in the native density or ca 0-10 feral males will be captured in 1982, depending on rate of increase of the native population density.

Assuming a 3 fold increase in population density (the rate of increase from 1979-1980) and a 1:1 sex ratio, the following calculations provide expected results of the 1981 release and of the proposed release in 1982.

Year	X-S:F Ratios	No.♂'s captured ITZ during peak flight		No♀'s 1/	No.♀'s reproducing
		Sterile	Feral	Feral	
1980 (7/31-8/12)	14.4:1	3193	237	237	15.4 X 3 fold increase=46.2
1981 (8/1-8/10)	128.6:1	3673	46.2 (50♂'s captured)	46.2 50♂'s	0.36 X 3 fold increase=1.08 or 0.39
1982 expected	3000:1	EST (3000)	1.08	1.08	0

1/ Assuming 1F:1M ratio for ever male trapped.

These calculations are based only on the numbers of males captured in traps which represents only a small proportion of the total feral and sterile populations. For example, the 237 feral males captured in 1980 were only 3%-10% of the total feral population (est. 2,370 - 7,900). Therefore the one expected feral male and female in 1982 could represent a population of 10 - 34 feral males and an equal number of feral females. Also, the expected density would be altered if the rate of increase is above or below a yearly threefold increase.

If the goal is eradication, the economical and biolgical advantages of initiating a program with higher S:F overflooding in the first year are clear. Given developments in rearing technology, including mechanization and the ability to rear predominately males, costs should decrease and make the technique more attractive.

Clearly this technique offers many potential advantages over standard eradication technology. Indeed, in environmentally sensitive areas where other technology is inapplicable, it is the only technique which has sufficent, supporting data to justify use with a high degree of confidence. Using the sterile male technique in combination with other strategies offers certain advantages. The technique complements treatments targeted at the immature stages e.g. pesticides, parasites, predators and mechanical control (destruction of larvae and/or egg masses). Integration of several of these may provide an eradication or suppression program that is cost efffective and environmentally sensitive.

Presently, the information available about the utility of the technique can only be extrapolated from the Berrien County pilot test. For the 1982 field season we plan to continue to release in Berrien County and initiate a replicate of the trial in another isolated population. This will provide more data on the utility of the technique, but will not begin to explore areas which could ultimately contribute substantially to our program. The 1982 objectives will include a proposal to initiate an inherited sterility (F_1 sterility) trial in an isolated infestation. The potential for this technique (described in another section of the report) is great. Preliminary data suggest the technique is viable. Knipling recently calculated that a release of substerile males at a ratio of 9S:1F would result in population suppression that would require a release of 79 totally sterile males: 1 fertile male. Because 4 or 5 years are required to test the approach, the sooner the essential tests are begun, the sooner implementation into the program can be expected.

Project Number: GM 0.2.8
Project Title: Factors Affecting Sexual Periodicity in Male Gypsy Moths
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: D.R. Lance, V.C. Mastro, T.M. ODell and G.L. McAninch

The competitiveness of sterile males can be reduced if their sexual activity is not in synchrony with that of wild males. In 1977 field studies with pheromone-baited traps, peak recapture of NJS males occurred 1-3 hours later than peak recapture of wild males. Subsequently, it was found that the sexual activity of NJS males would occur earlier in the day if the males had been held at relatively cool temperatures during the pupal stage. Unfortunately, cool temperatures also result in long developmental times. Before cooler pupal holding temperatures could be used in the sterile male program, age-dose irradiation tests would have to be completely redone, and pupal holding space would have to be increased. Further, it is likely that the flight clocks of wild males also are programmed by the temperatures which are encountered during the pupal stage. Ambient temperatures will vary from target site to target site and from day to day. Thus, optimal pupal-holding temperature would be different for each release.

Since the discovery of a mechanism to adjust male sexual periodicity, T. ODell and G. McAninch have been working to develop a practical means of utilizing this mechanism to improve the competitiveness of sterile male gypsy moths. In one series of tests, they have attempted to locate a "window", or short period of time, during which exposure to cool temperatures will cause a shift in activity rhythms. Males were reared under standard conditions (25°C) but were exposed to cold (15°C or 20°C) for 2 or more days during the pupal stage. When the exposure to cold occurred at or near the end of the pupal stage, male activity rhythms shifted somewhat, but not as much as they shifted when males were exposed to cold throughout metamorphosis. Thus, the temperature-sensitive shift in activity rhythm does not appear to be a "windowed" phenomenon; rather, it appears to be the cumulative effect of prolonged exposure to a given temperature regime.

Interestingly, differences in sexual periodicity might be reduced by the current methods of irradiating, shipping and releasing sterile males. For pilot tests, males were irradiated, packaged in a chilled container, and shipped as 8-11 day old pupae. They arrived at the release site and were placed in the field within ca. 24 hours of irradiation. With this system, 70-80% of males were exposed to ambient field conditions for 6-10 days prior to eclosion. Data from the Benton Harbor pilot test indicate that the 6-10 days at naturally fluctuating temperatures may eliminate much of the diffference in sexual periodicity between laboratory-reared and wild males. This hypothesis is being tested in actograph studies. Still, flight periodicity of sterile males appears to be more variable than the flight periodicity of the Benton Harbor males. More work will be conducted in this area.

In another series of actograph tests, gypsy moths were held under constant light from the time of hatch until adult eclosion. These insects also showed a distinct circadian periodicity of activity. Thus, if an internal circadian "clock" is set from photoperiodic cues, that setting must occur prior to hatch. Further tests will be run to determine if and when the internal clock is set.

Project Number: GM 1.2.1.
Project Title: Behavior and Ecology of Immature Gypsy Moths: Population Quality and Competitiveness of F_1-Sterile Individuals
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: D.R. Lance, V.C. Mastro, J. Elkinton and C.P. Schwalbe

When exposed to substerilizing dosages of radiation, insects typically produce a partial complement of more or less normally-developing offspring. For lepidopterans, it often is possible to find an irradiation dosage which causes complete sterility in the offspring of substerilized individuals. Currently, tests are being run to pinpoint a dosage which results in completely sterile F1 irradiated insects (see section GM in this report).

For sterile insect technique (SIT) projects, success hinges on the ability of the sterile insects to survive and mate (i.e., be competitive) in the field. The competitiveness of F1-sterile insects depends, in part, upon a number of aspects of larval behavior and physiology (e.g., survival on local host plants, ability to locate preferred hosts, ability to avoid predation and parasitization, resistance to disease, phenological synchrony with host plants, synchrony with normal larval development, etc.).

For the gypsy moth, larval behavior, development and survival vary from population to population. This variation is influenced by environmental factors such as food quality and population density. Population quality has been used as a catch-all term to refer to behavioral, physiological, and ecological differences among populations.

To estimate the competitiveness of F1-sterile larvae, we must understand how environmental factors influence population quality, and, specifically, we must understand the behavior and ecology of feral larvae in target populations. Further, a better understanding of population quality also could provide insight into such questions as: (1) is it necessary to apply control measures to a given population, (2) if so, what is the best control measure to apply, and (3) is it possible, through management practices, to "reduce" the quality of a gypsy moth population, thereby eliminating the need for control?

This report contains information on a series of preliminary studies on larval behavior and ecology. These studies are aimed at developing techniques for studying population quality and for assessing the feasibility of using F1 sterile gypsy moths as a method of control.

Study #1 - Synchrony of hatch in egg masses from various rearing, irradiation and chill treatments.

Before F1-sterile insects can mate with wild insects, the F1-sterile individuals first must survive to adulthood. Survival of early instars is impossible without suitable food, which is available only when hatch is in close synchrony with budbreak of host trees. Further, development of F1-sterile gypsy moths must be closely synchronized with wild insects.

Methods and Materials

On April 20, 1981, egg masses from various treatments were stapled onto both sides of a wooden "tent" which was held outdoors under a nylon canopy. The egg masses were examined daily (although occasional days were missed), and first instars were counted and removed. Egg mass treatments are summarized in Tables 1 and 2. Anaylses were run on the Julian date of 50% hatch for the various egg masses.

Results and Discussion

With F1 sterility, it may be feasible to control populations by releasing eggs which develop into completely sterile adults. To use this technique, we must know the chill (diapause) treatments which result in synchrony of hatch between released eggs and the eggs in target populations. In these tests, there was a significant inverse relationship between the length of the chill period and the time of hatch (Table 1). An acceptable degree of synchrony with hatch of wild eggs was produced by only a narrow range of chill periods, i.e., ca 170 days in the cold. A combination of relatively warm chill temperatures (8-9°C) and longer (>180 days) chill periods appears to result in a good deal of post-diapause development prior to the termination of the chill period. When chill temperatures were slightly lower (4-6°C), eggs which were chilled for ca. 220 days hatched slightly later than wild eggs (Table 2). Also, unusually long or short chill periods resulted in reduced hatch (Table 1).

Parental irradiation had little or no effect on the time of hatch (Table 2). Thus, if P1-substerilized males are released in an SIT program, their offspring should hatch in synchrony with the offspring of unirradiated males.

When egg masses were held over winter in an outdoor insectary, their hatch occurred slightly ahead of wild hatch (Table 2). This difference may have been caused by the selection pressures of laboratory rearing, or may have resulted from differences in microhabitat during the chill period.

These tests indicate that the timing of hatch is strongly affected by the length and temperature of the chill period. If sterile insects are to be released as eggs, great care must be taken to ensure that the hatch of released insects is in synchrony with both the hatch of wild insects and the budbreak of host trees. In other forest pests (i.e., the spruce budworm and the winter moth), asynchrony between hatch and budbreak has been shown to severely reduce the viability of the insects. It may be possible to synchronize the hatch of wild and released insects by placing F1-sterile eggs into the field as early as possible. Fortunately, parental irradiation was not found to affect the timing of hatch, and so the efficacy of releasing P1-substerilized males should not be reduced by an asynchronous hatch of F1 larvae.

Study #2 - Dispersal and activity of first instar larvae from several gypsy moth populations.

The dispersal, activity and viability of first instar larvae may vary with population quality. Theory suggests that such variation could have important implications both for the reproductive success of individuals and for gypsy moth population trends. This study was a preliminary investigation to determine whether, in fact, dispersal behavior and activity rates differ among first instar gypsy moths from different types of populations.

Methods and Materials

Egg masses were collected from 6 populations in Massachusetts. The characteristics of the 6 populations are listed in Table 3. Eggs were dehaired and held at 25°C until hatch. Newly hatched larvae were marked lightly with Day-glo powder and were released, in the early afternoon, onto the bases of small potted trees (3 red oaks and 3 red maples). The following evening, the trees were examined with a black light, and the presence of any marked larvae was noted. The trees were held outside, and the boles of the trees were banded with sticky tape to prevent larvae from crawling on and off of them.

In other tests, first instars from the different populations were placed into 3.7 litre wide-mouth jars. The jars were placed outside in a shaded location. For 15 min. of each hour from 0900 to 1600 hrs, movements of the larvae were traced onto the outside of the glass. At the end of the day, the larvae were removed, and distance was measured for each hour's movement using a "sticky-string" technique.

Results and Discussion

The proportions of first instars which remained on potted trees are shown in Table 4. Higher proportions of larvae remained on red oak (a preferred host) as compared to red maple (a less preferred host). Also, on red oak, larvae from "poorer" quality populations (i.e., outbreak and decline) dispersed significantly less frequently than did larvae from "higher" quality populations. On red maple, there were no significant differences in the proportions of larvae which remained on the trees.

This technique shows promise as a means of assessing the vagility and host-plant relations of first instar gypsy moths. However, the results of this particular series of tests may be unreliable for a number of reasons, e.g., transplantation stress on the trees, the unknown effects of marking on the behavior of first instars, and problems with releasing larvae onto the bases of the trees. These problems will be corrected before the tests are re-run.

In glass jars, there were no significant differences in the activity of larvae from the different populations. Possible differences may have gone undetected for 2 reasons: low sample size and high variability within populations. With this technique, obtaining a sufficient sample size would require time input beyond the value of the information which would be gained. This technique probably will not be used in the future.

Study #3 - Development and feeding rhythmms of gypsy moth larvae from different types of populations.

Larvae from different types of populations show qualitative differences in development and behavior. When compared to larvae in high-density populations, larvae in endemic populations develop slowly into large individuals. Also, larvae in endemic populations feed nocturnally, while larvae in high density populations feed throughout the day. These tests were designed to determine whether these developmental and behavioral traits can be transmitted from one generation to the next, or if these differences arise from more immediate environmental effects.

Methods and Materials

Larvae from 5 of the populations in Table 3 (the Ridge eggs were lost) were reared individually in 6-oz plastic cups. Larvae either were fed 3x weekly on B-4 artificial diet, or were fed daily on a diet of excised leaves (pin oak or red maple). Before being given to larvae, leaves were surface-sterilized in a weak (<0.5%) sodium hypochlorite solution for 5 min., washed in water for 5 min., and then partially dried. All insects were held at 25°C, 50-60% RH, and a 0500 to 2100 hours photoperiod.

Feeding rhythms of diet-reared larvae were monitored using a mechanical recording device (Fig. 1). Fifth or sixth instar larvae were placed in the feeding cages between 1400 and 1500 hrs, and left for 24 hours. The percentage of larvae which fed was computed for each hourly period from 1500 to 1300 hours the next day.

Results and Discussion

Table 6 shows pupal weights and developmental times for diet-reared gypsy moths from the 5 populations. There were no consistent differences in pupal weights among the different populations. Developmental times were significantly shorter for the laboratory strain than they were for the P_1 strains. This probably was the result of 21 generations of exposure to selection pressure in the laboratory. Differences in feeding rhythms also did not appear to carry over from generation to generation (Fig. 2A). In these tests, there appeared to be little or no differences in heritable characteristics among gypsy moths from different wild populations, except that the DAR larvae developed a little faster than the other larvae did. This suggests that qualitative differences among populations results primarily from more or less immediate differences in the field environment.

In general, larval development and survival was poor on natural foliage. This may have occurred because larvae were not hatched from eggs until 2-3 weeks after budbreak. No maple-fed larvae survived. On pin oak, only one NJS larvae and only ca. 15% of wild larvae survived to adulthood. There were no significant differences in development among foliage-fed individuals (Table 7). The relatively poor performance of foliage-fed NJS larvae indicates further potential problems in releasing F1-sterile egg masses as a control measure.

Study #4 - Density-dependent stress factors and gypsy moth population quality.

As outlined in Study #3, gypsy moths from different types of populations exhibit consistent qualitative differences in several developmental and behavioral parameters. This study was undertaken to determine if sub-components of density-related stress could be responsible for the qualitative differences between high and low density populations.

Methods and Materials

Developmental parameters and feeding rhythms were monitored for gypsy moths from several experimental treatments. Feeding rhythm determination is

described in Study #3. All rearing was done at 25°C, 50-60% RH, and 0500 to 2100 hours Pp. Larvae were hatched from Bay Rd. eggs (Table 3) which were dehaired and surface-sterilized prior to being used. The density-related stress treatments are as follows:

1. Control - insects were held at one per 6-oz plastic cup and were fed 3x weekly on B-4 diet.

2. 10/cup, left - larvae were reared at 10/cup. First instars were provided with 90 ml of B-4 diet per cup and were left to complete larval development without further feeding.

3. 10/cup, fed - each cup contained 10 larvae which were provided, 3X weekly, with a fresh chunk of B-4 diet.

4. SILK - Experimental larvae were reared at 1 per cup, and were fed 3x weekly. For this test, 4 sets of cups were used. At each successive feeding, each set of cups was treated in one of the following manners:

   Feeding period A: 5 larvae were placed in each cup. They were provided with diet.
   Feeding period B: The 5 larvae were left in the cups, but the diet was removed.
   Feeding period C: The 5 larvae were removed. An experimental larva was transferred into each cup, and it was fed.
   Feeding period D: Experimental larvae were removed. The cups then sat empty until the next feeding period, at which time they were started back at feeding period A.

   At each feeding period, one of the sets of cups each was treated as A, B, C and D. Thus, experimental larvae always were transferred into cups which previously had contained 5 starved larvae. These starved larvae apparently had been quite active and they typically spun large quantities of silk in their environment.

5. NEWCUP - Each larva was provided with a new, unused cup at each feeding period (3X weekly).

6. LCON - From instar III through pupation, 5 freeze-dried larvae (instar IV-V) were placed in each cup which contained an experimental larva.

7. STARVE - Insects were reared at 1/cup. They were fed on one day, and, on the next day, any remaining diet was removed. They were fed again on the following day, etc., throughout development. This was done to simulate feeding interruptions which may occur due to interlarval contact, defoliation of trees, and/or the high degree of intertree dispersal that occurs in high density sites.

8. VIRUS - Larvae were reared at 10/cup through the first 3 stadia. Upon molting to 4th instars, larvae were held for 24 hours without food, and then were given a small chunk of diet which had been innoculated with 10^6 PIB's of NPV. Larvae were discarded if they did not consume the entire chunk of diet within 24 hours. From instar IV through pupation, larvae were held individually in 6-oz cups and were fed 3X weekly.

9. VIRUS CONTROL - Larvae were treated as in #8, but the small chunk of diet was innoculated with distilled water.

Results and Discussion

Developmental parameters of experimental insects are shown in Table 8. Individuals which were reared at 10/cup were significantly smaller than those which were reared at 1/cup. However, developmental times did not vary significantly between 10/cup and control treatments.

Development of larvae from the SILK treatment was similar to the development of Control insects; unexpectedly, female NEWCUP insects did show reduced pupal weights and longer developmental times. Possibly, larvae which were placed into clean cups may have expended energy while searching for and/or reconstructing silk resting and feeding mats. Such expenditures of energy could affect development.

LCON insects also developed similarly to controls. On the other hand, STARVE insects developed slowly into small individuals. Small size is characteristic of insects in high-density populations; however, long developmental times are not.

There were no significant differences in development between VIRUS and VIRUS Control insects. 15% (4) of the VIRUS larvae died from NPV; all of the VIRUS Control insects survived from instar IV to adult.

For all except STARVE larvae, feeding rhythms were similar to those shown in Fig. 2A. On days following a day when STARVE larva had been provided with food, the STARVE larvae also showed this typical feeding rhythm. However, on days which followed a day when STARVE larvae had not been exposed to food, their feeding rhythms shifted: a large proportion of the larvae fed in the afternoon, and night feeding showed a single peak in the middle of the scotophase rather than the typical 2 peaks, one following "lights-off" and one following "lights-on" (Fig. 2B).

These tests indicate that density alone may not be sufficient to explain differences in population quality between gypsy moths in high and low density populations. Recent evidence indicates that defoliation of perennial plants can result in significant changes in foliar nutrient content and plant defensive chemistry. The relationship between leaf chemistry and population quality remains to be worked out. Also, the environment inside an XE-6 cup may be so different from the field environment that the effects of the stress treatments were more or less obliterated. Further, shifts in feeding rhythms possibly could be an immediate result of either interlarval contact or lack of suitable food. All feeding rhythm tests were run with 1 larva per cage and an ample supply of food. Clearly, much work is needed to explain density-correlated shifts in gypsy moth population quality.

Table 1. Relationship between length of the chill period and the timing of hatch for gypsy moth egg masses [1/].

Length of chill period	no. of egg masses	Mean days to 50% hatch [2/]	Mean length of hatch period (days)	Mean larvae per egg mass
79	6	32.8a	8.3	96
107	5	32.0a	15.6	172
136	6	29.3ab	12.8	430
164	6	23.0bc	18.0	497
188	6	12.8d	21.5	450
216	6	8.8d	12.1	213
244	6	14.3d	11.1	100
Wild	15	20.4c	12.2	317

1/ For chill treatment, NJS-F21 egg masses were held at 8-9°C. Wild egg masses were collected from Sandwich, MA in April, 1981.

2/ Number of days after April 20, 1981, when 50% of individuals had hatched from an egg mass. Means not followed by the same letter are significantly different at $P<0.01$, as determined by the Scheffe procedure.

Table 2. Effect of irradiation of male parent upon time of hatch in gypsy moth egg masses.

Age at Irradiation (Days after pupation) 1/	Chill treat-ment 2/	no. of egg masses	Mean days to 50% hatch 3/	Mean length of hatch period (days)	Mean larvae per egg mass
10-11	Inside	6	24.2a	5.7	91
8-9	Inside	4	23.0a	10.0	127
6-7	Inside	6	23.5a	7.0	226
Unirradiated	Inside	10	23.3a	7.7	229
10-11	Outside	6	18.5b	16.7	212
8-9	Outside	5	19.6a	13.6	246
6-7	Outside	6	19.7a	15.7	371
Unirradiated	Outside	10	18.8b	15.0	541
Wild	in situ	15	20.4a	12.2	317

1/ NJS-F20 males were irradiated as pupae at 8 krad. Wild egg masses were collected from Sandwich, MA, in April, 1981.

2/ Inside egg masses were held for ca. 220 days at 4-6°C. Outside egg masses were held in a screened insectary from Sept., 1980 to April 20, 1981.

3/ Number of days after April 20 when 50% of individuals had hatched from an egg mass. Means not followed by the same number are significantly different at $P<0.01$, as determined by the Scheffe procedure.

Table 3. Characteristics of 6 Massachusetts gypsy moth populations from which egg masses were collected.

Population name	Population location	Population classification	Estimated egg Mass density (per hectare) 1/	Mean egg size 2/
DAR	Goshen	Innocuous	<<10	1.22a
Bay Rd	Belchertown	Release/Outbreak	3,000	1.23a
Ridge	Belchertown	Outbreak	>10,000	no data
Cadwell	Pelham	Decline	2,000	1.21a
Jackson Hill	Montague	Decline	1,000	1.21a
NJS-F21	Otis AB	Laboratory	-	1.20a

1/ Estimated from counts of 4-1/100 ha. plots.

2/ Mean of 20 eggs from each of 10 egg masses.

Table 4. Proportions of first instar gypsy moths which remained on potted trees for >30 hours.

	Tree Species				
	Red Oak		Red Maple		
Source Population	# larvae released	Percentage of larvae recaptured 1/	# larvae released	Percentage of larvae recaptured	Percentage recaptured
Innocuous	65	26.2a	66	18.2a	22.1a
Release	63	28.6a	56	14.3a	21.8a
Outbreak	43	44.2ab	57	15.8a	28.0a
Decline (J.H.)	46	52.2b	59	10.2a	28.6a
NJS-lab-reared	42	33.3ab	44	22.7a	27.9a

1/ Within a column, percentages which are not followed by the same letter are significantly different, as $P<0.05$ as determined by the X^2 test.

Table 5. Activity of 1st instar gypsy moths from various types of populations.

Population type	n	Mean distance/larva/day (cm) 1/ Larval age 0 Days	1 Day	Mean	Mean dispersal attempts per larva per day (observed)
Innocuous	10	78.3a	149.7a	114.0a	1.5
Release/outbreak	10	56.0a	87.1a	71.5a	1.4
Outbreak	10	91.4a	108.8a	100.1a	2.3
Decline	5	41.5a	79.9a	60.7a	0.6
Laboratory	10	112.2a	82.7a	97.5a	3.4

1/ As measured in 3.8 L glass jars during eight 15-minute periods throughout the day. With a column, means not followed by the same number are significantly different at P<0.05.

Table 6. Developmental parameters for B-4 diet-reared gypsy moths from several populations.

Population type	Male development 1/ no. of Indivi-duals	Mean larval development	Mean pupal Wt(gm wet)	Female development 1/ no. of Indivi-duals	Mean larval development	Mean pupal Wt(gm wet)
Laboratory	32	30.1a	0.87b	31	33.3a	3.22a
Innocuous	8	32.8ab	0.75ab	15	36.6b	3.57a
Release/ outbreak	31	34.0b	0.79b	30	39.1c	3.53a
Collapse 1	37	34.6b	0.70a	30	38.6bc	3.54a
Collapse 2	17	35.4b	0.78ab	11	39.8c	3.57a

1/ Within a column, means not followed by the same letter are not significantly different at P<0.05, as determined by the Scheffe procedure.

Table 7. Developmental parameters for pin oak-reared larvae from several populations.

Population type	Male development 1/			Female development 1/		
	no. of Indivi-duals	Mean larval development	Mean pupal Wt(gm wet)	no. of Indivi-duals	Mean larval development	Mean pupal Wt(gm wet)
Innocuous	13	53.2a	0.32a	13	61.2a	0.92a
Release/ outbreak	8	53.3a	0.35a	18	62.2a	0.84a
Collapse 1	9	55.2a	0.39a	13	61.6a	0.99a
Collapse 2	17	53.1a	0.35a	12	61.8a	0.97a

1/ Within a column, means not followed by the same letter are significantly different at P<0.05, as determined by the Scheffe procedure.

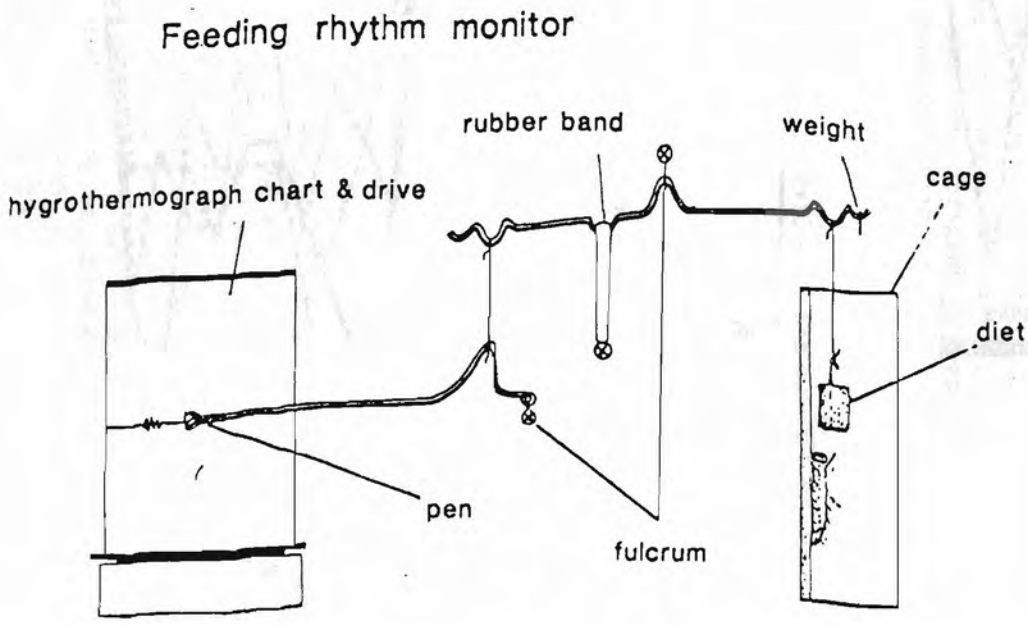
Table 8. Developmental parameters for laboratory-reared gypsy moths which were exposed to various stress factors.

Population type	Male development 1/			Female development 1/		
	no. of Indivi-duals	Mean larval development	Mean pupal Wt(gm wet)	no. of Indivi-duals	Mean larval development	Mean pupal Wt(gm wet)
Control	23	35.2a	0.759a	34	39.6a	3.29ab
10/cup,left	73	37.0a	0.640cd	67	40.7a	2.46def
10/cup,fed	49	37.2a	0.612d	41	40.1a	2.26f
SILK	15	34.7a	0.655bcd	14	38.9a	3.08abc
NEWCUP	9	36.0a	0.657abcd	18	42.0bc	2.76cd
LCON	13	34.4a	0.757ab	15	38.2a	3.48a
STARVE	9	42.1b	0.541d	19	46.9d	2.11f
VIRUS	11	37.5a	0.718abc	10	44.2c	2.92bcd
VIRUS Control	16	36.3a	0.707abc	13	44.5c	2.92bcd

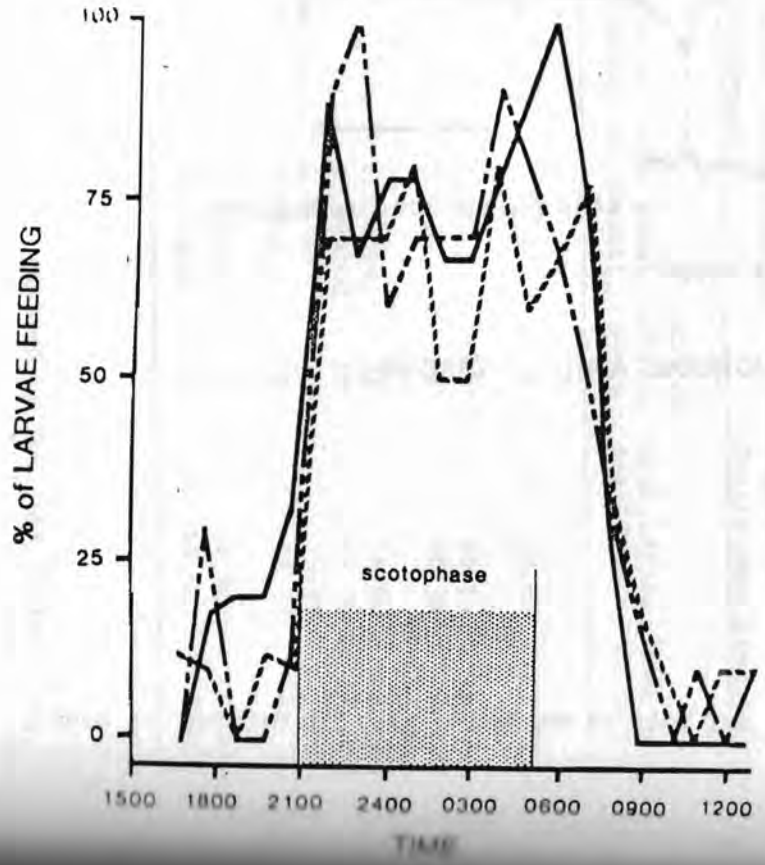
1/ See text for explanation of stress factors.

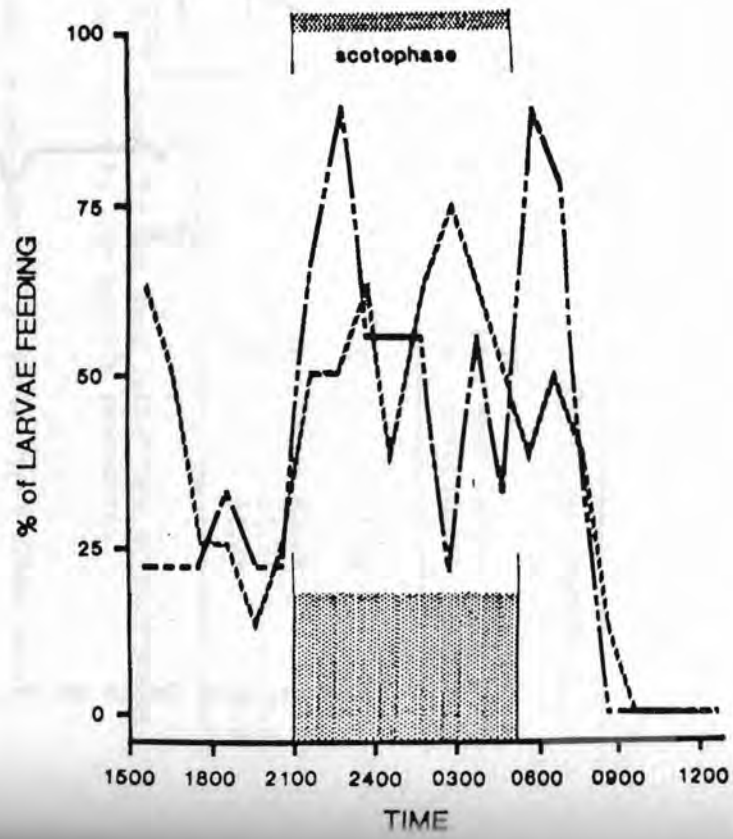
2/ Within a column, means which are not followed by the same letter are significantly different at P<0.05, as determined by Duncan's new multiple range test.

Figure 1. Mechanical feeding rhythm monitor for late-instar gypsy moth larvae.



Figures 2A and 2B. Feeding rhythms of late-instar gypsy moths in the laboratory. Fig. 2A: Larvae which we have taken, as eggs, from (1) Laboratory strain (solid line), (2) Innocuous population (evenly broken line), (3) Decline population (dash-dot-dot line). Fig. 2B: Larvae which were partially starved throughout development and (1) were starved prior to test (evenly broken line), or (2) were provided with food on day prior to test.





Project Number: Gm 1.2.2
Project Title: Sterile Male Technique-Application and Evaluation Studies
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Project Leader: V. C. Mastro, T. M. ODell, G. L. McAninch

INTRODUCTION

The following series of reports describes tests which had the general purpose of supporting the sterile male program. The objective of some of these trials was testing the quality of males currently used for the pilot study, while other tests were designed to evaluate new procedures or techniques which could potentially provide a more efficient or effective sterile male treatment. The following is the sequence in which these tests will be discussed.

I) Release methods. Objective-evaluate the merits of different release methods.

II) Evaluation of internal marking, radiation and packing treatments. Objective - quality control

III) Evaluation of adult males which had been reared on three larval diets. Objective - improve larval diet/lower cost.

IV) Evaluation of adult males which had been held under different temperatures in the pupal stage. Objective - determine if male flight periodicity could be altered by pupal holding temperature.

I. RELEASE METHODS

Sterile males used in the pilot study site (Berrien and Van Buren Counties, MI) were released by placing pupae (9-12 days old) in release cages from which they eclose and disperse over an eight day period. Release containers were designed, using results of predation studies, to minimize predation while not retarding eclosion or dispersal. To determine if other release methods are more efficient or result in a larger proportion of males eclosing, dispersing and actively searching for pheromone sources, a field study was conducted to evaluate four different release methods. This study did not attempt to determine what the causes of male losses were; the purpose was only to compare the proportion of released insects which were recovered (i.e. trapped at pheromone sources).

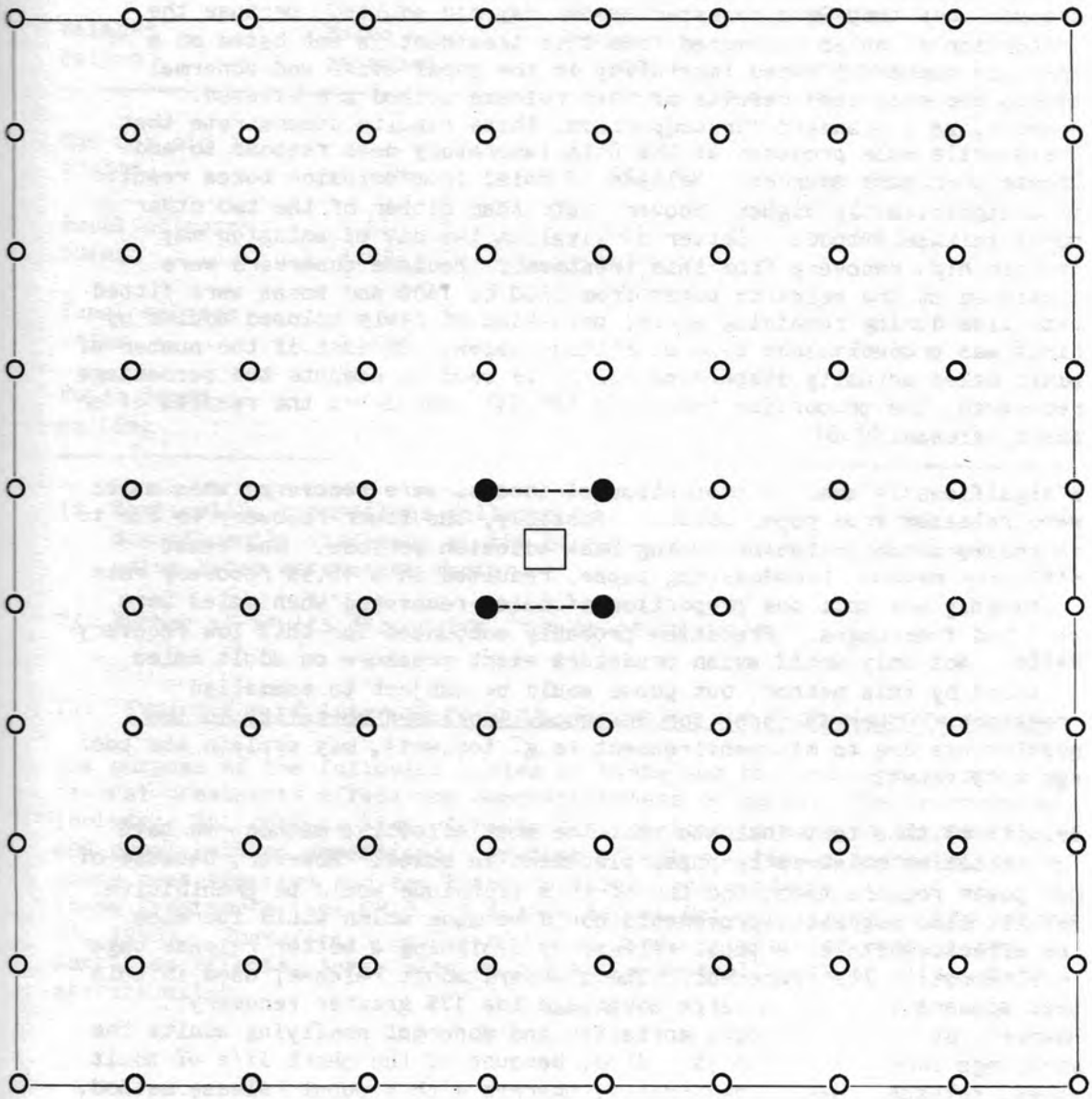
METHODS AND MATERIALS

Four different methods were used to release males from the center of a 10x10 grid of high-capacity traps (100 m intertrap spacing). The four release methods follow: 1) pupal release from standard release cages (described in GM 0.2.7) 2) pupal release from eclosion boxes 3) release by broadcasting pupae and 4) adult release (one day old males). Past experience has shown that adult release usually results in a large proportion of males trapped, therefore it was included as a standard. Insects released as adults were irradiated as pupae (8-11 days old), internally marked and held in 16 oz cups in an insectary until eclosion. Newly eclosed males were removed from pupal containers daily, marked with Day-glo powder and held in 16 oz cups (10 males/cup) until the following day when they were released. Adults were released over an eight day period (7/8-7/15) by placing cups containing males in the center of the plot (Figure 1), removing the lid and allowing males to disperse.

All insects used for the three pupal release methods were also irradiated as pupae (8-11 days old) and internally marked. Eclosion boxes (2'x2'x1'h), used standardly in competition plots, were placed in the plot center (Figure 1) and provisioned with pupae on July 8. Eclosion and dispersal were recorded continuously during peak eclosion hours, i.e. 0700 to 1600 EST. Secure lids were placed on boxes when observers were not present. As males eclosed, their antennae were dusted with Day-glo powder to distinguish them from males released by other methods. For the third release method, cages provisioned with pupae were hung from trees (1.5 m high) on four points (3 cages/point) surrounding the plot center (Figure 1). Approximately 272 pupae were placed in each cage and liberally dusted with Day-glo powder. Preliminary studies demonstrated that as males eclose from pupal cases which have been dusted, their antennae and thorax accumulate enough powder to mark them. Eclosion boxes and cages were removed from the plot on the morning of July 16, the last day of adult release. The fourth method of release was done by broadcasting pupae on the two east-west grid lines (100 m/ea) adjacent to the plot center (Figure 1). These pupae were not dusted with Day-glo powder but were internally marked. Care was taken not to damage the pupae when placing them but no effort was made to place them in concealed or protected areas.

High-capacity milk carton traps baited with (+) disparlure, (Hercon wicks) were placed on all points in the grid except the center four points. Traps were checked daily from July 9 through July 17. Any males captured were returned to the laboratory and the color of marking determined. Because the study area had a native gypsy moth population of moderate density, a large number of feral males were also captured.

Release Method Evaluation Plot



□ position of release boxes and adult male release site

● positions of release cages

----- area where pupae were broadcast

○ positions of high capacity traps

RESULTS AND DISCUSSION

Recapture rates for the four different release methods are presented on Table 1. As expected, a significantly larger proportion of males were trapped when they were released as one day old adults. Because the proportion of males recovered from this treatment is not based on a specific number of pupae (mortality in the pupal stage and abnormal adults are excluded) results of this release method are biassed. However, as a standard for comparison, these results demonstrate that the sterile male produced at the Otis laboratory does respond to and locate pheromone sources. Release of males from eclosion boxes results in a significantly higher recovery rate than either of the two other pupal release methods. Better survival on the day of eclosion may explain high recovery from this treatment. Because observers were stationed at the eclosion boxes from 0800 to 1600 and boxes were fitted with lids during remaining hours, predation of newly eclosed adults by birds was probably less than at release cages. In fact if the number of adult males actually dispersing (1233) is used to compute the percentage recovered, the proportion recovered (45.5%) approaches the results of an adult release.

A significantly smaller proportion of insects were recovered when males were released from pupal cages. Possibly, the lower recovery is due to increased avian predation during peak eclosion periods. The least efficient method, broadcasting pupae, resulted in a 16.5% recovery rate or roughly one half the proportion of males recovered when males were released from cages. Predation probably accounted for this low recovery ratio. Not only would avian predators exert pressure on adult males released by this method, but pupae would be subject to mammalian predators. Other factors, for instance, increased mortality in the pupal stage due to microenvironment (e.g. too wet), may explain the poor recovery rates.

Results of this test indicate that the most effective methods we have for releasing males is by pupal placement in boxes. However, because of man power requirements, the use of this technique would be prohibitive. Results also suggest improvements could be made which would increase the effectiveness of a pupal release by designing a better release cage or alternating its placement. The standard adult release, used in this test appears to offer a large advantage (ca 17% greater recovery). However, by including pupal mortality and abnormal nonflying adults the advantage decreases to ca 8%. Also, because of the short life of adult males, releases must be made daily; whereas with a pupal release method, because of a long eclosion period, releases could be made a few times a week. This test was run in only one area where a moderate density feral population was present (39,798 feral males were captured). Because adequate feral insects were available, predation in this study site may not have been as intense on released males. Results in a study site, where released males are the major component of the male population and the overall density of males is low, may show larger differences between release methods.

Table 1. Number of sterile males trapped when males were released using four different methods.

Release Method	No. of Males Released	No. of Males Trapped	Percent Recovery 1/
One day old adults	678	332	48.97 a
Pupal eclosion boxes	1,500 (1,233) 2/	561	37.40 b
Pupal release cages	3,272	1,050	32.10 c
Pupal broad-casting	1,500	248	16.53 d

1/ Vertically, proportions followed by the same letter are not significantly different at the 5% level according to Chi-square using Yates correction factor.

2/ Number of adults dispersing from eclosion boxes.

II. Evaluation of Internal Marking, Radiation and Packaging Treatments

The purpose of the following series of tests was to determine if any of several treatments affect the competitiveness of males. The treatments include: internal marking, irradiation, packing (simulated shipment) and pupal holding conditions. Production males released in the pilot study area (Berrien and Van Buren Counties) are subjected to all of these treatments (see GM 0.2.7 Annual Report October 1, 1979 - September 30, 1980). Therefore, results of these studies, while attempting to partition effects, serve also as a quality control check of pilot study sterile males.

Methods and Materials

All males used in the following study were NJ F-22 strain reared on B-4 diet (12/cup) under standard rearing conditions. Males were harvested as pupae (1 - 4 days after pupation using a procedure outlined in a previous report (GM 0.2.7 Oct. 1, 1979 - Sept. 30, 1980). The following four male treatments were reared on B-4 diet which contained calco red dye[1/] (1 g/10L). The addition of dye in the larval diet results in an internally marked (IM) adult. To separate possible effects of pupal holding conditions a group of IM males were held in a field insectary post-harvest while a similar group was held under standard rearing conditions (SRC). Two additional IM groups of males which were also held under SRC post harvest, were irradiated (15 krads) when 8 - 11 days old (i.e. 7 days after harvest). To separate the effects of packaging, one group was returned to SRC after irradiation, while the other group was placed in an insulated biomailer with two frozen blue ice packs for 24 hours. A fifth group of males which had been reared on B-4 diet without dye added was held in a field insectary post harvest and served as a control.

To summarize, the five treatments tested were:

1) Males which were reared on standard B-4 diet (no dye added) and held post-harvest in an insectary - "Control"
2) Males which were reared on B-4 diet with dye added and held post-harvest in an insectary - "IM - Held Outside"
3) Males which were reared on B-4 diet with dye added and held post-harvest under standard laboratory condition - "IM-SRC"
4) Males which were reared on B-4 diet with dye added, held post-harvest - SRC and irradiated - "IM-SRC-IR"
5) Males which were reared on B-4 diet with dye added, held post-harvest SRC irradiated and packaged "simulated shipment" "IM-SRC-IR-PKG".

When male pupae were 12 days old, all treatments were placed in eclosion boxes in the center of circular field plots. As males eclosed, their antennae were dusted with Day-glo powder, to distinguish between treatments, and then transferred to dispersal boxes. Eclosion and dispersal were monitored continuously from 0800 to 1600 EST. When observers were not present, eclosion boxes were sealed and dispersal boxes were fitted with wire mesh tops to discourage avian predators.

1/ Calco Oil Red A-1700, American Cyanamid Company

To compare the ability of males to locate pheromone sources, two concentric circles (70 m and 140 m radius) of traps were placed around the plot center. Modified delta traps baited with (+) disparlure (Hercon wicks) were placed 20 meters apart on the circumference of both circles which resulted in 22 traps on the inner circle and 44 traps on the outer circle. Traps were checked hourly from 0900 to 1600 for three days post-release. Any males captured were placed in labeled cups and returned to the laboratory for determination of the treatment (color of marking). The entire test was replicated three times.

Results and Discussion

Pupal holding condititons (outside vs SRC), internal marking or irradiation do not appear to affect the ability of males to locate pheromone sources (Table 2). In replicate 3, internally marked males (Treatment 3) were captured in a significantly larger proportion than control males held outside (Treatment 1) but not IM males held outside (Treatment 2). A significantly smaller proportion of males which had been packaged after irradiation were captured in the first replicate of this test. However, results of replicates of 2 and 3 did not detect any differences. Further studies will be undertaken to evaluate males held under the same and longer periods of cold treatment. The ability to hold males for longer periods under cold treatment would simplify shipping schedules and perhaps permit better utilization of males by stockpiling for release during peak feral flight.

In similar past comparisons between laboratory reared males and feral males, the former were captured in proportitons as great or greater than the latter. Albeit feral males were not included in this study, comparison with control males indicates that the production sterile male (Treatment 5) is competitive with its wild counterpart.

III. Evaluation of Males Reared on Three Larval Diets

B-4, the standard larval diet for gypsy moth, includes casein which provides a source of protein. Because the cost of casein has increased in recent years, alternate protein sources have been evaluated in rearing studies. One of these, a soy protein (Supro-610), when evaluated in rearing studies, compared favorably with a casein based diet. Before a change in the composition of the standard diet was made, males reared on the candidate Supro diet, with and without Calco red dye added, were evaluated in field comparisons.

Table 2. Proportion of male treatment groups captured in circular field plots.

	Replicate									
	One			Two			Three			
Male Treatment	No of♂ s Released	No of♂ s Captured	Percent Capture	No of♂ s Released	No of♂ s Captured	Percent Capture	No of♂ s Released	No of♂ s Captured	Percent Capture	$\bar{X}$ Percent Recovery
1) Control	1141	233	20.4b	455	39	8.6a	747	77	10.3a	13.1
2) IM-held outside	810	182	22.5b	17	3	17.6ab	88	9	10.2ab	16.8
3) IM-SRC	729	153	21.0b	669	51	7.6a	1087	153	14.1b	14.2
4) IM-SCR-IR	888	208	23.4b	536	76	14.2b	713	91	12.8ab	16.7
5) IM-SCR-IR-PKG	1186	191	16.1a	329	31	9.4ab	929	114	12.3ab	12.6

1/ Horizontally, proportions followed by the same letter are not significantly different at the 5% level according to chi-square using Yates correction factor.

Methods and Materials

Males reared on three larval diets (casein based, Supro based, and Supro based + calco red dye) were placed in eclosion boxes in the center of circular competition plots. Eclosion and dispersal were monitored continuously from 0800 to 1600 EST and newly eclosed males were dusted with Day-glo powder to distinguish between treatments. After 1600 hours dispersal boxes were fitted with wire mesh tops (1" x 1") to retard predation and eclosion boxes were removed from the plot. Modified delta traps, placed on two concentric circles (70 m and 140 m radii) were checked hourly for two days after male release. Captured males were returned to the laboratory for determination of antennal color (male treatment). In all, this test was replicated five times.

Results and Discussion

Males reared on soy-protein based diet were recovered in proportions as great as males reared on the standard B-4 diet (Table 3). Results of this test indicate that Supro appears to be an acceptable diet substitute. However, subsequent rearing tests using different lots of soy protein, have found differences in larval development synchrony and survival. Therefore, a uniform source for soy protein will have to be located before a change can be made.

Table 3. Proportions of males reared on different larval diets which were captured in circular field plots.

Treatment		Replicate 1(6/15)	2(6/18)	3(6/22)	4(6/23)	5(6/24)
Casein	No Released	109	106	368	257	154
	% Captured	26.4	24.5	3.8	12.5	34.4
Supro	No Released	228	155	353	251	278
	% Captured	43.8	31.6	7.1	18.5	36.0
Supro & Calco red	No Released	318	196	483	244	224
	% Captured	45.5	20.4	10.8	18.9	25.9

IV. Pupal Holding Conditions

Periodicity of sterile male flight is critical to male competitiveness (see GM 0.2.8). Results of earlier studies have shown that when male pupae are held under different temperature regimes, adult flight periodicity is altered. The tests described in the following section were an attempt to find a "window" where males could be held under cold treatment, effectively shifting periodicity of adult flight, without necessitating the development of new irradiatiton schedules or drastically increasing pupal development times (i.e. holding time). Even though the results indicated that changing pupal holding temperatures for short periods of time does not affect the activity rhythm significanttly, they also demonstrate that pheromone response (trap capture) was not adversely affected. In addition, more information on the competitiveness of the production sterile male (internally marked-irradiated and packaged) is presented.

In the following tests, males were placed, as pupae, in eclosion boxes in the center of circular competition plots. Eclosion, dispersal and trap capture data were collected using the same procedures as previously described in this report. The control treatment in all the following tests was the NJ standard strain males which had been harvested as prepupae and held outside in an insectary until release.

Series 1

In the first series of tests, 4 male treatments were evaluated: 1) NJSS-control, 2) Production sterile males (i.e. internally marked males, irradiated as pupae 8-11 days old and packaged), 3) Males held from the day of pupation for 8 days at 25°C, irradiated (15 krads), and then held from day 8 until eclosion began at 20°C (Day 8 - IR - 20°C), 4) Males held from the day of pupation for 8 days at 25°C, irradiated (15 krads) and then held from day 8 until eclosion began at 15°C. (Day 8 - IR - 15°C).

Results of these trials are not conclusive (Table 4). Recovery rates in all but the last replicate were low because of predation losses. Males held at either 15° or 20°C after irradiation generally performed as well as the control group or production sterile males in the first two replicates.

Table 4. Proportions of various male treatments recovered in circular competition plots.

Male Treatment	Replicate #1 No. Males Released	Replicate #1 Percent Captured	Replicate #2 No. Males Released	Replicate #2 Percent Captured	Replicate #3 No. Males Released	Replicate #3 Percent Captured
Control	161	6.9 a	129	2.3 a	94	46.8 c
Production Sterile Males	141	20.6 b	399	2.5 a	136	27.9 b
Day 8-IR-20°C	173	6.9 a	24	33.3 b	--	--
Day 8-IR-15°C	172	14.0 ab	87	16.1 b	742	17.4 a

1/ Vertically, proportions followed by the same letter are not significantly at the 5% level according to Chi square using Yates correction factor.

Table 5. Proportions of various male treatments in circular competition plots.

Male Treatment	REPLICATE 1		2		3		4	
	No. Males Released	Percent Captured	No. Males Released	Percent Captured	No. Males Released	Percent Captured	No. Males Released	Percent Captured
Control	348	47.4 a	303	29.0 a	264	18.9 a	437	15.3 b
Day 10-IR-15°C	463	36.7 b	674	19.7 b	-	-	-	-
Day 8-IR-15°-25°C	-	-	-	-	364	14.0 a	488	21.0 a
Short Diapause	-	-	262	19.5 b	294	13.9 a	656	13.7 b

Series 2

Control males used as a standard in this series were treated in the same manner as the previous series. Also included were production sterile males. In the first two plots the following male treatment was included: males held at 25°C from pupation until 10 days old, irradiated (15 krads) and transferred to 15°C until eclosion began (Day 10-IR-15°C) In the second two plots another treatment was tested: males held at 25°C from pupation until 8 days old, irradiated (15 krads) and transferred to 15°C - day 8 thru 10 and transferred back to 25°C on day 11 (Day 8-IR-15°-25°C). Included in 3 of the four tests were males from a strain of gypsy moths that required only 45 days to complete diapause (short diapause). The strain which is used extensively in laboratory experiments would be useful in field studies where the results require evaluation of egg masses.

Results of this series of trials indicate that males from the short diapause strain are nearly as competitive as control males. The proportion of these males recovered was always less than the control treatment but only significantly less in the second replicate. Before use of these males is initiated for release, further testing will have to be undertaken to determine if other characteristics (i.e. periodicity of flight) are comparable.

Trials involving male treatment with various temperature regimes again provided insufficient data for conclusions. Although significantly smaller proportions of Day 10-IR-15°C males were captured than control m-ales, males in the group treated Day 8-IR-25°C appear to have performed as well as controls. Periodicity of captured data from these field plots was inconclusive, however. Actograph analysis described in another section of this report indicates a shift in activity periodicity does occur.

Project Number: GM 1.2.3
Project Title: Location of Female Moths by Released Males:
(a) Longevity of Males Within a Trap Array;
(b) Trap Capture of Mated Male Moths
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leader: Dave Simser, Wendy Ratcliffe, Debra Jacobs and Maurice Huard

Introduction:

In separate experiments, aspects of mate location by released male gypsy moths were investigated. Test (a) was designed to indirectly measure longevity of released male moths within a 4 ha site by the percentage of female moths mated daily within the sites. Mating incidence was compared between sites arrayed with milk carton (MC) traps (treatment) and those without (control). Test (b) was designed to demonstrate (hourly) mating and capture intervals of male moths released within an array of MC traps and female moths.

Each experiment was conducted during June 22-26, 1981, on Otis Air National Guard Base, MA. Site locations typically consisted of oak spp (predominant), pitch pine, and mixed hardwoods, with understory, although site terrain varied. Feral population size varied within and between sites, consisting primarily of third and fourth instar larvae. No native adult gypsy moths were present during this period.

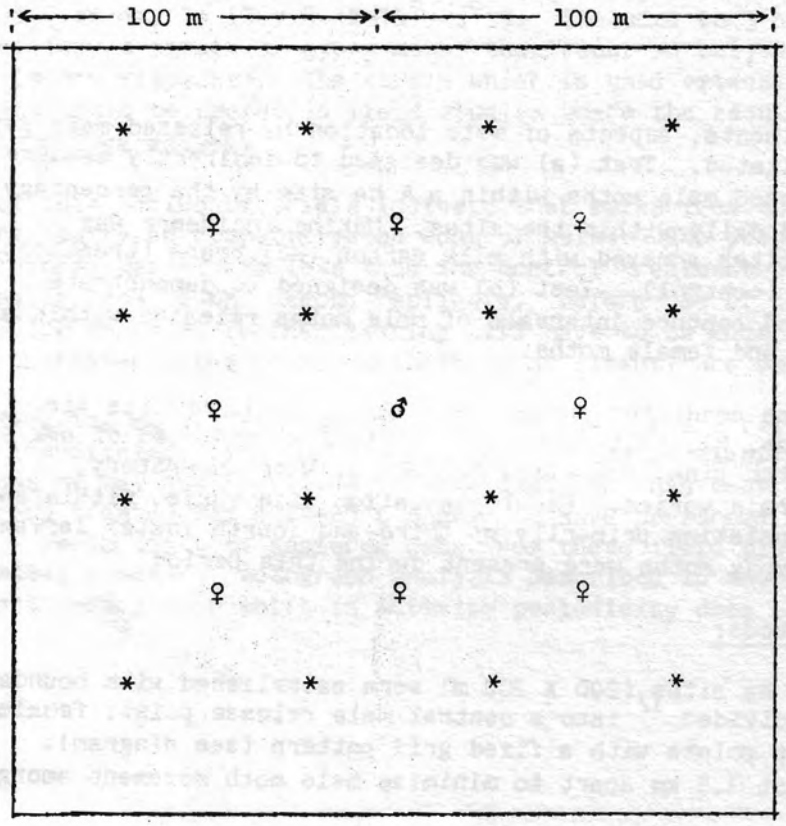
Materials and Methods:

Test (a): Four 4 ha sites (200 X 200 m) were established with boundary markers, then subdivided [1/] into a central male release point, female stations, and trap points with a fixed grid pattern (see diagram). Sites were at least 1.5 km apart to minimize male moth movement among study areas.

Sexed pupae were held in separate environmental chambers until adult emergence. [2/] Healthy adults were collected as they eclosed and held separately. [3/]

At the onset of test (a), all moths were removed from storage and acclimatized ca 2 h to ambient conditions. Eighty male moths (20 per 16 oz cup) were liberated within each site. Dispersal was facilitated by gently shaking each cup after opening.

Female placement (8 moths/site) followed male release in treatment and control sites by allowing each female to crawl into a teepee. Calling behavior usually commenced within 5 minutes. Liberation of males, and placement of females was concluded by 1200 on day 1. Thus, males were released at a 10:1 male:female ratio, and each female was surrounded by 4 traps (in the treated plots). Treatment sites with traps were checked at 1500, on day 1, and the number of males captured per trap was recorded.



♀ = female stations
♂ = male release point
* = MC trap points

Diagram of treatment release site. Control sites were identical, but without MC traps.

Females or egg masses were collected and females replaced for 4 consecutive 24 hour intervals. Traps were checked daily. Two replicates were conducted.

Collected female moths were held under constant conditions (see footnote 1) until oviposition or moth mortality ensued. At the termination of the experiment, dead moths were discarded, and all egg masses were placed in coded (site, placement date, position) glassine envelopes, and incubated ca 45 days, under described conditions, for embryonation. Egg masses were then dehaired, and eggs were examined for embryonation, a positive sign of mating. See GM 1.2.4 for a description of comparison between embryonated vs non-embryonated, or parasitized, eggs.

1/ The central male release point was at ground level, in diffused sunlight. Female stations were constructed by fixing wooden shelters (teepees) at breast height on host tree boles. Each trap point consisted of a single milk carton trap, baited with 500 ug (+) disparlure (Andrulis 1981 synthesis, impregnated in laminated plastic wicks by Herculite Products Inc.), and suspended at 1.5 m, above ground level, away from foliage or branches.

2/ Male pupae were held at 24 C, 70%RH, 16:8L:D and females, at 25 C, 50%RH and 16:8L:D.

3/ Adults of small relative size, with incomplete wing development, or with observable physical deformities were discarded. Male moths were placed in 16 oz cups (20 moths/cup) and stored 24 h at 12 C, 50% RH and 16:8LD. Female moths were placed individually in 2 oz plastic creamers, and held at described conditions.

Test (b): One 4 ha site was established, and moths were stored and handled as described above, with the following exceptions. Moths (n=80) were released daily at 0900 in a 10:1 male:female ratio; females were placed immediately thereafter in stations. Stations were checked hourly, and pairs <u>in copula</u> were lightly dusted with fluorescent powder (daily change of colors), and permitted to complete copulation. Males dispersed soon after mating and females were replaced with virgin females. Females were observed and traps were emptied each hour; captured males were stored in coded plastic envelopes. They were later examined under UV light for signs of fluorescent powder. In all, 4 replicates of hourly checks were conducted.

Mated female moths were killed and discarded, and any deposited egg masses were soaked in hexanes, then discarded.

Results:

Test (a): Once released, male moths are exposed to predation and other factors. Survivors may remain within, permanently disperse from, or leave and re-enter a site repeatedly. We assumed that 25% of the released population was removed during a 24 h period, due to these agents. Within treatment sites, daily moth capture further reduced the number of male moths. Based upon assumed and actual reduction of the male population, the number of moths available to locate and mate females was calculated (Table 1).

Table 1. Male populations based upon actual and assumed losses. Assumed loss was calculated after reduction by trap capture.

Hours Post Release	No. Males	No. Males Captured	Assumed Loss (25%)	Estimated Population
0-24	80	29	13	38
24-48	38	2	9	27
48-72	27	1	7	19
72-96	19	0	5	14

Although the percentage of captured male moths was high during the inital 24 h period (36%), this rate did not increase substantially during the next 72 h (to cumulative 40%), as the majority of male moths were captured within 6 hours following their release. Estimated population (Table 2) demonstrates the effect of trap capture, as the control site population is nearly double that of the treatment site.

Table 2. Estimated male populations up to 96 h post release.

	Hours Post Release			
	0-24	24-48	48-72	72-96
Control	80	60	45	34
Treatment	80	38	27	19

Greater numbers of male moths within a site (calculated) resulted in higher pecentages of mated female moths (Table 3). The results also illustrate the tendency of males to remain within an area for several days following emergence (release).

Table 3. Percentage of female moths mated at various intervals following the release of male moths. Females were replaced daily. (Also see Appendix).

	Hours Post Release			
	0-24	24-48	48-72	72-96
Control	92.0	61.5	57.1	40.0
Treatment	100.0	66.7	37.5	20.0

Values are average of two replicates.

Placement of traps at high density (4/ha), therefore, served to reduce mating of released moths over a 4 day period. However, trapping did not eliminate mating. Male moths remained within the study area and mated females up to 96 h after their release.

Test (b): Low numbers of mated female moths, and few captured males hampered data analysis. However, no mated males (dusted) were among the 22 moths captured in traps. Also, multiple mating by previously dusted moths were not observed.

Appendix: Percentage of recovered female moths which were mated in test (a). Values in parentheses are total numbers of observations in 2 replicates.

Hours Post	Percentage	
	control	treatment
0-24	92.0 (13)	100.0 (14)
24-48	61.5 (13)	66.7 (15)
48-72	57.1 (14)	37.5 (16)
72-96	40.0 (10)	20.0 (15)

Project Number: GM 1.2.4
Project Title: Mass Trapping of Male Gypsy Moths. II. Effect of Male Release Ratios on Trapping Success and Female Mating
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: Dave Simser, Maurice Huard, Debra Jacobs, Wendy Ratcliffe

Introduction:

The previous study (GM 0.2.11 (Oct. 1979 - Sept. 1980)) demonstrated the effect of varying trap distribution on % mated female moths, and on the proportion of male moth capture, with the number of released males and placed females kept constant within each site.

In the present study, the number of placed female moths, trap density, and trap distribution were kept constant within sites, while the number of males released into sites was varied (0.5, 1.0, 5.0 and 10.0 to each female). Control sites without traps were compared. The influence of male release ratios on % mated females, male capture by trap arrays before and beyond placed females, and mating success of female moths with and without intercepting traps was evaluated. Like study GM 0.2.11, experimentation was conducted during mid-June, with feral populations consisting primarily of 3rd and 4th stage larvae.

Experimental Design:

Four male release ratios were evaluated in treatment and control sites. Daily (AM) central releases of male moths within each site preceeded female moth placement (AM) and recovery, followed by sequential trap checking (PM). A 2:1 trap-female ratio was utilized, with each female surrounded by four equidistant traps. Recovered females were held at constant laboratory conditions until oviposition or mortality ensued. Following a 45 day incubation period, all egg masses were dehaired and examined for embryonation, which is indicative of successful mating. The aforementioned objectives were then investigated through data and statistical analysis.

Site Establishment and Preliminary Preparation:

Eight areas (separated by at least 1.5 km) within Otis Air National Guard Base, MA were delineated as study sites. All sites were positioned at western or northern boundaries of the base. Typically, each site was predominated by oak spp of varying maturity and canopy density. Other deciduous species, including beech, hickory, and maple also were present in some sites, as were several coniferous species. Understory was generally light with occasional patches of shrubby or woody vegetation. Feral populations varied greatly, silva ranged from dense forested areas to sparse power line vegetation, and terrain differed between and within sites.

Boundaries (200 X 200m = 4 ha) were flagged with day glo fluorescent pink tape, then subdivided into trap points, female monitor stations, and a central male release area (Fig. 1). Each female monitor station (8 per site) was prepared by suspending a wooden shelter, commonly referred to as a teepee, at waist height on branches of oak spp with bole diameter of at least 20 cm (8 inches).

Standard APHIS milk carton traps were constructed, and equipped with a (+) disparlure source [1/] and moth killing agent [2/], then placed at trap points within treatment sites. Each trap was suspended at chest height, at a trap point within the array, from a limb or branch, and away from the bole or obstructing foliage. All traps were placed in appropriate plots at least 16 h prior to beginning the test.

Pupae were sexed and held in separate envioronmental chambers [3/]. Emerged adults were placed in 16 oz cups (20 males/cup; 10 females/cup) for 24h prior to use, when the required number of daily (replicate) insects were totaled. Any additional moths collected during that 24h period were discarded in sealed containers; thus, the age of moths released (males) and placed (females) during each replicate of the study was constant. Only healthy adults were selected.

Experimental Procedure

A crew member was assigned a treatment and control site for a specific ratio. During a (daily) replicate, males were released at appropriate ratios within sites; all releases were made at ca 0900. Males dispersed from holding containers readily. Each crew member then placed females [4/] (n = 8) at monitor stations before similar placement in the comparative site. All females were placed, or recovered, by 1100. Consistent and uniform trap checking was conducted in treatment sites (1500); traps were checked in sequential order, and the number of captured male moths was recorded.

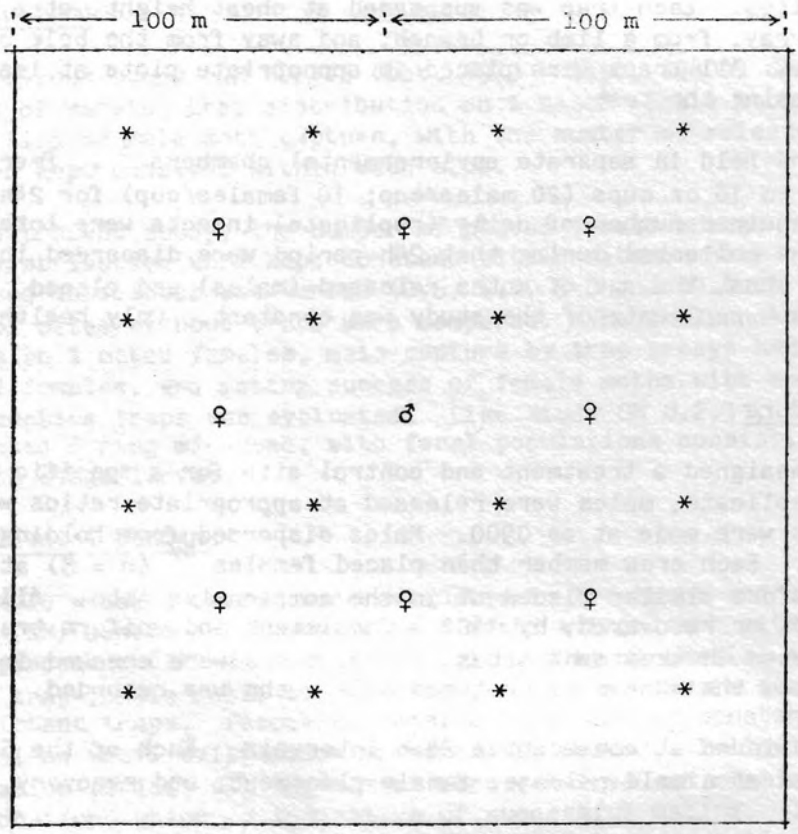
This format was continued at consecutive 24 h intervals. Each of the 5 replicates consisted of a male release, female placement, and recovery, and trap check.

1/ Each trap was equipped with a standard 1981 (+) disparlure wick containing 500 ug active material.

2/ Moths entering a trap were exposed to a 2 inch plastic strip impregnated with DDVP.

3/ Males were held at 12 C, 70% RH, 16:8 LD; Females were held at 24 C, 60% RH, 16:8 LD.

4/ Females were allowed to crawl from cups into teepees. Recoveries of placed females was conducted concurrently.



♀ = female stations
♂ = male release point
* = MC trap points

Fig. 1. Treatment site for male release test. Control sites were identical, but without traps.

Female Recovery, Storage, and Mating Confirmation:

During each replicate, female monitor stations within a site were examined. Generally, placed female moths did not crawl from the shelter within the test period. Often, a deposited egg mass was recovered whole by teasing it from the substrate. Recovered material was held separately in 2oz plastic creamers under constant conditions. Recovered female moths exhibited no distress within the creamers, as unmated females continued calling behavior. One hundred percent mortality of females occured ca 5 days following the final recovery, at which time egg masses or spewed eggs were placed within labelled glassine envelopes, and incubation was continued. No deposition of eggs was recorded as a non mating.

All eggs were examined ca 45 days post incubation; setae were vacuumed from eggs, which were then examined (30X) and rated. Unembryonated eggs appeared pale, with shrunken chorions; eggs parasitized by Ooencyrtus kuwanai, an encyrtid microhymenopteran, appeared shiny and black within the chorion; embryonated eggs appeared tumescent, dark, with discernable larval head capsules. A single embryonated egg within a mass reflects a positive mating; however, embryonation within masses was readily apparent by a majority of eggs. Ratings of all treatment and control sites were tabulated, and this parameter (% mated females) was analyzed with regards to influence by male release ratios, trap capture of males, and trap placement.

Results:

Effect of male release ratio on mating

Increasing the male release ratio correspondingly increased the percentage of mated female moths in treatment and control sites (Fig. 2). Generally, the presence of MC traps reduced % mating from the control, except at the highest (10:1) ratio tested. Analysis of comparative ratios (see Appendix 1) yielded highly significant differences between the lowest (χ^2 = 9.71**) and highest (χ^2 = 9.57**) ratios tested, while intermediate ratio comparisons were not significantly different (χ^2 = 2.00 for 1:1 release ratio; χ^2 = 2.16 for 5:0).

Intercept vs direct female placement

Although % mating of intercept and direct female (stations) increased with increasing male release ratios (Appendix 2), no significant differences between ratios were evident. However, % mating was lower in all cases at intercepting points, possibly indicating a disruptive effect due to trap placement.

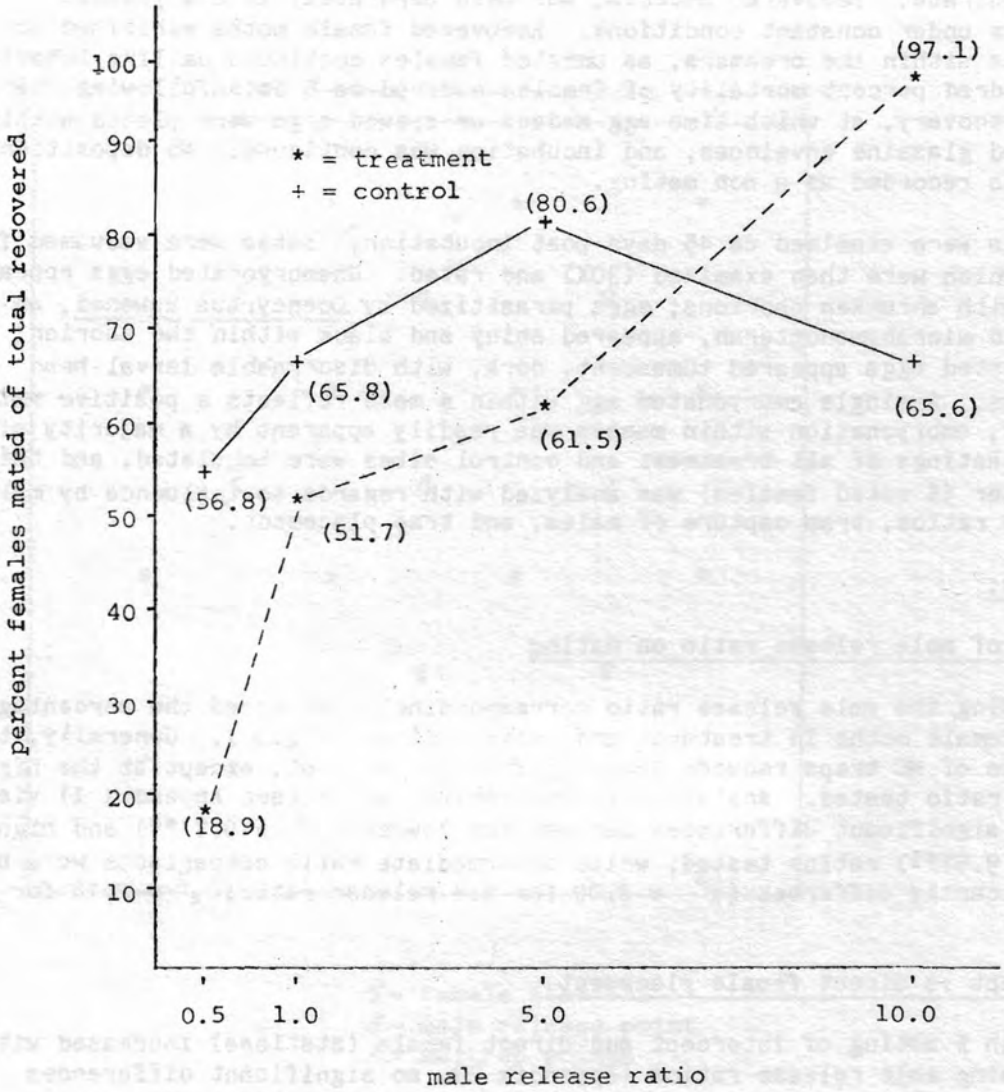


Fig. 2. Percentage of females mated by several male release ratios. Parentheses indicate actual percentage.

Inner vs outer trap trap rings

Because of the alternating 'rings' of traps and females design, males could be captured by four traps within the female ring, or twelve traps beyond. Numbers of captured males increased with increasing male release ratios. Total numbers of captured males were similar at the 5:1 and 10:1 ratios, but inner traps captured larger numbers of moths on a per trap basis (Appendix 3).

Traps at the four tested ratios captured 10%, 27.5%, 24%, and 22.5%, respectively, of the total number of released males.

Discussion

Preseason testing requires cautious interpretation of results because seasonal differences in temperature, photoperiod, and other factors may affect behavior of adult moths. However, data were consistent among replicates and are believed to be indicative of what would occur in more natural settings.

The chosen density of placed females represents a sparse population (defined as <10 females/ha), and was constant at all male release ratios. Male behavior, which is expected to be influenced by female density, presumably remained similar within all sites. The high percentage of mated females at the lowest sex ratio demonstrates the efficiency of males in locating and mating females within a relatively large area. Additionally, males tended to remain near the release point, rather than dispersing out of the study area.

Placement of traps within a site generally reduced the percentage of female moths that mated. Anomolous results at the highest ratio where more mating occurred in the trapped plot may be due to site topography rather than male (or female) behavior. Males within this control site were released into an open, disturbed area where they could have promptly dispersed before encountering female produced pheromone. Pheromone from traps may have kept males in the area, increasing the chances of mating.

The density of traps represents a design intended to prevent mating; even with few males released, mating was suppressed but not eliminated. At higher release ratios, mating was not suppressed below 50%. As such, deployment of traps into a sparse infestation probably does not reduce the degree of successful matings significantly. The presence of traps did decrease mating somewhat, however.

Appendix 1. Effect of male release ratios on % mated female moths (total of 5 replicates).

Male release ratio	Mating frequency (No. mated/no. recovered)	% mated	X^2
.5:1 control	21/37	56.8	9.71**
.5:1 treatment	7/37	18.9	
1:1 control	26/38	65.8	2.00 NS
1:1 treatment	14/29	48.3	
5:1 control	25/31	80.6	2.16 NS
5:1 treatment	24/39	61.5	
10:1 control	21/32	65.6	9.57**
10:1 treatment	35/36	97.1	

Appendix 2. Percentage of mated females directly in line with released males, versus females intercepeted by MC traps.

Male release ratio	frequency	% mated	X^2
.5:1 direct	5/20	25.0	0.364 NS
intercept	2/17	11.8	
1:1 direct	10/15	66.7	1.68 NS
intercept	5/14	35.7	
5:1 direct	13/19	68.4	0.283 NS
intercept	11/20	55.0	
10:1 direct	18/18	100.0	0.0 NS
indirect	17/18	94.4	

Appendix 3. Number of captured males by traps placed at "inner" and "outer" rings. Values are totals of 5 replicates.

Ratio	Total # captured		# per trap	
	inner	outer	inner	outer
0.5	0	2	0	0.16
1.0	10	1	2.5	0.08
5.0	23	25	5.8	2.1
10.0	49	41	12.3	3.4

Project Number: GM 1.2.5
Project Title: Application of Mating Disruption and Mass Trapping Techniques on Small (0.1 ha) Plots
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: Dave Simser, Joseph Argentine, Maurice Huard

Gypsy moth populations in residential areas are a major nuisance of homeowners, leading to utilization of many available commercial products, or home remedies for relief. Such solutions may have a placebo effect, but actually accomplish little, if any, population suppression. Ultimately, public perception of the problem may be tarnished by repeated control failures, and future measures may be based upon emotion, rather than rationale.

Currently, experimental techniques attempt suppression by reducing the mating success of moths, either thru mass trapping or mating disruption. Each technique relies on disparlure, the synthetic sex pheromone.

The objective of this study was to compare mating reduction by mass trapping, mating disruption, and a combination of techniques. Treatments were applied in sparse, increasing and heavy gypsy moth populations in sites representative of an average property lot (0.1 ha). Female moths were placed within sites, and the number mated compared, to determine the effectiveness of treatments on populations of each density. Capture of male moths in (+)-disparlure baited traps was also compared.

Methods and Materials

Estimates of population densities were conducted on Otis Air National Guard Base, MA during June, 1981. Forested areas were typical of Cape Cod sand plain communities, consisting of dominating oak species and scattered hardwoods and conifers. Generally, undergrowth was limited to members of the heath family. Population category selection was based upon relative densities, and direct measurement of population density was not conducted. Selected sites were at least 5 km apart, and gypsy moth densities among sites varied greatly.

Site density estimates

The sparse population was in an area on the western border of Otis ANG Base. Preliminary surveys revealed no egg masses on host trees or sheltered sites; no defoliation was observed through the canopy. Bark flaps and burlap bands placed on host trees revealed several (<10) late stage larvae. A 1 ha site was established within a solid stand, with level terrain.

Another 1 ha site in a building population was located on a NW slope on the northwestern border. Large egg masses (600+ eggs/mass) were conspicuous. Groups of 20-50 late stage larvae were observed beneath burlap bands during mid day. Defoliation was estimated at 50% prior to the pupation period. Infection by viral wilt (NPV) was estimated at 30%. Species of parasites observed were: Apanteles melanoscelus, Blepharipa pretensis, and Brachymeria intermedia. Additionally, predation by unidentified nymphal pentatomids was observed.

The peak population occurred at the northeastern border of the Base. Contiguous egg masses were apparent on tree boles and crotches. The canopy had been 90% consumed and large larvae were migrating and feeding on other available vegetation. Large, dense mats of caterpillars clustered under burlap bands, tree crotches, and elsewhere. Viral disease was high (>90%), but we noted isolated patches of viable pupae throughout the site. A 1 ha site, divided by meandering roads and trails was established. Within each site, four 0.1 ha (30 x 30 m) subplots were established. Moth activity was confirmed by capture of feral males in milk carton (MC) traps baited with 500 ug (+) disparlure.

Treatment design (see diagram) and materials were:

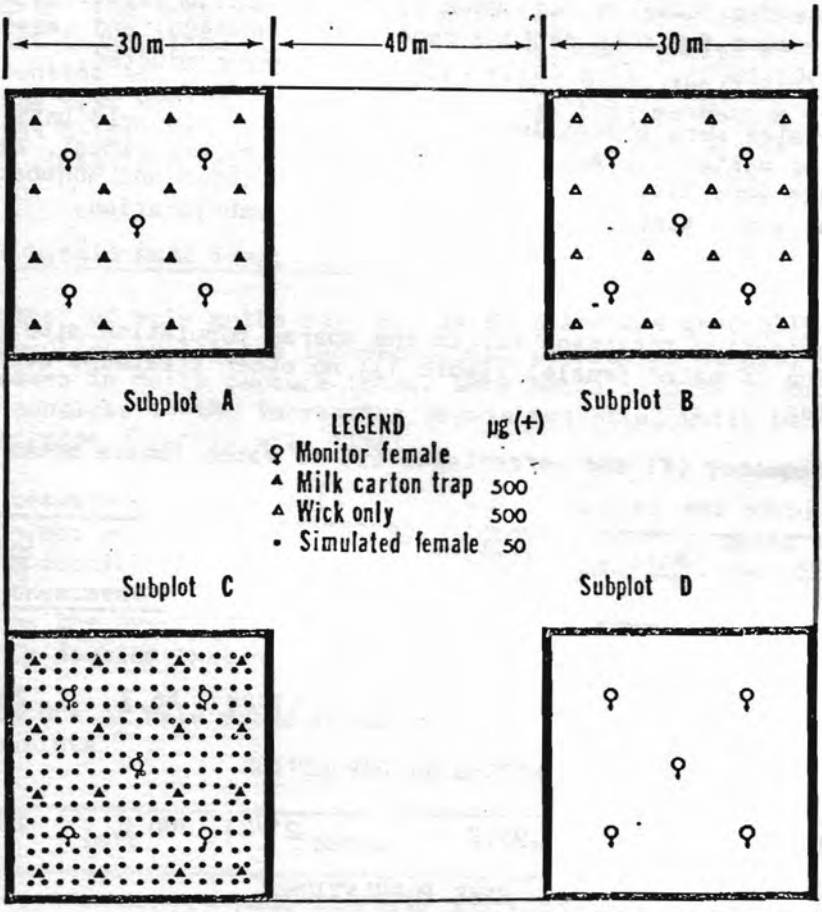
Subplot (a): mass trapping. Five equidistant female monitor stations (teepees) were placed on host tree boles ca 1.2 m above ground level. Sixteen standard MC traps were arrayed in a 10 x 10 m grid, thus surrounding each female station with four traps. Each trap was suspended 1.5 m above ground level, away from obstructing foliage or branches.

Subplot (b): mating disruption. Five female stations were arrayed. A 500 ug (+) disparlure wick (1981 standard) was stapled at 2.0 m above ground level on a host tree bole. Sixteen wicks were arrayed in a 10 x 10 m pattern, surrounding each female station with four equidistant wicks.

Subplot (c): combination. Five female stations were arrayed. Standard MC traps were suspended on a 10 x 10 m grid. Pseudo-female moths were fabricated by cutting a Whatman filter paper disc into quarters and charging each section with 50 ug (+) disparlure (1980 formulation). A pseudo-female was positioned tip upward and stapled at 2.0 m above ground level on a host tree bole. Two hundred and twenty five pseudo-females were placed in a 1.5 x 1.5 m array.

Subplot (d): control. Five female stations were arrayed.

Subplot position was randomized within sites and all preparation was completed by 1300 on 12 July 1981.



Design and placement of materials within each population site.

Testing Procedures

Field work was conducted simultaneously in the 3 sites. Female moths (laboratory reared 24-48 hr old) were placed at monitor stations by 1000; traps were checked for moth capture recorded (1300-1400) and females were recovered (1500-1600). Five daily replicates were conducted.

Recovered females were placed in 2 oz plastic cups, and held until oviposition or mortality. At the termination of the experiment, egg masses or spewed eggs were placed in coded glassine envelopes and incubated for ca 30 days at which time they were examined for embryonation.

Results:

Only the combination treatment (c) in the sparse population site resulted in a reduction of mated females (Table 1); no other treatment substantially reduced mating.

Table 1. Frequency (f) and percentages (%) of mated female moths.

Mass Trapping		Mating Disruption		Combination		Control	
f	%	f	%	f	%	f	%
			SPARSE POPULATION				
23/24	96.0	22/23	96.0	11/24	46.3	23/24	96.0
			INCREASING POPULATION				
23/24	100.0	20/21	95.2	21/21	100.0	25/25	100.0
			PEAK POPULATION				
20/20	100.0	24/24	100.0	20/20	100.0	18/21	85.7

1/ Values are totals of 5 replicates; frequency denominator represents number of recovered females or egg masses; numerator represents number of embryonated egg masses.

Mating incidence in the combination subplot may have increased if females had been left in the field longer. The high percentage of mated females in all other subplots demonstrates the effectiveness of male moths in locating (and mating) calling females.

Although the emission rate of (+) disparlure from commercial wicks is relatively constant over a prolonged period (treatments a and b), (+) disparlure applied to filter paper wicks (c) is emitted at a rate greatly influenced by temperature. The emission profile of such wicks demonstrated this rate during the test period. The amount of airborne material released into a subplot ranged from 80 ng/hour to 160 ng/hour. Males were frequently observed orienting to, and attempting copulation with, individual wicks throughout the test period. This indicates that this mechanism of mating disruption was point source confusion, rather than point source camouflage or habituation. Details of gas chromatographic analysis of wicks is detailed in Appendix 1.

Male Capture Within Mass Trapping and Combination Subplots

The daily number of male moths captured in MC traps was generally consistent within a subplot; trap capture increased with population density (Table 2). Slight increases in daily capture totals were noted in mass trapping and combination subplots in the increasing population site, while other totals tended to decrease slightly with time.

Differences between capture totals in the mass trapping and combination subplots were not apparent, although the average number of moths captured in mass trapping subplots in sparse and peak populations was greater than the combination treatment totals. This trend was reversed in the increasing population, as the average number of moths captured in the combination subplot nearly doubled that of the mass trapping subplot.

Table 2. Capture of male moths in mass trapping and combination subplots 2/.

Replicate	Date	Sparse	Increasing	Peak
		MASS TRAPPING SUBPLOTS		
1	13 July 1981	21.2	5.4	99.8
2	14 July 1981	10.1	13.1	203.5
3	15 July 1981	8.3	16.9	133.1
4	16 July 1981	7.5	25.3	104.0
5	17 July 1981	2.9	34.3	83.8
$\bar{x}$		10.0	19.0	124.8
		COMBINATION SUBPLOTS		
1	13 July 1981	10.8	12.3	97.9
2	14 July 1981	4.1	14.7	120.9
3	15 July 1981	2.9	32.8	85.4
4	16 July 1981	3.1	38.2	95.2
5	17 July 1981	1.3	72.3	84.6
$\bar{x}$		4.4	34.1	96.8

2/ All male capture totals are averages of 16 MC traps per replicate.

Discussion

Males captured in the peak population site were markedly smaller than those captured by traps within the sparse population. Some males captured in the increasing population were small, but the majority were comparable in size to those from the sparse population. However, male moth size did not seem to affect mating incidence; mating in control subplots averaged 96.0, 100.0 and 86.0% in the sparse, increasing and peak populations, respectively.

The techniques employed to limit mating did not substantially result in suppression at any population density. Reduction of mating to 46% (subplot c, sparse population) probably would not decrease subsequent generations.

Application of these techniques by homeowners would not serve as an effective gypsy moth control approach. At best, utilization of certain products will monitor activity of male moths within an area, but will not decrease subsequent generations.

Appendix 1 Analysis of residual (+) disparlure content on filter paper wicks

Preparation: The amount of (+) disparlure applied to a wick was verified by gas chromatographic analysis.[4/] Wicks were placed in the field and removed at intervals. Each of two wicks were placed in 10 ml n-hexane for ca 16 h at -12C. Vials were then returned to room temperature (ca 22C) and vortexed for ca 1 min. Wicks were removed and washed with an additional 5 ml n-hexane. Solvent was evaporated under N_2 to 0.5 ml. An aliquot of this material was analyzed and the resultant peak height compared to that of a known amount of disparlure. All calibrations were corrected to T_o = 50 ug disparlure. Amounts of disparlure emitted daily were expressed as % of total amount applied at T_o.

4/ Gas Chromatograph Tracor 550 Column: 3% OV-1 on 80/100 Gas Chrom. Q; 6' m x 4mm I.D. glass
Temperature: (^oC) Oven, 188; detector, 230, inlet 225; outlet 250.
Flow Rates: H_2 = 70 cc/min
Air = 1.2 ft^3/hr
N_2 (carrier) = 75 cc/min

Project Number: GM 1.2.6
Project Title: Evaluation of Mass Trapping for Control - Appleton, WI
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leader: C. P. Schwalbe

In 1975 a standard detection survey in Outagamie Co. Wisconsin resulted in the capture of 5 male moths in the city of Appleton. The 1976 delimitation survey (16 traps/mi^2) with racemic disparlure found 8 moths (6 in one trap and 1 each in 2 traps). These moths were within 1/4 mile of the 1975 positive traps. Visual survey in November, 1976 yielded 3 larval exuviae and 1 pupal case. In 1977, a small amount of (+) disparlure was available and the 1976 survey was essentially duplicated except that 1/3 of the traps contained 100 µg (+)-disparlure (on cotton wicks) and the remainder were baited with the racemic mixture. The center square mile of the project area contained 36 traps; 26 moths were captured in this survey, all within the center square mile. Based upon these findings, it was concluded that a reproducing population existed in the area and was confined to no more than 640 acres.

At this juncture there was virtually no information on the effectiveness of trapping in controlling sparse populations. Since (+) disparlure was scarce, it was decided to trap the area again in 1978 with 84 traps per square mile, with the intent of further defining the boundaries of the infestation in preparation for a future eradication attempt. Twenty males were captured in 5 adjacent traps and one was found in a trap several hundred meters from the "core". This survey unequivocally located the center of the infestation and it was estimated to be no more than 50 acres in size.

In 1979 it was proposed that mass trapping be evaluated as a method for eradicating this population and the project area was trapped at the rate of 3 traps baited with (+) disparlure per acre. Seven moths were captured in 6 traps. When the trapping program was repeated in 1980 and 1981, no additional moths were captured and we conclude that the infestation has been eliminated.

While it may not be particularly convincing that trapping was responsible for the disappearance of this infestation, no other means on control were used. No larvae or egg masses were collected and no spraying was performed. Therefore, trapping, in concert with natural mortality causing factors is believed to have resulted in the elimination of the infestation.

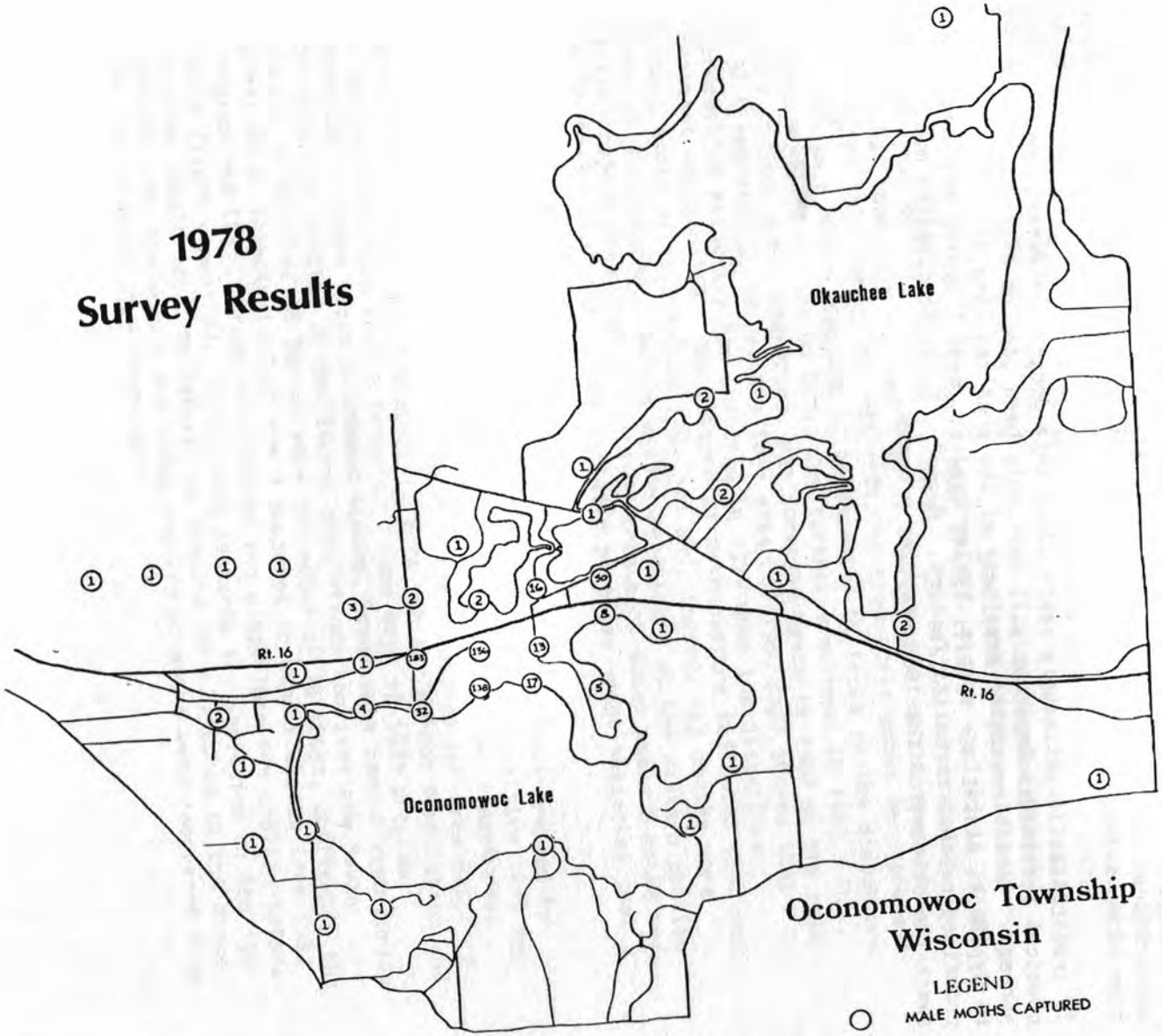
Project Number: GM 1.2.7
Project Title: Gypsy Moth Mating Disruption - Oconomowoc Wisconsin Demonstration Project
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: Charles P. Schwalbe

The 1977 gypsy moth detection trapping program in Wisconsin resulted in the capture of several male moths in Oconomowoc Township. The delimitation survey in 1978 captured ca 700 moths over a large area and led to the location of egg masses and larvae. These survey results are shown on the attached map (1978 survey results) and formed the basis for designating treatment plots for an eradication program utilizing non-chemical control methods. In 1979, two treatments of gypcheck ($25X10^6$ potency units/acre) and a single treatment of Hercon flakes containing disparlure (20 grams disparlure/acre) were applied to ca 425 acres (see map of 1979 Program Results). The evaluation trapping program that year only yielded 4 moths, all outside of the sprayed area. Since this survey did not indicate that adult moths were present (at least in numbers adequate to be detected by traps in the disparlure treated area) there was no basis for further treatment in 1980. The entire project area, therefore, was surveyed again in 1980 at the rate of 32 traps/mi2 Those results (see attached 1980 map) showed that reproduction evidently had occurred in 1979 when the disparlure treatment was applied. The pattern of moth capture indicated that only 3 small spots of infestation remained and, indeed, egg masses were found at 4 locations. No egg masses were found at the western portion of the project area (out of the 1979 treatment zone) where 6 moths were caught in 3 traps, but, these isolated positive traps suggested that a small population persisted there.

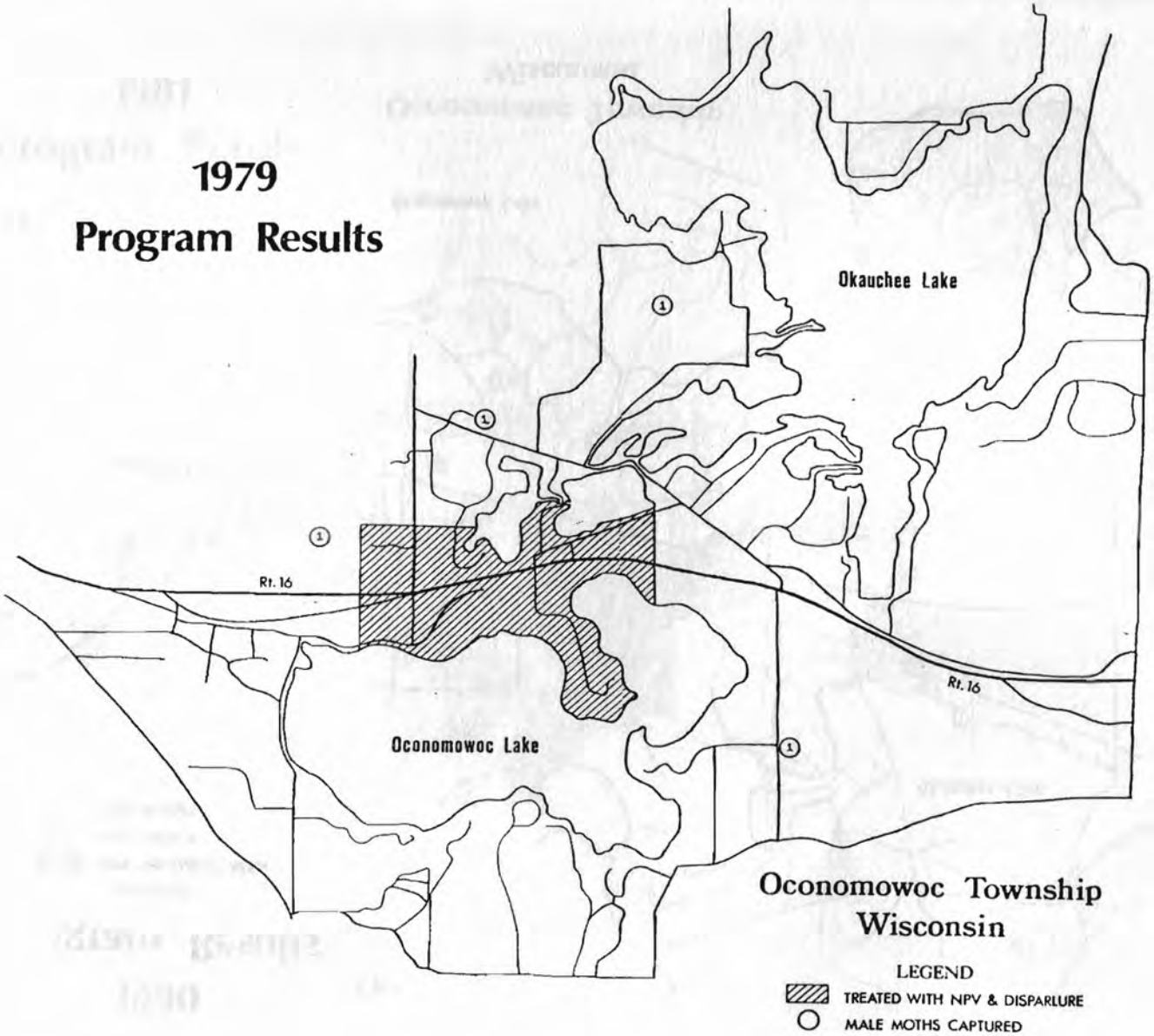
Because egg masses had been discovered, small spots were treated by hydraulic sprayer with Sevin XLR. Areas surrounding positive 1980 egg mass sites and a suspect western area (where 6 moths were captured) were sprayed. Three larger areas were then proposed for treatment: 2 were to receive 20 grams disparlure/acre in the Hercon formulation and 1 was to be trapped with 3 traps/acre (see maps of 1981 program results). Within these treatment blocks, trees were banded with burlap to establish whether or not larval populations survived the Sevin spray. A total of 85 larvae were collected at 18 sites, confirming the presence of insects in 2 of the 3 proposed treatment blocks (see 1981 program results). Traps were placed and disparlure was applied before adult flight commenced. Adult moth captures are indicated on the 1981 Program Result map. As before, no insects were captured in the areas treated with disparlure and large numbers of moths were recovered from the plot trapped at 3 traps/acre.

Following the 1978 survey, the infestation was estimated to cover ca 425 acres. Provided that no insects are detected in the 1981 disparlure spray areas in the future, the only remaining site probably infested is ca 30 acres bordering Okauchee Lake. Other tests have shown that mass trapping may be an effective method for eradicating sparse infestations and we propose trapping the entire project area (425 acres) at 3 traps/acre in 1982. Such a program will reveal any remaining areas of infestation and, presumeably, exert additional control. At this point, additional disparlure sprays are not planned and, while we feel that control has been effected by the disparlure applications, definitive evidence for eradication with the mating disruption method has not been obtained.

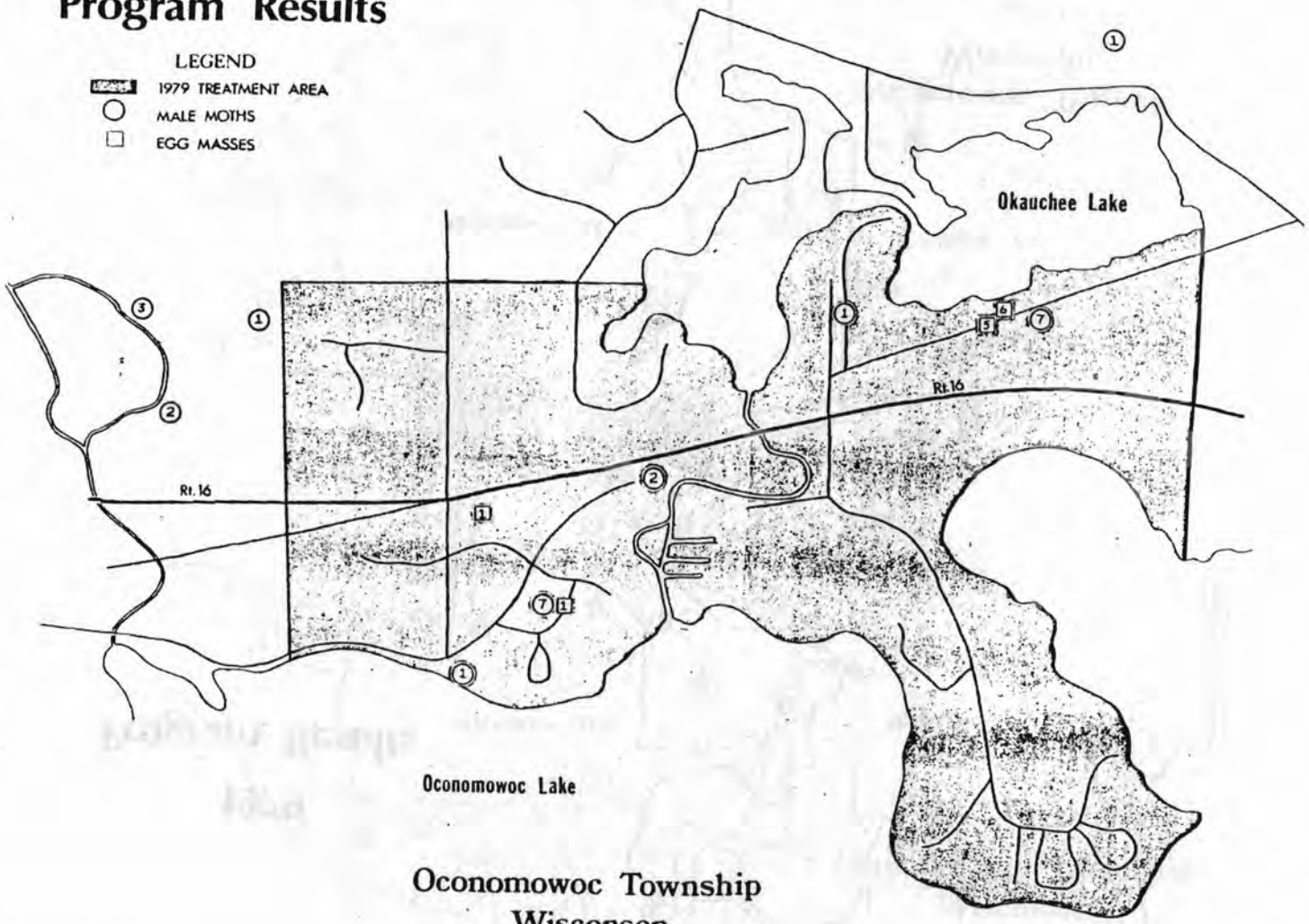
If eradication is ultimately achieved by this program, however, the chemical pesticide component will have only been used on every small acreage. Control over the remainder of the area can then be ascribed to Gypcheck, disparlure sprays, burlap banding, mass trapping and other undefined natural mortality factors. However, those conclusions must await the outcome of the 1982 trapping program.


1978
Survey Results
Okauchee Lake
Oconomowoc Lake
Rt. 16
Rt. 16
Oconomowoc Township
Wisconsin
LEGEND
MALE MOTHS CAPTURED

1979
Program Results

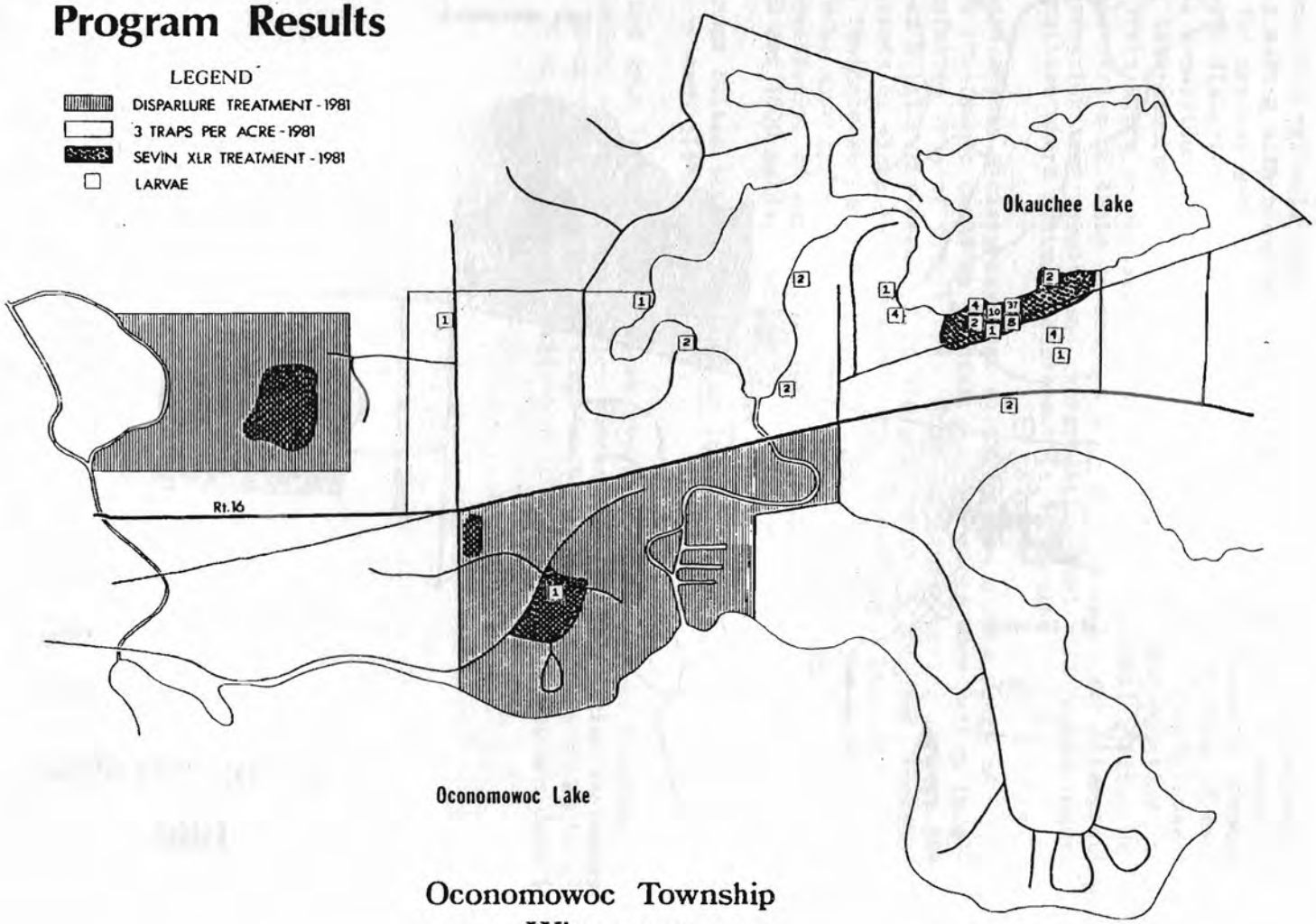


1980
Program Results



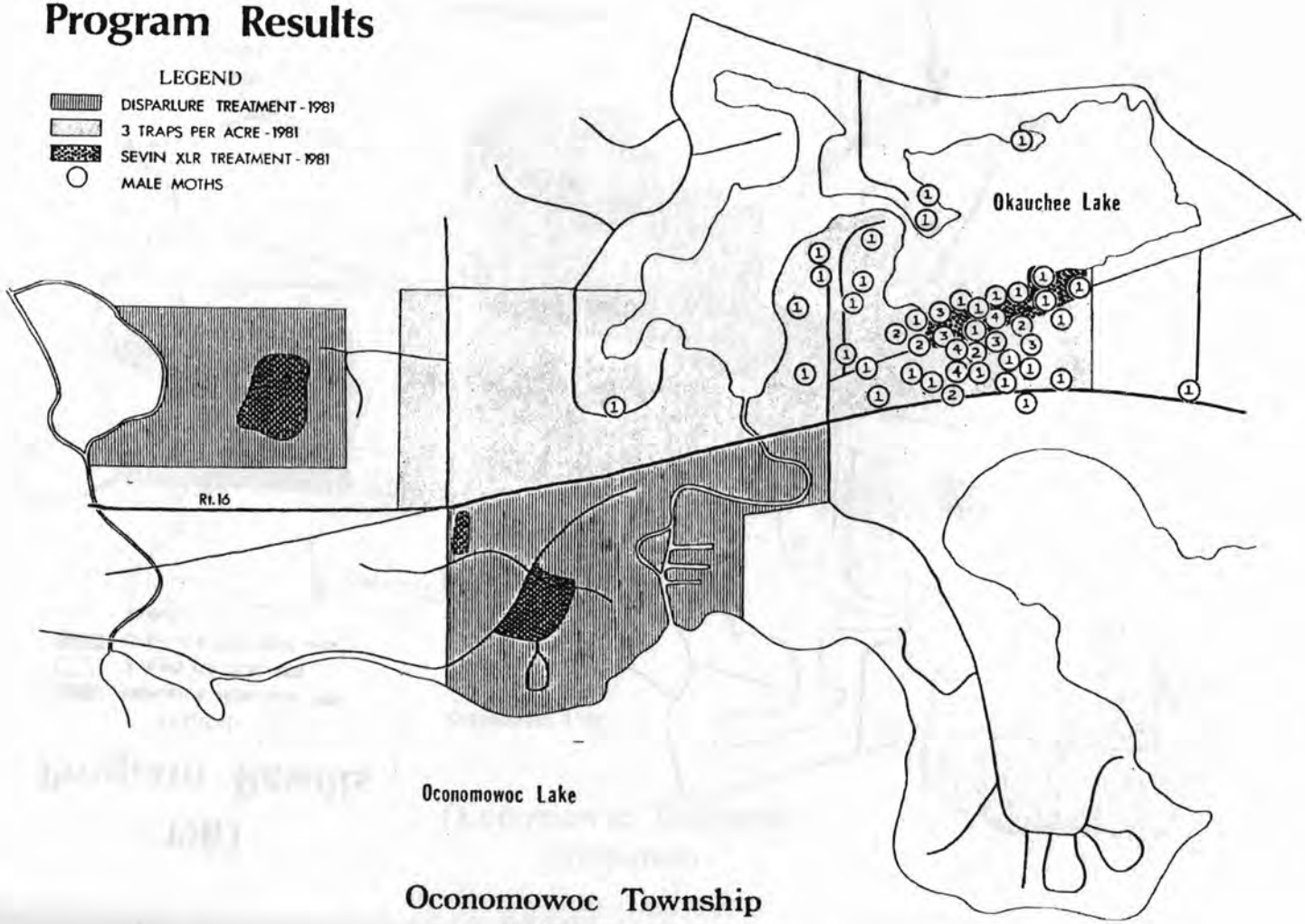
Oconomowoc Township
Wisconson

1981
Program Results



Oconomowoc Township
Wisconson

1981
Program Results



Oconomowoc Township
Wisconson

Project Number: GM 1.2.8
Project Title: Gypsy Moth Mating Disruption: Behavioral Aspects
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leader: C. P. Schwalbe

Development of a reliable and biologically meaningful method of disrupting gypsy moth mating with broadcast applications of disparlure has been underway for several years. Trap catch can be easily reduced by treating an area with disparlure, but a correspondingly reduced incidence of mating in natural populations has not been conclusively shown, mainly because of seemingly insurmountable sampling difficulties at the low population density levels where we envision the technique finding application. We undertook a series of tests to develop a behavioral basis for interpreting mating disruption test results and extrapolating what will happen under sparse densities where actual measurement is difficult.

It was necessary to miniaturize test plots so that a wide range of disruptant treatments could be studies. One ha plots were set up in a heavily infested area of Cotuit, MA. Transect lines running through the plots at 10 m intervals resulted in 100 grid points. Selected atmospheric concentrations of disparlure were achieved by stapling 1 inch wide strips of Hercon laminate at each grid point. Thus, 5, 50 and 500 grams of disparlure/ha were applied by installing strips 2.5, 25 and 250 inches long, respectively, at each grid point. Disparlure release was estimated at 15, 150 and 1500 mg/day/plot.

Sixteen traps baited with 1, 10, 100 or 1000 ug (+) disparlure were installed in each plot.

Traps baited with 1-1000 ug disparlure typically yield a dose response curve in which, as bait concentration increases, trap catch also increases, to a peak of ca 100 ug. Catch in traps baited with 1000 ug declines to about the 10 ug level. This is evident in the pretreatment capture data in Table 1.

Table 1. Capture of male moths in traps baited with 1-1000 ug (+) disparlure before and after treatment of test plots with 5-500 g racemic disparlure/ha. (Cotuit, MA 1981).

Disruptant Treatment	Trap Bait (ug)	Average Daily Trap Catch Pretreatment	Posttreatment	% Change
Control	1	27.5	28.2	+ 1.0
	10	66.7	69.8	+ 1.1
	100	106.0	103.4	- 2.4
	1000	88.7	61.6	-30.6
5 g/ha	1	148.8	6.8	-95.4
	10	312.2	48.8	-84.4
	100	488.0	121.0	-75.2
	1000	384.8	89.2	-76.8
50 g/ha	1	79.3	1.8	-97.7
	10	171.3	8.8	-94.9
	100	231.7	70.6	-69.5
	1000	146.3	52.8	-63.9
500 g/ha	1	60.5	0	-10.0
	10	233.2	0.6	-99.7
	100	400.8	3.6	-99.1
	1000	183.7	9.0	-95.1

When a plot was treated with 5 g racemic disparlure/ha, we noted a general reduction in trap catch from pretreatment levels. Traps baited with 1 ug were suppressed greater than those with 10, 100 or 1000 ug and it should be noted that, after treatment, the 1000 ug traps caught fewer moths than the 100 ug traps.

Increasing the application rate to 50 g/ha resulted in a further overall reduction in trap catch and, in this case, catch in the 1 and 10 ug traps was reduced appreciably. Traps baited with 100 ug still captured more than any other trap type.

When the disruptant treatment was 500 g/ha the trap catch pattern changed dramatically. Catch in 1, 10 and 100 ug baited traps was reduced 99-100%. The traps with 1000 ug, for the first time, captured more than other bait concentrations.

It appears that pheromone communication disruption is at least partially caused by masking attractant sources and that the more concentrated the attractant source, the more background chemical (disruptant) that is required to make it "disappear" chemically. As long as sources can be perceived, male moths apparently can resolve concentrations and respond accordingly. Thus, at low disruptant levels, the higher bait concentrations are recognized and optimum concentrations resolved. At high disruptant levels, fewer attractant sources can be recognized as such.

It could have been possible that the reason trap catch was reduced was that fewer moths remained in the plot following treatment, perhaps being repelled from the plots by the massive amounts of chemical present. Males were censused in the center 10 X 10 m sector of each plot periodically and were found present in approximately equal numbers in the 3 treated plots as shown in Table 2. Density in the control plot was somewhat less than in treated plots.

Table 2. Average number of male gypsy moths observed in center 100 m^2 of 1 ha plots treated with varying amounts of racemic disparlure (Cotuit, MA 1981).

	Control	5 g/ha	50 g/ha	500 g/ha
Number of observations	18	18	18	17
Number males/census	1.1	2.3	2.1	2.9

There were large numbers of moths in the plots, then, that were not being captured in traps, and tests were conducted to determine levels of mating activity. Female moths were placed in the plots and collected at 3-4 day intervals. Results are tabulated in Table 3.

Table 3. Mating incidence of female gypsy moths in 1 ha plots treated with varying amounts of racemic disparlure (Cotuit, MA, 1981).

	Control	5 g/ha	50 g/ha	500 g/ha
Percent mated	100	93.5	65.5	16.0
Percent not mated	0	6.5	34.5	84.0

In the control plot, all of the sentinel insects were either mated or lost but in treated plots, mating incidence was clearly reduced. At least 6% were unmated in the 5 g/ha plots, 34% in the 50 g treatment and 84% were found unmated and calling in the 500 g plots. This was a very unusual sight, with males present in great abundance and virgin, calling female moths commonplace. Native unmated females could be easily seen throughout the 500 g plot but searches in the environs around that plot failed to yield any.

Observers were stationed in the plots to monitor how long it took for males to locate and begin mating with females.

Table 4. Precopulatory and copulatory periods of female gypsy moths in plots treated with varying amounts of racemic disparlure (Cotuit, MA 1981).

	Control	5 g/ha	50 g/ha	500 g/ha
Precopulatory period (min)	103	74	77	-
Copulatory period (min)	64	80	60	-

These results were intriquing in that females in treated plots were found mating as soon after release as those in the control plot (that is, 75-100 minutes after they were placed). There were just fewer cases of mating in the treated plots and, in fact, none were observed in the 500 g treatment plot. The duration of those observed copulations were also similar at ca 60-80 minutes.

These results suggest that communication disruption in the gypsy moth is effected by chemical camouflage and that, given a high enough atmospheric concentration of disparlure, pheromone emitted by female moths is imperceptible above the background. However, even in the 500 g treatment plots some females were mated. Exactly what cues males used to locate those females is uncertain but it probably is of a very short range nature which they successfully use in dense populations where the probability of chance encounter is high. Therefore, disruption of this chemical communication in sparse populatiton densities where chance encounter is rare should result in effective mating disruption. Additional information on behavioral observations performed during the course of this work will be included in the next report.

Project Number: GM 1.2.9
Project Title: Comparison of Delta and MC Traps for Detection Survey
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: C. P. Schwalbe and NER, Area V, District 2

There has been considerable controversy over which trap design is best suited for detection survey programs. While the delta trap is less costly and easier to handle, using the MC trap may be worthwhile if it ensured a higher probability of detection. Available reports on trap efficiency are somewhat conflicting; furthermore, these traps have never been compared purely in a detection context where populations are very low and traps are widely spaced. To settle this question, it was decided to alternate trap types in the detection survey in Michigan in 1981. This survey took place primarily in the southern 3rd of the state which was presumed uninfested. Results, excerpted from the Cooperative Crop Monitoring Service Gypsy Moth Trapping Program Summary prepared by H. Russell, D. Miller, S. Gage and T. Prokrym, are summarized below.

Table 1. Statewide summary of delta and MC traps used in detection survey. Michigan, 1981.

	MC Traps	Delta Traps
Zero Catches	2241 (98.29%)	2455 (94.79%)
Positive Catches	39 (1.71%)	135 (5.21%)
Total Moths Caught	49	191
Catch per Positive Trap	1.26	1.41
Total Traps Recovered	2280	2590
Total Traps Missing	163 (7.15%)	203 (7.84%)

Table 2. Frequency of the number of moths caught in delta and MC traps in detection survey. Michigan, 1981.

Number Moths	MC Traps		Delta Traps	
Captured	Number	Percent	Number	Percent
0	2241	98.29	2455	94.79
1	32	1.40	100	3.86
2	5	0.22	25	0.97
3	1	0.04	7	0.27
4	1	0.04	0	0.00
5	0	0.00	0	0.00
6	0	0.00	1	0.04
7	0	0.00	2	0.08

These results indicate that delta traps are more effective in detection survey than MC traps because the probability of detection is greater. A larger proportion (significant at 99% level of confidence by Chi-square analysis) of delta traps was positive and the number of moths per trap was greater. Trap capacity was not a factor in this test since no traps (MC or delta) captured more than 7 moths, within the capacity limits of both designs.

Project No. GM 1.2.10
Project Title: Evaluation of Pheromone Dispensers as Trap Baits
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: E.C. Paszek, C.P. Schwalbe, B.A. Bierl-Leonhardt

The primary objective of this study was to determine the activity of Hercon and Zoecon pheromone dispensers loaded with varying amounts of (+) disparlure. Ten replicates of the following dispenser designs were aged outdoors 5/14 - 7/22/81 in 1/2 gal MC traps.

1. 5x50 mm strips containing 100, 200, 300, 400, 500 and 750 ug/dispenser and aged 1, 2, and 3 months.

2. 5x25 mm strips containing 500 ug/dispenser and aged 1, 2 and 3 months. This design was used in the 1980 survey program.

3. Zoecon rubber septa loaded with 100, 200, 400, 600 ug/dispenser and aged 1 and 2 months.

Five replicates of each of the above 29 aged treatments and 11 freshly baited unaged treatments were placed on 7/22 in 1/2 gal MC traps and field bioassayed in a randomized placement at Sandwich, MA in a 330' grid plot. Traps were spaced 165' apart on the grid lines. The remaining 5 replicates of the 29 aged and 11 unaged treatments were retained for GLC analysis. One treatment of 5 cotton wicks impregnated with 100 ug Andrulis-ES 81-59 pheromone used in the 1981 experimental dispensers (No. 1 and 3 above) were used as a control.

Results

The data indicated that there were no substantial effects of varying pheromone concentration or size of the laminate dispensers (Table 1). Gas chromatographic analysis of the unaged dispensers indicated that very little lure was lost from the laminates during the outdoor aging. The lure content for the 0, 23, 50 and 79-day aging periods for the 5x50 mm 1981 and the 1980 survey wicks are shown in Table 2. The total moth catch for all the aging periods shows no pattern. The 1980 survey wick performed essentially as well as any of the 5x50 dispensers containing 100-800 ug.

The rubber septum dispensers that were aged for several time periods captured very few moths. No lure was detected in the analysis of these rubber septa that originally contained 100-600 ug. Every septum, however, contained the olefin precursor (2-methyl-cis-7-octadecene) in proportion to the original disparlure content in the unaged dispensers. Steck et al (Environ Entomol 8, 732-733, 1979) reported that rubber septa contain an aniline compound which reacts with aldehyde pheromones and that this compound could be removed with alcohol.

Table 1. Average number of moths captured in traps baited with various pheromone dispenser designs.

Dispenser	Nominal Lure content (ug)	Days Aged Before Bioassayed 0	23	50	79
Hercon 1981 Experimental (5x50 mm)	96	437	534	859	688
"	216	342	642	590	505
"	287	505	540	559	612
"	398	343	400	622	610
"	522	416	604	539	649
"	800	382	439	382	558
Zoecon Rubber Septa	100	7	11	3	-
"	200	7	11	14	-
"	400	9	28	21	-
"	600	32	26	23	-
Hercon 1980 Survey (5x25 mm)	500	467	516	480	495
Cotton wick	100	299	-	-	-

Table 2. Results of GLC analysis of pheromone dispensers.

Dispenser	Nominal	Lure content of dispensers after Bioassay 0	23	50	79
Hercon 1981 Experimental (5x50 mm)	96	83	90	64	85
"	216	183	138	222	138
"	287	307	246	230	209
"	398	427	349	359	417
"	532	644	581	439	556
"	800	737	660	561	653
Zoecon Rubber Septa 1/	100	21	-	16	-
"	200	46	-	31	-
"	400	96	-	67	-
"	600	110	-	92	-
Hercon 1980 Survey (5x25 mm)	500	216	165	182	231
Cotton wick	100	Not aged or analyzed			

1/ Values reported are for olefin precursor (see text).

Project Number: GM 1.2.11
Project Title: Aging Study of Salvaged 1980 (+) Disparlure Dispensers.
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: E. C. Paszek and C. P. Schwalbe

The objective of this study was to bioassay salvaged 1980 (+) pheromone dispensers. Dr. B. A. Bierl-Leonhardt analyzed samples of used dispensers and reported that ca 50% of the pheromone remains in the dispenser after a season in the field. This suggests that dispensers could be used more than 1 season.

Used 1980 (+) lure dispensers were aged outdoors in MC traps for two aging intervals, 60 days (5/21 - 7/13/81) and 30 days (6/19 - 7/13/81). Ten replicates of each were placed in the field on 7/13/81 with a used 1980 lure control that was stored in a freezer and not aged outdoors in 1981 and an unaged 500 ug 1981 (+) survey lure control. Traps were checked 3 times. Results are summarized below.

Total number of moths captured in 10 traps baited with salvaged 1980 (+) disparlure samples and with unaged 1981 (+) disparlure controls.

Replicate	60 day 1980 reused	30 day 1980 reused	0-day 1980 reused	0-day 1981 unaged
1	2,620	2,592	2,308	1,245
2	4,588	4,560	4,072	2,848
3	5,951	5,848	5,672	3,805
Total	13,159	13,000	12,052	7,898

Conclusion:

Aging the used 1980 dispensers for another 60 days did not affect the catch. There appears to be a substantial difference between the 1981 and 1980 dispensers. The 1981 dispensers tested in this study were not as attractive as the 1980 dispensers.

Project Number: GM 1.2.12
Project Title: Comparison of Thuron Dog Flea Collar and Hercon Laminated Insect Tape
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: E. C. Paszek and C. P. Schwalbe

Objective

The 3g (18.6% DDVP) Zodiac Flea Tag sold by Zodiac Pet Products was used in 1979 as a killing agent in MC Traps. It was discontinued and replaced in the pet market with a more effective flea killing formulation, the Thuron Dog Flea Collar, which contained 8.37% DDVP. Tests done in the greenhouse in 1979 indicated that a 3.5" piece of this flea collar was effective in killing gypsy moths in MC traps. It was used in the 1980-1981 MC traps as the replacement for the flea tag. In 1980, Hercon Division of Health-Chem Corporation formulated a new product, a laminated insect tape containing 10.2% DDVP, (44.5 mg/in^2). This Hercon insect tape was bioassayed in the greenhouse in 3 sizes, 1x4", 1x2" and 1x1" and it showed potential as an effective killing agent for use in the MC trap.

Table 1. Greenhouse bioassay comparing Hercon insect tape and Thuron flea collar.

Killing Agent	Size of Killing Agent	Avg. no. minutes immobilize moths
Hercon Insect Tape	1X1"	13.2
Hercon Insect Tape	1X2"	9.3
Hercon Insect Tape	1X4"	6.6
Thuron (1981) Flea Collar	*3.2" long*	*6.5*

These Hercon insect tapes are flexible and are easily stapled inside MC traps. The Thuron Flea Collar strips have to be stapled on a twist-em plant tie and then suspended inside the MC trap.

An aging study of the Hercon Insect Tape and the Thuron Flea Collar was conducted in the spring of 1981. These agents were aged outdoors in individual MC traps for 10 weeks, 6 weeks and 2 weeks from 5/7 - 7/14/81. They were then compared in 5 replicates in Sandwich, MA in a field bioassay with unaged controls. The traps were placed in a 330' grided plot with the traps spaced 165' apart on the grid lines.

Results:

Table 2. Average of number moths captured/trap in 5 replicates.

Aging Period (Weeks)	Thuron 3.2"	Hercon 1X4"	Hercon 1X2"
10	104	45	77
6	177	91	63
2	180	97	84
0	275	214	171

Conclusion:

The most effective killing agent is the 3.2" strip of the Thuron Dog Flea Collar which was used as the killing agent in the large capacity MC traps in 1981. The 1x4" laminated Hercon insect tape was effective in the 0-day (unaged) treatment. It captured an average of 214 moths. However, after aging 10, 6 and 2 weeks, the number of moths captured steadily diminished. Hercon insect tape loses its effectiveness as a killing agent more readily than the Thuron Flea Collar after prolonged field use.

Project Number: GM 1.2.13
Project Title: Preseason Bioassay of 1982 Production Lots of (+) Disparlure
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: D. R. Lance, V. C. Mastro, C. P. Schwalbe

Five lots of synthetic gypsy moth pheromone, (+) disparlure, were bioassayed for activity in a grapefruit grove between February 9-17, in Brownsville, TX. A circular plot design (95.5 m radius) with 30 trap stations (20 m between stations) was used to bioassay 3 concentrations (1, 10, 100 ug) of each lot of material. Treatments (5 lots X 3 concentrations = 15 treatments) were randomly placed on each half of the circular plot. The treatment positions were randomized daily. Because of the release characteristics of cotton dental rolls, wicks were changed every three days. Sources for the lots of material used in the bioassay were:

1) J. Plimmer (ARS) - "standard"
2) Andrulis Research Corp. "80-81" - material used in the 1981 detection program
3) Andrulis Research Corp. "81-28"
4) Hercon Corp. "8102 M"
5) Hercon Corp" 8101 S"

Males were released from the center of the plot when temperatures were expected to become warm enough for male flight (ca 21°C). Traps were checked daily and the numbers of captured males were recorded. In all, 6 replicates were run.

Results and Discussion:

Mean numbers of males captured for all lots and concentrations of pheromone are presented on Table 1. Anaylsis of the data indicates that there were no detectable differences among sources of pheromone. In other words, all sources of pheromone appeared to be equal in attracting males into traps. Differences among disparlure concentrations were significant. Although the dose-response relationship does not appear to be sharply defined for the two highest concentrations, differences in weather (temperature) could explain some of this variation. Also, the significant difference among replicates can be partly explained by the release of different numbers of males for the various replicates, and by temperature fluctuations (21°C - 34°C daily high) which occurred during the test period.

Table 1. Mean number of males captured in traps baited with three concentrations of five sources of (+) disparlure.

Pheromone Concentration	Standard J. Plimmer	Andrulis 80-81	Andrulis 81-28	Hercon 8102M	Hercon 8102S
1 ug	13.92	21.67	9.08	14.75	9.50
10 ug	65.33	31.92	57.75	27.67	46.67
100 ug	41.42	43.75	54.50	51.58	53.17

		F	Sig. of F.
ANOVA	Main Effects	3.045	0.001
	Lot (Source)	0.192	0.942
	Dose	6.715	0.002
	Replication	3.859	0.003

Project Number: GM 1.2.14
Project Title: Pre-season Evaluation of (+) Disparlure Prior to 1981 Field Use
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leader: Dave Simser

Objective:

Commercially prepared (+) disparlure must display field activity (male moth attraction) comparable to that of standard formulations prior to purchase and use in survey and detection programs. In this study, several lots of (+) disparlure (prepared by Andrulis Research Corp., Bethesda, MD) were compared with (+) and (±) standards. Trials were conducted in Brownsville, Texas during February 1981, utilizing released laboratory reared male gypsy moths. Disparlure activity was measured by comparing the percentage of moths recaptured in milk carton traps baited with the various formulations.

Methods and Materials:

All disparlure formulations were received from these sources:

Disparlure Treatment	Source
A	Pooled samples 1/ from lots 80-14, 80-4, 80-24 received 6 February 1981 from Andrulis Research Corp.
B	Pooled samples from lots 80-21, 81-59, 81-60 received as in A.
C	Sample from lot 80-30C received as in A.
D	Sample from 1980 standard (+) formulation prepared by Dr. Donald Farnum, Michigan State University, and utilized in 1980 survey and detection programs.
E	Sample of (+) disparlure received 2 February 1981 from Dr. Meyer Schwarz SEA-AR, Beltsville, MD.
F	Sample of racemic (±) disparlure used in 1980 detection surveys.

1/ Samples were pooled in equivalent amounts (1 μg/μl) based on cis-isomer content of the various neat lots.

All samples were diluted in n-hexane to 1 ug/ul, and were analyzed with a Tracor 550 gas chromatograph.[2/] Comparison of sample peak height with those of standard concentration confirmed consistent quantification of all test solutions. Samples were then sealed in ampules and stored until use.

A circular field plot (r=114.5m; ITD= 20m; 36 points) was established within a citrus orchard in Brownsville, Texas during February, 1981.

At the onset of the experiment, 100 ul aliquots of the (+) disparlure (100 µg) samples and 200 ul of the racemate were loaded into cotton dental wicks. Each wick was suspended by copper wire within a milk carton trap. In addition, each trap was equipped with a plastic strip impregnated with DDVP as the killing agent. Each of the six treatments was represented by six baited traps. The circular plot was divided into six 60° arcs; thus, each treatment appeared in one arc of the circular plot, and treatments were randomized within these arcs. All traps were suspended ca 1.5 m above ground level, away from obstructive foliage or branches.

Male moths were transported to Brownsville as pupae, which were held in cardboard boxes under room conditions until emergence. Moths were collected daily and held 24 h (25 ♂♂ per 16 oz container) until release, to insure wing development and to standardize release ages. A total of 5 releases were made at 0930 (500 moths/release) at consecutive 24 h intervals. The central release point was ground level in indirect (shaded) sunlight. Moths readily dispersed from each 16 oz holding container. However, the remainder were gently shaken from the containers to prompt dispersal and limit avian predation.

Traps were checked at 1730 following the second release, and at 24 h intervals thereafter (4 replicates). Each arc of 6 (treatment) traps was collected following checking, and suspended at random at trap points at the succeeding arc, with those traps checked, collected, and replaced. Thus, each of the treatments was randomized throughout the circular plot, within a 60° arc, at 24 h intervals.

Numbers of moths captured were recorded, and daily percent recoveries were transformed to arcsine for statistical analysis.

2/ 3% OV-1 on chromosorb w/HP 80/100 mesh, 6' x 1/4" glass column, oven 188 °C, inlet 225 °C, detector (FID) 250 °C.

Results:

Results are summarized below (Figure 1) and demonstrate that all samples of (+) disparlure captured significantly more moths than the racemic (±) formulation (F).

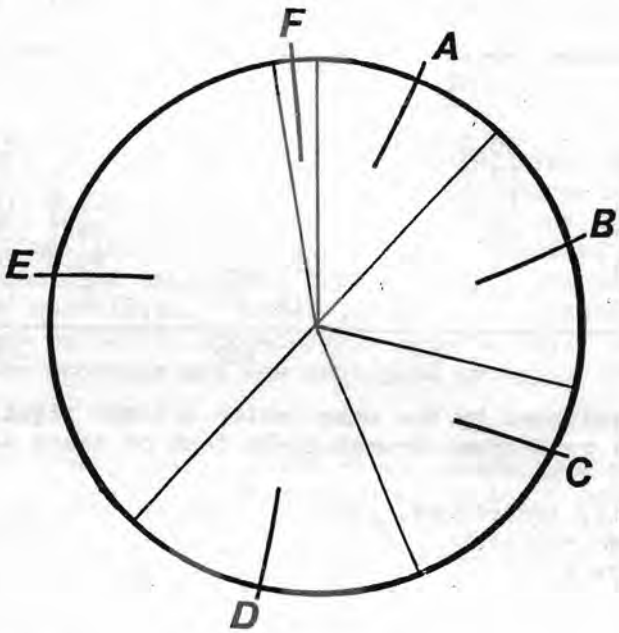


Fig. 1. Proportion of gypsy moths recaptured per lure of total number recaptured. See text for treatment codes.

Daily recapture percentages were consistently low, probably due, in part, to the unnatural setting and conditions. In addition, daily temperatures did not rise above 75 °F for extended periods, which may have hampered moth dispersal and orientation to traps.

Although formulation (E) recaptured the greatest number of moths, this total was not significantly different from recapture by other (+) lots. Recapture of moths during replicate 3 (see appendix) was relatively high, due to a temperature increase following the morning in which males were released. Because each treatment was represented by only six traps, a single trap capturing an unusually high number of moths would skew the total number per treatment disproportionately higher. This effect was borne out in this study, as two (E) traps captured 25 and 22 moths. Again, statistical comparison of the average number of moths captured during the entire study revealed no differences between (+) enantiomer lots.

The low number of recaptured moths in this study dictates that additional in-season testing be conducted before a conclusive evaluation be made. However, each lot of (+) disparlure did demonstrate attractiveness to male moths. Additional testing was conducted during the 1981 field season, details of which may be found in GM

Appendix. Recapture of released male gypsy moths by traps baited with various disparlure lots.

Treatment [1/]		Replicate [2/] 1	2	3	4	Total
A	a	18.2 (4)	30.8 (8)	7.4 (9)	13.8 (4)	17.6 (25)
B	a	22.7 (5)	19.2 (5)	9.0 (11)	34.5 (10)	21.4 (31)
C	a	22.7 (5)	13.6 (3)	14.8 (18)	13.8 (4)	16.2 (30)
D	a	18.2 (4)	30.8 (8)	15.8 (19)	24.1 (4)	22.2 (35)
E	a	18.2 (4)	4.5 (1)	50.8 (62)	13.8 (4)	21.8 (71)
F	b	0 (0)	4.5 (1)	2.5 (3)	0 (0)	1.8 (4)
Total		22	26	122	26	196

1/ Treatment codes followed by the same letter are not significantly different ($P \leq .05$) by Student-Newman-Kuels test of means separation.

2/ Figures are % daily recaptures; parenthesized values are actual numbers of moths captured per replicate. Percentages were transformed to arcsine for statistical analysis.

Project Number: GM 1.2.15
Project Title: Field Bioassay of Standard and Experimental (+) Disparlure (July 13-28, 1981)
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: Dave Simser, Wendy Ratcliffe, Debi Jacobs

Introduction

The practice of monitoring the spread of existing gypsy moth populations, and discovery of new, and potentially damaging, outbreaks requires a large number of standard traps baited with the synthetic sex pheromone, (+) disparlure. Availability of this material has been limited, due to expenses in synthesizing this pure, optically active enantiomer in more than very limited quantities. However, commercial interest has intensified the search for new synthetic procedures. Several novel syntheses have been developed, and the products are now available to applicable federal agencies.

Prior to purchase, commercially prepared (+) disparlure must be field tested for attractiveness to male moths. Large scale production of (+) disparlure (by Andrulis Research Corp., Bethesda, MD) for use in 1981 survey programs was preceeded by assay of sample production lots in a preseason, male release experiment (see GM1.2.14) which demonstrated (+) disparlure activity. However, spurious results can occur during preseason tests, due to variables such as changing weather patterns, irregular wind speeds, and other unseasonal conditions; lots must also be field assayed during the natural male flight period. Such assay provides a basis for confirmation of lure activity (or inactivity).

In this report, the current (+) disparlure bait (disparlure incorporated into a wick dispenser) was compared with experimental preparations. Separate experiments were conducted from July 13-18, 1981, the peak period of male flight in Cape Cod populations. Comparisons were based upon the number of male moths captured in standard survey traps, baited by the various disparlure formulations.

Methods and Materials

Sources of (+) disparlure are listed, and were received in neat or diluted concentrations. Dilution of materials was standardized to 1 ug disparlure/1 ul n-hexane, and quantified by gas chromatographic analysis; materials were then held at -12°C until preparation and incorporation into standard USDA milk carton (MC) traps. The 1981 commercially prepared (+) disparlure was received in field ready form: 500 ug of material incorporated into a three layer, plastic laminate wick. Foil packets containing 250 wicks were also refrigerated until use.

A forested tract on the northern perimeter of Otis Air National Guard Base was selected, and a field grid site was established, consisting of a 10x10 point array, with 25 m between trap points. Site selection was based on these criteria: 1) a light to moderate natural population (no apparent defoliation, few observed egg masses or larvae); 2) accessibility and level terrain; and 3) dominance by mature, preferred host species (Quercus spp) with a canopy ca 18 m in height. The study site was adjacent to areas with moderate to heavy populations. Thus, male populations during the test would be large enough to provide trap capture totals necessary for statistical analysis of data, but not so large as to confuse interpretation of results by exceeding trap capacities. In addition, milk carton traps baited with n-hexane or other unattractive solvents often capture considerable numbers of moths in heavily defoliated areas, due to contamination by trace amounts of (+) disparlure during handling and preparation, and by the large numbers of actively searching male moths contacting virtually every available surface within the area.

Prior to the onset of Test 1, monitor traps were placed at the site boundaries, and at points within the site interior. Traps were checked daily, which served to indicate levels of moth activity around and within the site. When trap captures were consistently high (20-25 males per trap), the first experiment was initiated.

Selected disparlure lots were removed from refrigeration and held until ambient (ca 20°C), then loaded into cotton dental wicks (when applicable) by syringe. Following solvent evaporation, a charged wick was suspended by copper wire within a milk carton (MC) trap, along with a 2 inch (5 cm) plastic strip impregnated with DDVP; each wick was positioned central to all (12) entry ports . Standard 1981 wicks were handled similarly. Treatment groups of 10 MC traps were placed in plastic trash bags to minimize cross contamination prior to field placement.

A representative trap from each treatment group was randomly placed at a point along a survey line on a host tree ca 1.5 m above ground level, and away from obstructing foliage or branches. This process was continued until all traps were suspended at grid points (1400 h on July 13, 1981). Thus, each treatment was represented somewhere along each survey line.

Traps were checked at five consecutive 24 h intervals and re-randomized. Each check (replicate) consisted of opening a MC trap spout, and recording numbers of captured males. Any escaping males were included in the recorded counts. Emptied traps were then resealed and taken down. When an entire line was checked and collected, all those traps were randomly relocated on a subsequent line.

The 4 day interval between tests possibly allowed dissipation of residual disparlure, and attracted additional males moths into the study site. Residual activity may have affected further testing in this area.

After disparlure treatments were selected, Test 2 was initiated on July 22, 1981. All preparatory, placement, and recording procedures were identical to those described in Test 1. The final (fifth) replication was completed on July 27, 1981. All traps were collected and all boundary ribbons were removed from the site, returning it to a pristine state.

Numbers of captured males in each test were compared by analysis of variance, and significant differences of mean numbers of captured males separated by Duncan's Multiple Range Test.

Disparlure Sources for Test 1, July 13-18, 1981.

Treatment Code	Disparlure per wick (ug)	Wick type [1/]	Source of disparlure
A	500	a	1981 (+) survey wick. Disparlure lots [2/] ES-80-14, 80-4, 80-24.
B	100	b	aliquot of lot 80-4 cis content = 94.6%
C	100	b	aliquot of lot 80-24 cis content = 92.0%
D	100	b	aliquot of lot 80-21 cis content = 96.0%
E	100	b	aliquot of lot 81-59 cis content = 100%
F	100	b	aliquot of lot 81-60 cis content = 100%
G	100	b	aliquot of lot 80-30C cis content = > 92.0%
H	100	b	sample of (+) disparlure prepared by Dr. M. Schwarz, USDA, ARS, Beltsville, MD
I	-	b	blank control 100 ul n-hexane per wick. (J.T.Baker Chemical Co., Phillipsburg,NJ)
J	500	a	1980 (+) survey wick [3/]

Disparlure Sources for Test 2, July 22-27, 1981.

Treatment Code	Disparlure per wick (ug)	Wick type	Source of disparlure
K	500	a	1981 (+) wick see A, and footnote.
L	100	b	sample of (+) disparlure received from J.T.Baker Chemical Co., Phillipsburg, NJ
M	500	b	received as in L
N	100	b	received as in H
O	500	b	received as in H
P	500	b	sample of precursor (disparlure intermediate) received as in H
Q	500	b	sample of racemic disparlure 4/ GC x 3549
R	100	b	pooled (equivalent amounts) of lots 80-14, 80-4, 80-24
S	-	b	blank solvent control as in I
T	100	a	modified 1981 (+) wick 5/

1/ Type a wicks were manufactured by Hercon Products Controlled Release Division and consisted of a plastic laminate (5 x 25 mm) impregnated with 500 ug (+) disparlure.

Type b wicks were cut from standard dental wicks to ca 3 mm, soaked in n-hexane and air dried prior to disparlure incorporation.

2/ All 'lot' designations refer to production lots of (+) disparlure by Andrulis Research Corp. Those listed in treatment A were pooled prior to impregnation into wicks.

3/ Standard 1980 (+) wicks were prepared by Hercon Products with (+) disparlure supplied by Dr. D. Farnum, Michigan State University, East Lansing, MI.

4/ Because of the racemic lure content, 1000 ul (±) was added per wick to approximate 500 ug of (+) disparlure.

5/ Standard 1981 (+) wicks were modified by slicing a wick to 1/5 size (5 x 5 mm) in order to approximate a lure concentration of 100 ug.

Results

Test 1. Examination of Test 1 results (Fig. 1 and Appendix 1) reveals surprising differences among treatments. The standard 1981 (+) wick (A) captured significantly fewer moths than any disparlure lot (B-H) or the 1980 (+) wick (J); the total was not significantly different from the number captured by the control (blank) trap (I). Treatments B-H and J captured similar numbers of moths. Capture of moths was remarkably consistent within treatment groups for the duration of this test, as mean standard errors ranged from 0.31 to 2.90. The total number of males captured did not markedly increase with consecutive daily replications, as might be expected by normal moth emergence. This was due to a rapid change in weather patterns from warm sunny conditions, to increasing cloudiness, decreasing temperature, and increasing RH. Because concentrations between the standard (+) wick (500 ug) and other comparative treatments (100 ug) were disimilar, equivalent concentrations were selected for comparison in Test 2.

Test 2. Examination of test 2 results (Fig. 2 and Appendix 2) again yielded surprises. First, the number of males captured by the standard 1981 (+) wick (K) was not significantly different from those captured by the blank (S), racemic (Q), or precursor (P). All disparlure formulations applied to cotton wicks captured significantly greater numbers of male moths (L-O, R). The modified 1981 (+) wick (T) captured significantly less moths than disparlure treatments L, N, and R (comparison of 100 ug amounts per wick).

Interestingly, comparison of treatments L-O demonstrated greater attractivity by traps baited with a lower concentration of material (100 ug) than by those baited with 500 ug. Also, the disparlure precursor (P) possibly demonstrated an antagonistic effect by repelling, rather than attracting, moths, as average catches were consistently lower than blank (S) totals.

Larger numbers of moths were captured during this test, although average daily treatment captures were again consistent (SE $\bar{x}$ = 0.16 to 2.37). The increase in total numbers of males captured may have been due to emergence patterns, and an increase in daily temperature during this period.

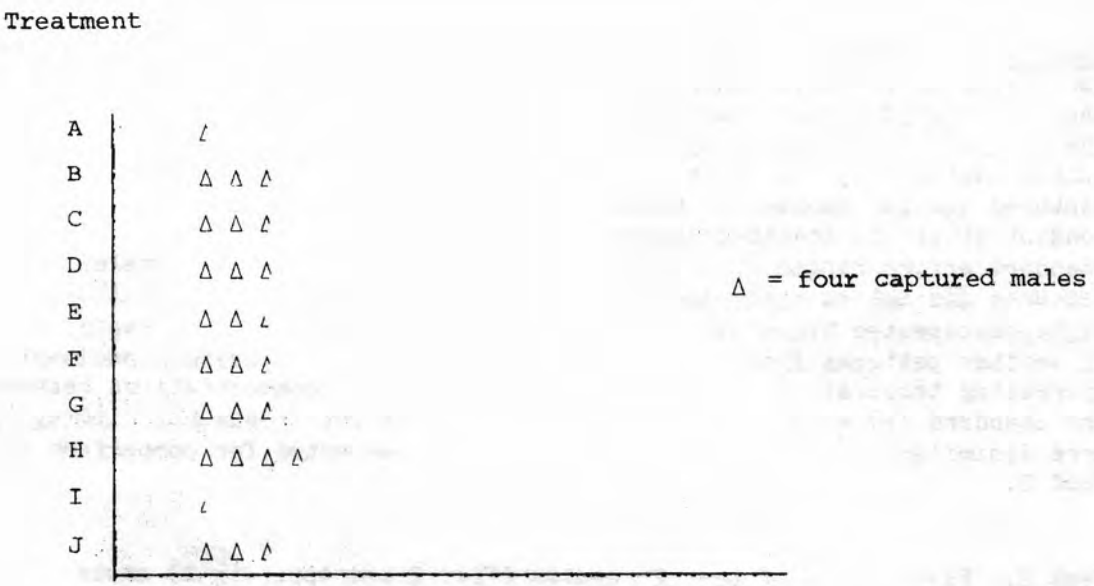


Fig 1. Number of male moths captured by treatments during test 1 (July 13-17, 1981). Values are averages of five replicates, with ten traps per treatment per replicate.

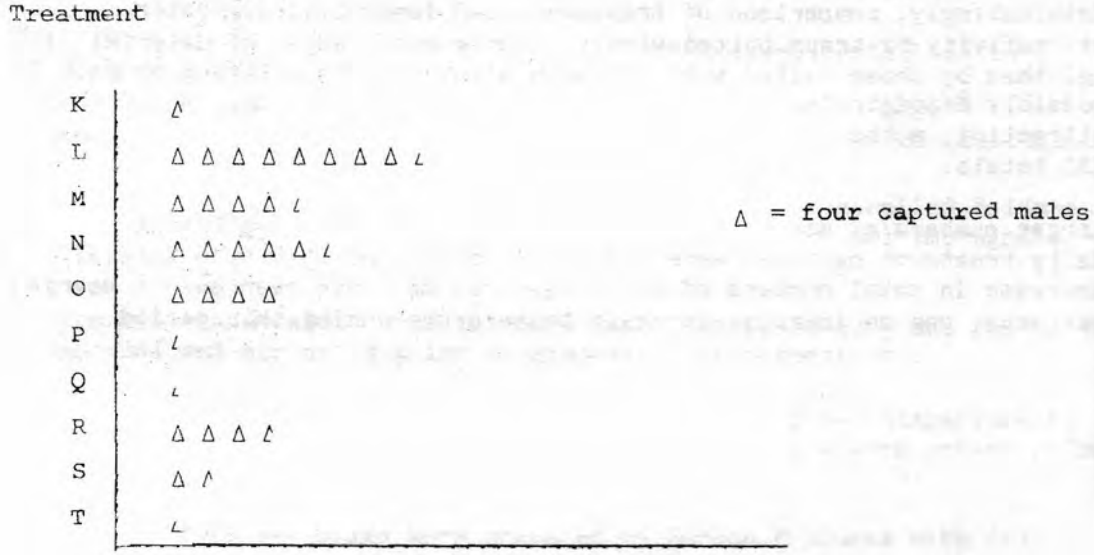


Fig 2. Number of male moths captured by treatments during test 2 (July 22-26, 1981). Values are averages of five replicates, with ten traps per treatment per replicate.

Discussion

These tests compared standard 1981 (+) wicks to the previous years', and to disparlure lots added to cotton dental wicks; in each, the standard wick displayed little activity. Variables in wick emission rates (cotton vs plastic) may be reflected by varying trap catch. However, the 1980 (+) wick utilized an identical controlled release substrate (as 1981) but captured far more male moths.

Response by male moths is affected by emission rates of disparlure; ideally, a wick should emit at a rate similar to that of calling female moths. Possibly, disparlure emission from cotton wicks, which is highly correlated to temperature, came closer to approximating pheromone release patterns than the commercial wicks, which are designed to release disparlure at a constant rate for a prolonged (season) period. Were this the case, all commercial wick types should capture similar numbers of moths, a condition not reflected by test results.

Residual analysis of aged and unaged (T_o) standard wicks (Appendix 3 and 4) illustrates a possible explanation for relatively low attractiveness of these wicks. Initially, wicks contained an average of ca. 410 ug (T_o); wicks field aged for more than a week surprisingly also contained an average of 410 ug. Because we used 1981 wicks directly from freezer storage, it is possible that such baits were not releasing much disparlure during the test period. Our tests were terminated after 5 days. This explanation would account for disparities in results of various field tests utilizing these wicks, as older wicks displayed activity, while fresh wicks did not. In the second experiment, 1981 (+) wicks were altered (Treatment T) by slicing them to 1/5 size (to approximate 100 ug (+) disparlure). These wicks captured significantly more males than traps baited with unaltered 1981 (+) wicks. Reservoirs of disparlure within wicks could be exposed by this alteration, accounting for the higher trap totals.

The disparlure used to charge 1981 (+) wicks was demonstrated to attract moths and could not itself account for relatively low capture totals.

Perhaps wicks used in mass trapping or mating disruption studies should be field aged for optimal attraction during the peak, and critical, period of male flight. Detection and survey programs would not require timed placement, as standard traps are routinely placed in the field prior to the flight season, and would therefore be attractive throughout this period.

Appendix 1. Male capture by MC traps baited by various disparlure lots (Test 1 - 13 July - 17 July 1981).

Treatment 1/	Amount Disparlure Per Wick (ug)	Replicate 2/ 1	2	3	4	5	Total X
A	500	3.0	3.2	2.1	2.3	1.5	2.4
B	100	13.7	14.5	10.6	9.5	4.6	10.6
C	100	9.6	14.6	13.1	12.1	2.8	10.4
D	100	16.7	15.2	11.7	8.1	3.4	11.0
E	100	14.9	14.0	9.0	11.5	3.3	9.7
F	100	11.6	14.1	10.3	11.5	4.3	10.4
G	100	17.4	13.5	7.2	6.7	6.2	10.2
H	100	21.5	21.5	15.1	15.3	5.7	15.8
I	-	4.1	1.5	0.5	0.3	0.0	1.3
J	500	12.6	17.0	8.9	10.0	3.7	10.4

1/ See Materials and Methods for treatment codes

2/ Values are replicate averages (10 traps per treatment) of male moth capture during 24 h.

Appendix 2. Male capture by MC traps baited by various disparlure lots. (Test 2 - 22 July - 26 July 1981)

Treatment	Amount Disparlure Per Wick	Replicate 1	2	3	4	5	Total
K	500	4.8	2.9	2.3	3.2	4.7	3.6
L	100	36.1	24.9	28.4	33.3	42.4	33.0
M	500	16.0	16.4	15.4	18.9	22.9	17.9
N	100	13.7	20.8	19.1	24.7	27.5	21.2
O	500	14.6	10.8	6.9	13.2	18.1	12.7
P	100	0.7	0.8	0.6	1.3	1.4	1.0
Q	500	1.4	2.0	1.3	1.2	0.6	1.3
R	100	15.8	12.7	10.5	16.3	16.8	14.4
S	100	2.4	9.3	5.7	7.0	12.0	7.3
T	-	1.3	1.7	2.8	1.1	4.1	2.2

1/ See Materials and Methods for Treatment Codes

2/ Values are replicated averages (10 traps per treatment of male capture during 24 h.

Appendix 3. Gas chromatographic analysis of bioassayed dispalure lots (quantitative).

GC Conditions:

Temperature (°C)	
Detector	225
Oven	188
Inlet	225
Outlet	250
Column	3% OV-1 80/120 Chromasorb Q on glass 6' X 1/4" ID

Aliquots of sample concentrations were analyzed for lure content prior to bioassay; concentrations were compared to standards by peak height, and standardized if necessary.

Appendix 4. Quantative gas chromatography of 1981 (+) wicks, aged and unaged.

Time Aged (Hours)	x	# Wicks Assayed
0	412.5	4
184	410.7	10
1200	190	2

(See Appendix 3 for CG conditions.)

Analytical Procedure: Collected, field aged 1981 (+) wicks were soaked separately in 10 ml n-hexane for 24 h at 2°C. Solutions were warmed to room temperature (ca 21°C), then vortexed; wicks were removed, rinsed with 2 ml n-hexane, and the sample evaporated (N_2) to 0.5 ml. Additional wick soaking yielded virtually no traces of residual disparlure; only trace amounts (< 10 ng) of disparlure were lost during the evaporation process.

Project Number: GM 6.3.6
Project Title: Establishing Standards
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leaders: John Allen Tanner and Bernadine P. Weeks

This project has been combined with project number GM 8.3.4 (Evaluating the Sampling Techniques Used to Establish Standards and Monitor Production) to form GM 1.3.1 (Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Rearing Facility).

Project Number: GM 7.3.4
Project Title: Insect Production and Distribution
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: L. F. Kennedy and J. J. Baker

The primary objective of the rearing facility of this laboratory is to produce sufficient quantities of all stages of the gypsy moth life cycle in support of research projects at this laboratory and at several universities and experiment stations in the United States and foreign countries. The following is a summary of FY 1981 production and distribution of such material.

A. Productiton

Infested	7,044,903	Neonates
Eggs refrigerated	103,758	Masses

B. Distribution

1. Methods Development

Insecticide screening	350,622	L-2
	156	L-3
	3,236	L-4
	4,400	L-6
Total	358,414	Larvae
Sterile Male Summer Project		
Male pupae	903,159	Male pupal
Male prepupae	121,026	Male prepupae
	1,024,185	Total males
	38,800	Females
Total	1,062,985	
Periodicity studies		
	148,098	Prepupae
Sterile Male Grand Total	1,211,083	
Pheromone studies	7,000	Adult males
Virus Production	672,000	L-4
Grand Total	679,000	L-3 & L4

2. ARS

Virus Tests	326,282	L-2
	7,678	L-3
	360	L-4
	2,520	L-5 & 6
	14,700	Neonates
	1,030	Male pupae
Total	352,570	Insects

3. Cooperators:

Michigan State University - R. Carde East Lansing, MI	35,000 21,000	Male pupae Female pupae
University of Massachusetts - J. Elkinton Amherst, MA	83,229 17,000	Male pupae Female pupae
Agricultural Research Center - R. Webb Beltsville, MD	34,000 11,950	Male pupae Female pupae
University of Maryland - M. Ma College Park, MD	16,000	L-5 + 6
University of Maryland - P. Barbosa College Park, MD	18,000	Female prepupae
University of Massachusetts - C. Yin Amherst, MA	13,000	Larvae (3 + 4)
England - J. Kennedy	1,900 1,900	Male pupae Female pupae
New Jersey Dept. of Agriculture - R. Chianese Trenton, NJ	3,050	Egg masses
U.S. Forest Service - T. ODell Hamden, CT	1,270	Egg masses
U.S. Forest Service - Wallner Hamden, CT	200	Egg masses
Boston University - L. Driver Boston, MA	143	Egg masses
Connecticut Agricultural Experiment Station Anderson	20	Egg masses
Boston University - B. Wang Boston, MA	120	Egg masses
University of NY - S. Hornung Syracuse. NY	840	L-3 + 4
Pennsylvania University - W. Yendol University Park, PA	136	Egg masses
Division of Forest Pest Management - R. Fusco Middletown, PA	180	Egg masses

The insects used by Otis Laboratory and several cooperators plus adults used for maintaining colony totaled 3,052,838. This is approximately 43% of insects infested.

Sterile Male Pupal Harvest 1981

Michigan 60 day total - All Red Dye - 37% recovery

Male pupae	479,930	= Daily average of 7,999
Male prepupae	69,936	= Daily average of 1,166
Total males	549,866	= Daily average of 9,164
Female pupae	18,000	= Daily average of 300

Otis, MA 74 day total - 43% recovery

Male pupae	423,229	= Daily average of 5,719
Male spins	50,090	= Daily average of 676
Total males	473,319	= Daily average of 6,396
Female pupae	14,800	= Daily average of 200

Project Number:	GM 8.3.4
Project Title:	Evaluating the Sampling Techniques Used to Establish Standards and Monitor Production
Report Period:	October 1, 1980 - September 30, 1981.
Report Type:	Final
Project Leaders:	John Allen Tanner and Bernadine P. Weeks

This project has been combined with project number GM 6.3.6 (Establishing Standards) to form GM 1.3.1 (Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Rearing Facility).

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, 1980 - September 30, 1981
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 successfully substituted for casein in small research tests. It has also been tested under mass rearing conditions with no adverse effects on the growth and development of the P_1 generation. (Tanner and Weeks 1980). However, before Supro-610 is substituted for casein in the B-4 diet, the growth and development of the F_1 generation should be examined.

Methods and Materials:

P_1 Generation:

Two 60 liter batches of B-4 diet 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 8 minutes and visually checked for consistency. Mixing was continued, if necessary, until the diet ingredients were completely mixed. 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 teatment/replicatiton.

The insects were allowed to develop to the pupal stage at 25.5°C, 50% RH, and a 15L:9D photoperiod. The pupae were held at for 25.0°C, 50% RH, and a 15L:9D photoperiod until adult emergence occurred. Both growth chambers were known to have a 2-3° C temperature difference between the upper and lower level during the summer rearing program. Larval and pupal developmental rates varied significantly between the upper and lower levels. To minimize this effect, each rack was divided into three levels (upper, middle and lower). Each level contained 8 trays (240 cups). Complete developmental and reproductive data were taken from insects at each level. Approximately 25 egg masses were obtained from each level/treatment replication, and embryonated 28 days. The egg masses were chilled 180 days at 6-8°C.

F_1 Generation:

Developmental data were taken on F_1 progeny of insects reared on diet containing Supro-610 or casein. One half of the F_1 neonates from each treatment were reared on diet containing Supro-610 as the protein source. The remaining F_1 neonates were reared on diet containing casein. This resulted in the following four protein source combinations:

Protein Source P_1 Generation	Protein Source F_1 Generation
Supro-610	Supro-610
Supro-610	Casein
Casein	Supro-610
Casein	Casein (Control)

The F_1 neonates were also separated by the rearing cart levels the parent population was reared on. The F_1 neonates from each P_1 rearing cart level were likewise divided and reared on the top, middle or bottom portion of the rearing cart. This resulted in the following rearing rack combinations for each protein source combination:

Rearing Rack Level P_1 Generation	Rearing Rack Level F_1 Generation
Top	Top
Top	Middle
Top	Bottom
Middle	Top
Middle	Middle
Middle	Bottom
Bottom	Top
Bottom	Middle
Bottom	Bottom

These rearing cart level combinations were established in spite of the fact that the rearing cart level had no effect on the development and reproduction of the P_1 parents (Tanner and Weeks 1980). The P_1 parents were reared during the fall and winter months. Production is at its lowest level during these months and the rearing rooms were not filled to capacity at this time. This allowed a freer movement of air in each rearing room, and resulted in less of a temperature gradient between the top and bottom portion of the rearing cart. In contrast, the F_1 generation was scheduled to be reared during the late spring - early summer peak production period. In previous years, a temperature gradient of 2-3°C was observed between the upper and lower portion of a rearing cart. Insect development also varied between the upper and lower section. If this situation does occur during the rearing of the F_1 generation, the above rearing rack level combination will enable us to partition this effect out of the satistical analysis.

The development of 40 neonates (4 cups with 10 neonates/cup) were followed for each protein source - rearing rack location combination. A total of 360 neonates were used for each protein source combination/replication. Three replication were conducted, each commencing at weekly intervals. All three replications were conducted in the growth chamber which orginally held the P_1 parent pupae (25.0°C, 50% RH, 15L:9D).

The developmental and reproductive data taken for the parental generation and the F_1 generation consisted of the following measurements. Each measurement is preceded by the letters P, F or P-F which indicates if the measurement was made in the parental (P), the $F1(F)_1$, or in both generations (P-F).

Generation measurement taken	Measurement
1) P-F	Percent establishment of nonates on diet.
2) P-F	The mean larval stage on the 7, 14 and 21 post infest date (PID).
3) P-F	The mean male and female pupal DT_{50}.
4) F	The mean male and female pupation curve.
5) P-F	The mean male and female pupal weights on the 35th PID.
6) P-F	The percent male and female pupal deformity.
7) P-F	Percent survival of neonate to pupae.
8) P-F	The mean male and female adult DT_{50}.
9) P-F	The percent male and female adult deformity.
10) P-F	The percent male and female adult recovery.
11) P-F	The percent survival of neonate to adult.
12) P-F	The sex ratio.
13) P	The percentage of mating pairs.
14) P	The mean number of eggs laid/female.
15) P	The percent hatch of eggs chilled 120, 150 and 180 days.

Results:

The developmental and reproductive data have been collected for both the P_1 parent and F_1 generations. The data for the P_1 generation was presented in the 1979-1980 laboratory report (Tanner and Weeks 1980). The data for the F_1 generation are currently being analyzed. The final results observed for both the P_1 and F_1 generation will be presented in the next laboratory report.

References cited

Bell, R. A., C. Owens, M. Shapiro and J.G.R. Tardif. 1980. Development of Mass Rearing Technology, Chapter 6.5 in C.C. Doane, Ed. The Gypsy Moth: Research Towards Integrated Pest Management, U.S. Dept. of Agri. Tech. Bull. 1584 (In Press).

Tanner, J. A. and B. P. Weeks. 1980. Substituting Supro-610 for casein in the Gypsy Moth Diet (B-4) under mass rearing conditions, APHIS, Laboratory Report October 1, 1979 - September 30, 1980, 184-188.

Project Number: GM 1.3.1
Project Title: Evaluating the Development and Reproduction of Insects Produce in the Otis Methods Development Rearing Facility
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: John Allen Tanner and Bernadine P. Weeks

The purpose of this project is to measure the quality of insects 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.

Since the last report, a major modification has been made in the quality control techniques. Previously, development and reproductive data were collected daily and compared with standards developed from data collected in early 1979 (Tanner and Weeks 1980). A comparison, such as this, is vague. The only statement that can be made, is that the performance of the insects has improved, deteriorated, or remained the same as the performance of the insects in 1979. No accurate statement can be made as to the probable cause(s) for the changes in the performance of the production.

To help identify the agent(s) which cause a change in the development of the production insects, an internal standard (Chambers 1975, 1977; Huettel 1976) was incorporated into the quality control program. The internal standard is insects that are reared under the best conditions presently available in the rearing facility. Variables normally encountered in the production program are held as constant as possible when rearing the internal standard insects. This enables us to measure the effects of the variable on the production insects and to make changes in the rearing techniques when possible.

Insects reared solely for colony maintenance (colony insects) are currently serving as the internal standard. Colony insects are placed on diet, once a week, at weekly intervals. Their development is compared to production insects started on diet at the same time the colony insects are started. Both the colony and production insects are reared in the same controlled environmental room.

Colony insects are reared on diet made from dietary ingredients that are preweighed at the beginning of each rearing year (September 1) to ensure a complete years supply. The ingredients are stored in a freezer (-15°C) until needed. This ensures that each dietary ingredient is selected from one lot number. This reduces the variation in colony insect development that may be associated with differences in the quality of dietary ingredients obtained from different lot numbers.

Production insects are reared on diet made from dietary ingredients that are weighed out on a day to day basis. During the course of a rearing year, each dietary ingredient may come from several different lot numbers. This may result in increased variation in the development of production.

Colony insects are reared at 8 neonates/diet cup with 30 diet cups on each rearing tray. Each rearing tray is placed in a single layer in each rearing truck section. Production insects are started 10 or 12 neonates/diet cup with 30 diet cups being placed on each rearing tray. Three rearing trays are stacked in each rearing truck section.

Developmental data and, in the case of the colony insects, reproductive data, are collected with the modified Tanner - Houle procedure (Tanner and Buck 1979a and 1979b, Tanner and Weeks 1980). Data are collected on the colony insects, the production insects started 12/cup, and production insects started 8 neonates/cup. This last group is a special group of production insects which enables us to observe both the effects of density and diet on the development of the gypsy moth. Table 1 compares the developmental data of colony insects started 8 neonates/cup and production insects started 8 or 12 neonates/cup. Table 2 lists the major developmental differences observed.

Table 1. Developmental data of colony insects (8 neonates/cup) and production insects reared at the 8 or 12 neonates/cup densites.

Parameter	Number of Neonates/cup		
	Colony	Production	
	8	8	12
Mean Larval Stage			
D-7	2.12 ± .19 b	2.06 ± .16 b	2.16 ± .22 a
D-14	3.90 ± .15 a	3.91 ± .17 a	3.95 ± .17 a
D-21	5.44 ± .11 a	5.45 ± .18 a	5.44 ± .12 a
Pupal Weights (gms)			
Males	0.73 ± .04 b	0.75 ± .04 a	0.65 ± .04 c
Females	2.19 ± .14 b	2.34 ± .16 a	1.93 ± .18 c
Pupal DT_{50}			
Male	26.3 ± .8 a	26.4 ± 1.2 a	26.3 ± 1.2 a
Female	28.3 ± 1.0 a	28.7 ± 1.2 a	28.6 ± 1.4 a
% Pupal Deformity			
Males	19.6 ± 7.5 a	17.7 ± 8.6 a	15.6 ± 7.5 a
Females	84.9 ± 9.4 a	87.2 ± 8.8 a	80.8 ±10.5 a
% Survival (Neonates to pupae)	88.1 ± 5.4 b	91.0 ± 7.9 a	74.4 ± 12.9 c
Adult DT_{50}			
Males	40.2 ± 1.1 a	40.2 ± 1.2 a	39.9 ± 1.4 a
Females	39.6 ± 1.1 a	39.7 ± 1.1 a	39.4 ± 1.3 a
% Adult Emergence			
Males	99.2 ± 1.7 a	99.0 ± 1.3 a	99.1 ± 1.5 a
Females	92.0 ± 6.5 b	92.8 ± 5.6 b	98.2 ± 2.6 b
% Adult Deformity			
Males	1.2 ± 1.5 a	1.0 ± 1.3 a	1.3 ± 2.6 a
Females	18.5 ± 7.8 a	19.6 ± 7.9 a	10.4 ± 6.5 b
M:F Sex Ratio	50:50 3.4 a	50:50 3.4 a	51:49 4.4 a
Neonate to Adult Survival			
Males	87.5 ± 6.9 a	86.0 ± 14.7 a	75.8 ± 15.0 b
Females	80.8 ± 6.7 b	84.7 ± 8.7 a	73.7 ± 13.6 c
% Contamination			
Bacteria	0 a	0 a	0 a
Fungus	0.06 0.2 a	0 a	0.33 1.4 a
Virus	0.15 0.3 a	0.18 0.6 a	0.28 1.0 a

1/ Means within a row not followed by the same letter are significantly different at the .05 level.

Table 2. Significant developmental differences observed between the New Jersey colony (8 neonates/cup) and the New Jersey production insects reared 8 or 12 neonates/cup.

The colony insect had:

1) A lower mean larval stage on the 7th PID than production insects reared 12/cup.

2) Lower male and female pupal weights than production insects reared 8/cup but higher weights than production insects reared 12/cup.

3) A lower percent survival to the pupal stage than production insects reared 8/cup but a higher percent survival than production insects reared 12/cup.

4) A lower percent adult emergence and a higher percent of deformed females than production insects reared 12/cup.

5) A lower percent survival, neonate to adult females, than production insects reared 8/cup but a higher percent survival than production insects reared 12/cup.

Colony and production insects started at the same 8 neonate/cup density had similar growth curves. For the first 7 days their growth rates were slower than the production insects started 12 neonates/cup. The growth rates were similar for all three groups after the initial 7 day period.

Colony pupae had lower mean weights than production insects started at the same 8 neonates/cup density, but higher mean pupal weights than production insects started at the 12 neonates/cup density. Figures 1 and 2 show the weekly trend in pupal weights during the 1980-81 rearing year. The low pupal weights obtained at the 12 neonate/cup density is consistent for the whole rearing year. This would be expected as higher neonate densities normally result in smaller pupae (Leonard 1968). Comparing the pupal weights of colony insects to the pupal weights of production insects started at the same neonate/cup density (8) shows no particular weekly trend (Figs. 1 + 2). Colony pupae tended to be heavier at times while production insects tended to be heavier at other times. The production insects tended to have periods where the mean pupal weights were extremely high but no probable cause can be identified at this time.

All three groups tend to have periods of high pupal weights and low pupal weights (Figs. 1 + 2). This phenomenon is more prevalent at the lower neonate/cup density.

Percent survival to the pupal stage and to the adult stage was lower for production insects started at 12 neonates/cup than insects from the other two groups. Colony insects had a lower percent survival to both stages than the production insects started at the same density. Adult female emergence was higher and adult female deformity lower with insects started at the higher neonate/cup density.

The sole function of the colony insects is to produce enough egg masses to provide for the next generation needs. These are the only insects that are allowed to reproduce in our rearing facility, thus, reproductive and eclosion data are only collected on the colony insects.

To determine if there has been a change in the availability of neonates in the following generation, the reproductive and eclosion data collected in 1981 were compared with similar data collected in late 1980 (Table 3). Two reproductive differences occurred between the 1980 and 1981 rearing year. There was a reduction in the percentage of mated females and a reduction in the number of eggs deposited/female during the 1981 rearing year. The eclosion data were similar for both years.

Table 3. Reproductive and egg eclosion data of colony insects reared in 1980 and 1981.

	1981	1980[1/]
% Mated Females	89.2 ± 7.7	95.4 ± 4.2
# Eggs deposited/female	1004 ± 98.5	1059 ± 80.5
% Hatch		
120 day chilled eggs	91.1 ± 5.8	92.0 ± 4.5
150 day chilled eggs	86.4 ± 23.3	-
180 day chilled eggs	89.6 ± 5.2	88.0 ± 5.5
Time to 50% Hatch		
120 day chilled eggs	9.2 ± 1.6	8.7 ± 1.0
150 day chilled eggs	4.4 ± 1.0	-
180 day chilled eggs	1.6 ± 0.5	1.4 ± 0.3

1/ Insects reared June 11, 1980 to August 29, 1980.

The reduction in the mean percent mating (Table 3), was due to the gradual decrease in the average weekly percent mating, during the course of the rearing year (Fig. 3). This period of declining average weekly percentage may be part of a cycle that varies between periods of high and low mating. A cycle is evident in Figure 4 which shows the average weekly number of eggs deposited/female The reduction in the mean number of eggs deposited/female (Table 3) was due to a period where low number of eggs were deposited/female. This period was actually a continuation of a low period that started at the end of the 1980 rearing year. The mean number of eggs deposited/female gradually increased during the middle portion of the 1981 rearing year, and eventually reached a level which corresponded to the higher number of eggs deposited/female during the 1980 rearing year.

Cycles of low and high measurements are also indicated in the pupal weights (Fig. 1 and 2) and in other quality control data (Tanner personnel observation). The reason for these cycles is presently unknown.

In 1980 and most of 1981 mating was accomplished by placing 25 pairs of adults into each mating container. One week after mating, the egg masses were harvested, and the percent mated and the number of eggs deposited/female determined.

In mid-1981 the mating technique was modified. Mating pairs were selected in their pupal stage. Twenty-five pairs of pupae were placed into each mating container. The adults were allowed to emerge and mate. The percent mated and the number of eggs deposited/female was determined one month after the pupae were placed into the mating container.

The reasons for making this change were to hasten the placing of mating pairs into the mating container and to reduce the amount of time personnel are exposed to gypsy moth hairs.

To determine if the new mating technique had any effect on the percent mating or the number of eggs laid/female, the 1981 data were compared according to the technique used to select mating pairs. The results are presented in Table 4.

Table 4. Percent mated females and the number of eggs deposited by as affected by the stage the designated mating pairs are in at time of selection.

	Stage	
	Pupae	Adult
% Mated females	88.0 ± 8.9	90.7 ± 7.3
No. of eggs deposited/female	1031.9 ± 107.8	972.6 ± 79.2

A slight reduction in the percent mating occurred when the mating pairs were selected in the pupal stage. Conversely, selecting the mating pairs in pupal stage resulted in considerably more eggs deposited/female.

Gypsy moth egg masses are normally refrigerated for 180 days before being incubated for use in colony maintenance and production. Most of the egg masses are refrigerated in an automated refrigerator (8.2°C to 4.9°C). In this refrigerator all the egg masses are mechanically rotated throughout the refrigerator to insure that they are all exposed to similar conditions.

A small portion of each weeks production of egg masses are stored in a standard walk-in refrigerator (5.7-3.8°C). These masses serve as a "back up" if something happens to the egg masses located in the automated refrigerator.

Quality control data are taken on egg mass samples selected from each refrigerator. These data enable us to predict the amount of hatch and hatch rate pattern that will be encountered in production and to identify times of poor hatch so that corrective actions can be taken.

Five egg mass samples are selected from both refrigators for each week of production. Hatch tests are conducted on each sample after 120, 150 and 180 days refrigeration. Percent hatch (based on embryonated eggs only) and HT_{50} (incubation time to 50% Hatch) are determined for each sample period. The results for egg masses produced by insects reared during the 1980-81 rearing year are presented in Tables 5 and 6.

Table 5. Percent hatch of gypsy moth eggs chilled (120, 150 and 180 days) in the automated refrigerator compared to similar eggs chilled in a walk-in refrigerator.

Chilling Period Days	Percent Hatch 3/ Automated 1/	Walk-in 2/
120	92.9 ± 5.0 a	90.9 ± 7.2 a
150	92.0 ± 3.7 a	87.3 ± 6.0 a
180	91.5 ± 4.6 a	69.9 ± 13.6 b

1/ Temperature range 8.2°C to 4.9°C

2/ Temperature range 5.7°C to 3.8°C

3/ Means within a row or a column not followed by same letter are significantly different at the .05 level (Newman Keuls multiple range test).

Table 6. Egg incubation time to 50% hatch (HT 50) of gypsy moth eggs chilled 120,150 and 180 days in the automated refrigerator compared to similar eggs chilled in the walk-in refrigerator.

Chilling Periods (Days)	HT 50 (days) 3/ Automated 1/	Walk-in 2/
120	9.6 ± 1.2 a	7.8 ± .7 b
150	4.5 ± 1.8 b	6.2 ± .9 a
180	1.6 ± 0.3 b	5.2 ± .4 a

1/ Temperature range 8.2°C to 4.9°C
2/ Temperature range 5.7°C to 3.8°C
3/ Means within a row not followed by the same letter are significantly different at the .05 level (Newman-Keuls multiple range test).

Chilling the eggs for 180 days did not effect the percent hatch of eggs stored in the automated refrigerator but did effect the hatch of eggs stored in the walk-in refrigerator (Table 5). Refrigerating eggs for 180 days, in the walk-in refrigerator, results in a drop in the percent hatch from 87.3 % to 69.9%.

Lengthening the chilling period reduced the HT_{50} for egg masses chilled in either refrigerator (Table 6). This is a typical response of gypsy moth eggs to chilling (Masaki 1956). Reduction in the HT_{50}'s was more pronounced with eggs stored in the automated refrigerator. After 120 days of chilling, the eggs stored in the walk-in refrigerator has a HT_{50} that was 1.8 days sooner than eggs stored in the automated refrigerator. After 150 days of chilling, the situation was reverse. Egg masses chilled in the automated refrigerator reached their HT_{50} 1.7 days sooner than egg masses chilled in the walk-in refrigerator. After 180 days of chilling, egg masses chilled in the automated refrigerator reached their HT_{50} 3.6 days sooner than the egg masses chilled in the walk-in refrigerator.

The 180 day chilling of gypsy moth eggs, in the walk-in refrigerator, is detrimental to the eggs. It is recommended that either the conditions within the walk-in refrigerator be change to coincide with the conditions found within the automated refrigerator, or that a new refrigerator be used that can be programmed to the conditions found in the automated refrigerator.

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Figure 1. Mean male pupal weights observed during the 1981 rearing year for colony insects reared at the 8 neonates/cup density and production insects reared at the 8 and 12 neonates/cup densities.

Figure 2. Mean female pupal weights observed during the 1981 rearing year for colony insects reared at the 8 neonates/cup density and production insects reared at the 8 and 12 neonates/cup densities.

Figure 3. The percentage of mated females (colony - 8 neonates/cup) observed weekly during the late 1980 and the 1981 rearing season.

Figure 4. The mean number of eggs deposited/female (colony - 8 neonates/cup) observed weekly during the late 1980 and the 1981 rearing years.

Figure 1. Mean male pupal weights observed during the 1981 rearing year for colony insects reared at the 8 neonates/cup density and production insects reared at the 8 and 12 neonates/cup densities.

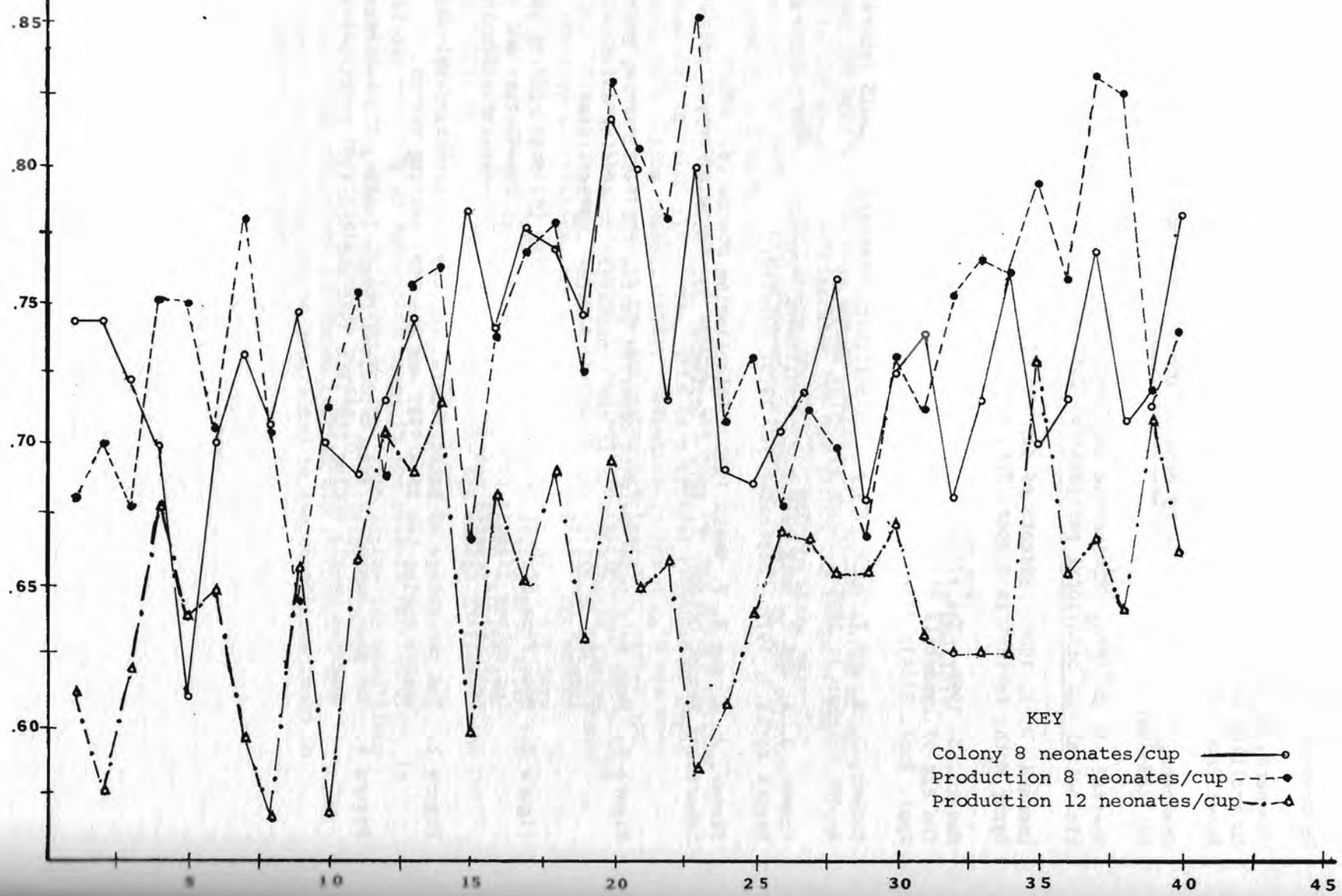


Figure 2. Mean female pupal weights observed during the rearing year for colony insects reared at the 8 neonates/cup density and production insects reared at the 8 and 12 neonates/cup densities.

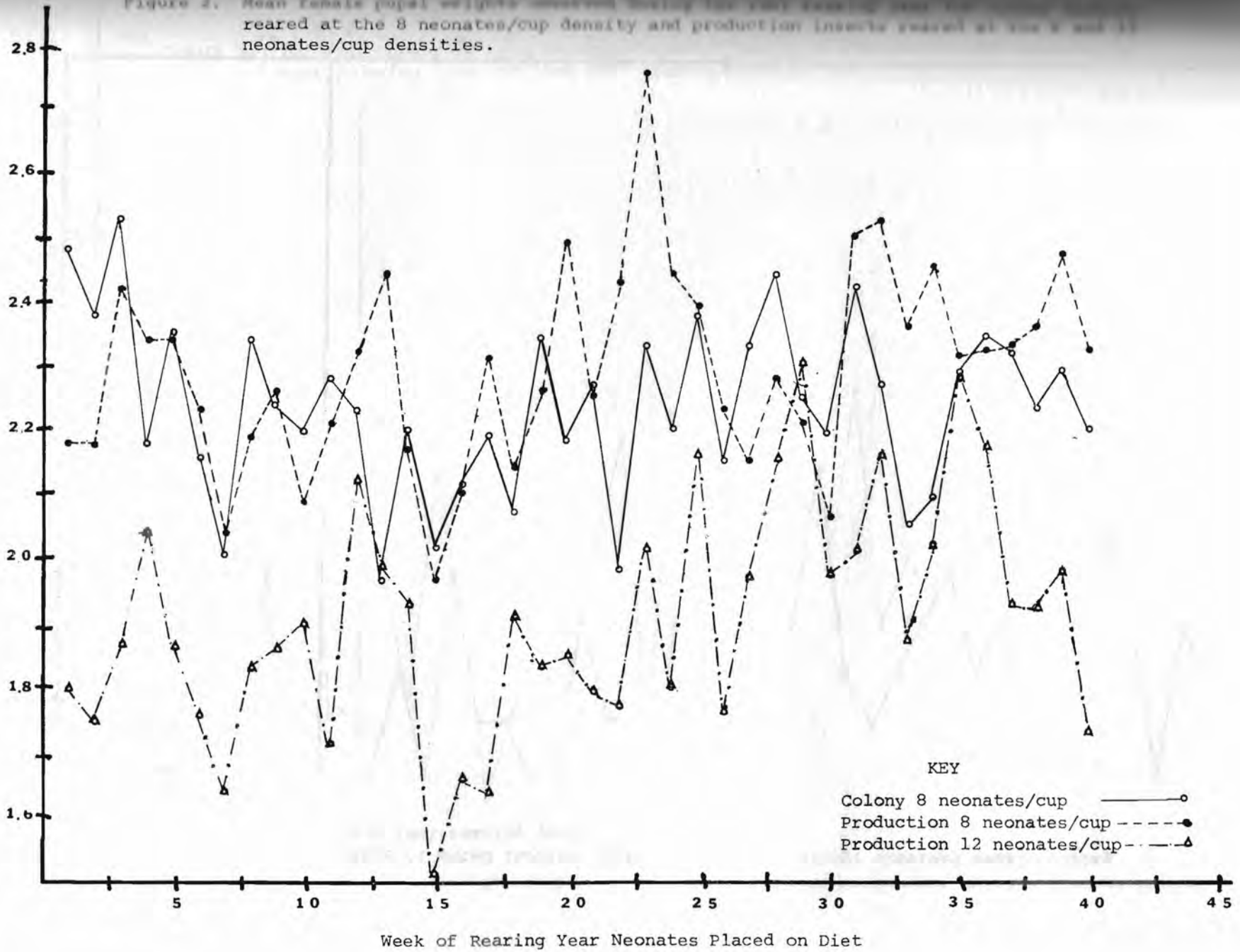


Figure 3. The percentage of mated females (colony - 8 neonates/cup) observed weekly during late 1980 and 1981 rearing years.

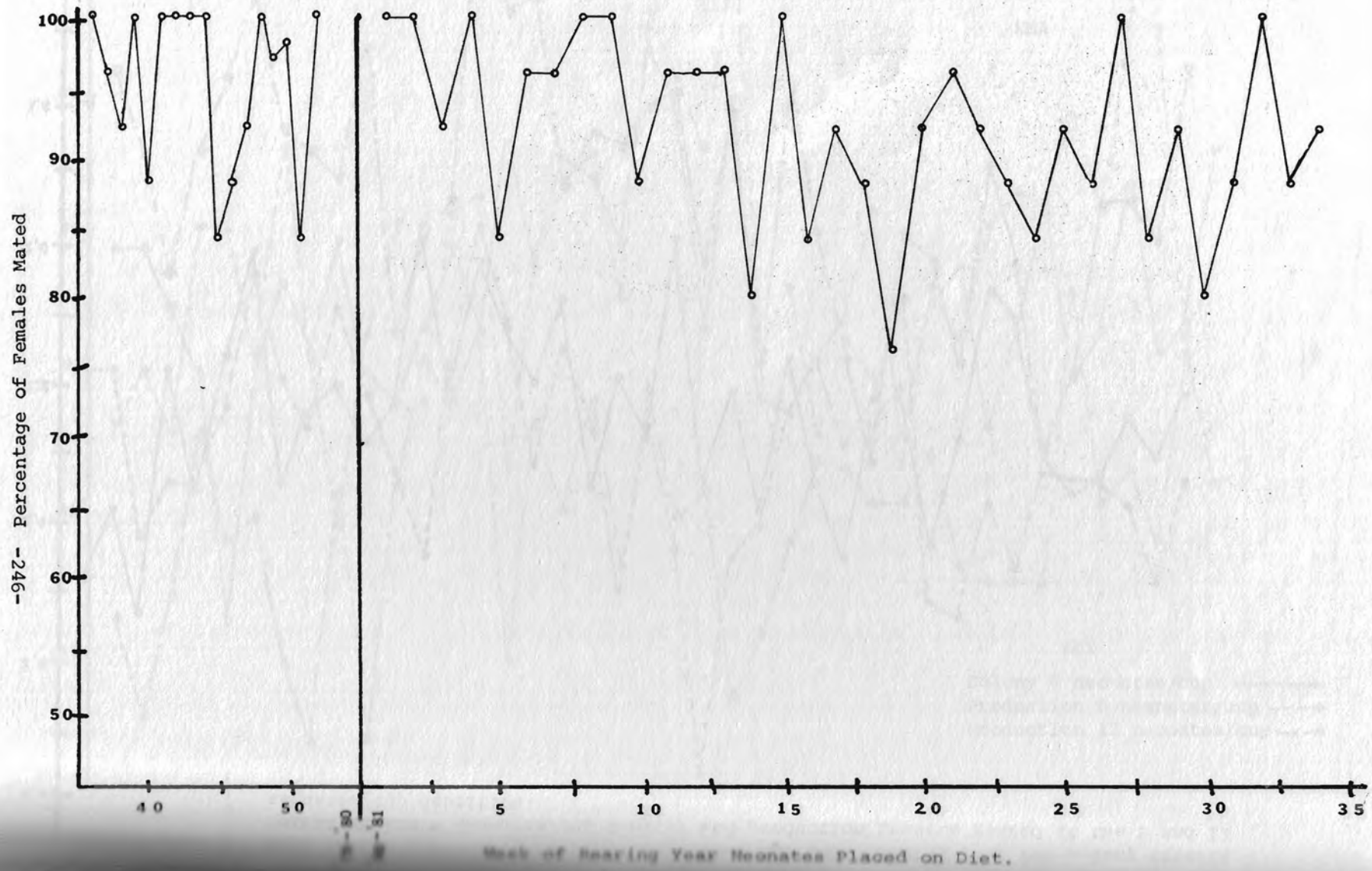
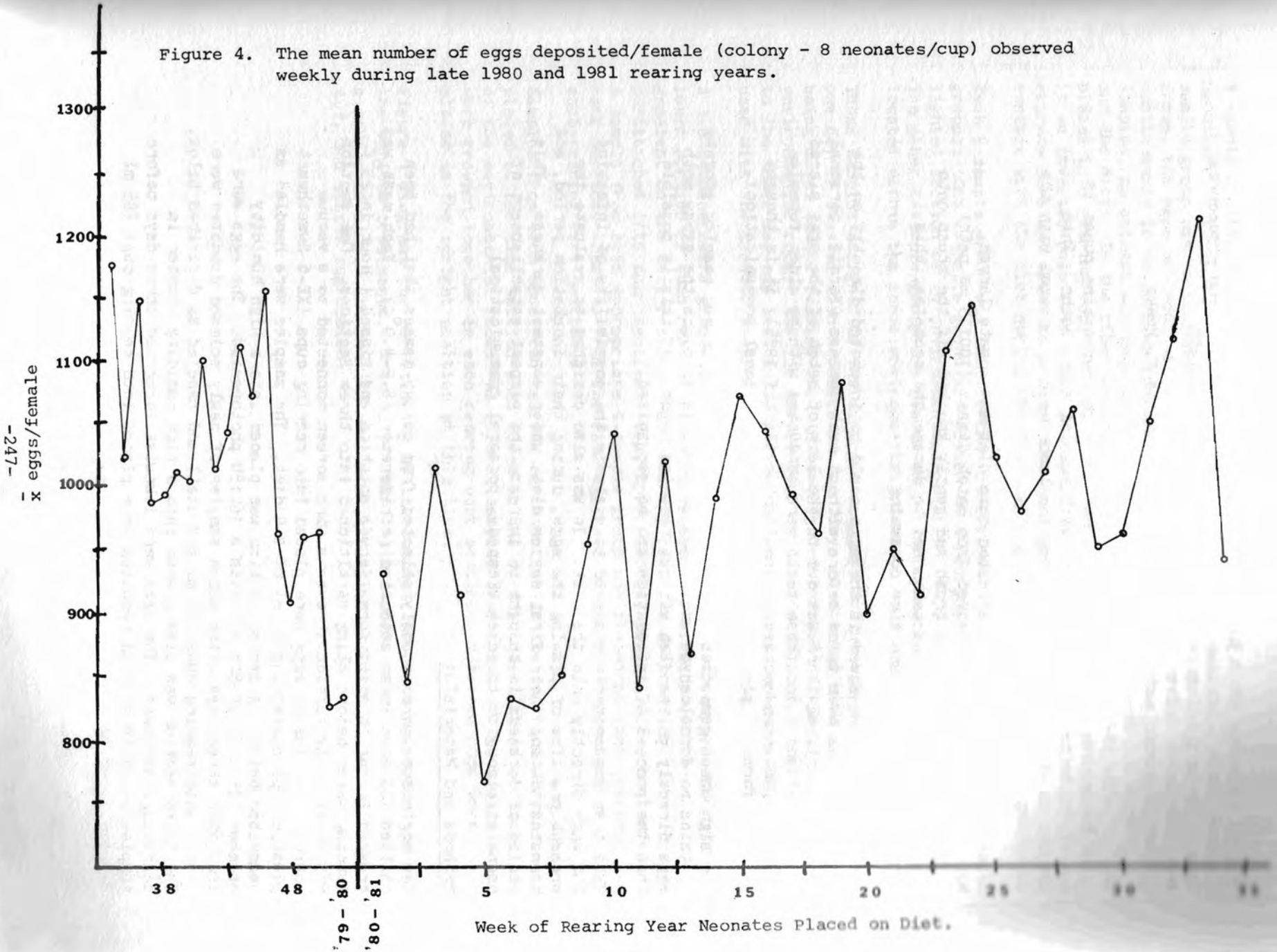


Figure 4. The mean number of eggs deposited/female (colony - 8 neonates/cup) observed weekly during late 1980 and 1981 rearing years.



Project Number: GM 1.3.2
Project Title: Development of Mechanical Egg Infestation Procedures
Report Period : October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: John Allen Tanner and Bernadine P. Weeks

Labor devoted to infesting insects onto diet and harvesting pupae is one of the major costs associated with rearing gypsy moths. This project is concerned with reducing the labor cost associated with the infesting of larvae onto diet.

Gypsy moths are presently infested onto diet as neonate larvae. Neonates are carefully removed from hatch dishes (100X15 mm petri dishes) with a camel hair brush and gently knocked off the brush onto diet. Since care must be taken not to damage the neonates, this operation is tedious and time consuming.

Much time could be saved if the eggs could be incubated directly on the diet. This has been tried before without much success (Tardif, personal communication). Failure was due to the lack of hatch of the eggs placed on the diet and since those tests were conducted with an older formulation of diet which contained formaldehyde (Tardif 1975), it is thought that the formaldehyde killed the eggs (Bell, personal communication).

The high wheat germ diet (B-4 diet) presently used in our rearing program contains no formaldehyde and it may be possible to place the gypsy moth eggs directly on the diet without reducing hatch. If this is possible, then the process of infestation can be mechanized.

This test was primarly designed to evaluate the possibility of infesting the eggs directly onto the diet. It was also designed to evaluate the present practice of placing the eggs, during their incubation period, and neonates, during their first day on diet, under constant darkness. This period of darkness is thought to increase the percent establishment of neonates placed on the diet (Kennedy, personal communication).

Methods and Materials:

Ten egg masses were randomly selected from colony masses that had been chilled 180 days in an automated refrigerator (8.2-4.9°C). Each mass was treated 1 hour in a 10% formaldehyde solution and rinsed 1 hour in 25°C running water before being partitioned into three sections. One section was dehaired by rubbing it over a wire screen connected to a vacuum source. Samples of eggs were placed into rearing cups (XE-6 Sweetheart plastic cup) containing 85 ml of B-4 diet. The samples were handled as described below. A second section was placed into a high humidity chamber (25 1°C, 70-80% RH) with a 16L:8D photoperiod. The eggs were incubated three days after which samples of newly eclosed neonates were placed into rearing cups (85 ml B-4 diet) and handled as described below. The third section was also placed into a high humidity chamber in continuous darkness. The eggs were likewise incubated three days before samples of newly eclosed neonates were placed into rearing cups (85 ml B-4 diet) and handled as described below.

A sample consisted of either 10 embryonated eggs or 10 newly eclosed neonates. A total of 16 samples were drawn from each section. Each 16 sample group was partitioned into two 8 sample groups. In one 8 sample group, the eggs or neonates were placed into rearing cups that were held continuously in an upright postion (diet on bottom). In the case of egg samples, no attempt was made to prevent direct contact between the eggs and the diet. In the other 8-sample group, the eggs or neonates were placed into rearing cups and held one day (neonates) or four days (eggs) in an inverted postition (diet on top). In the case of egg samples, extreme care was taken to prevent the eggs from coming into direct contact with the diet during the four day egg incubation period.

Each 8 sample group was then further partitioned into two 4-sample groups. One 4-sample group was placed on a rearing rack located in the lighted section of an environmental room (25 1°C, 50-60% RH, 16L:8D). The other 4-sample group was placed on a rearing rack in a darkroom located within the above environmental room.

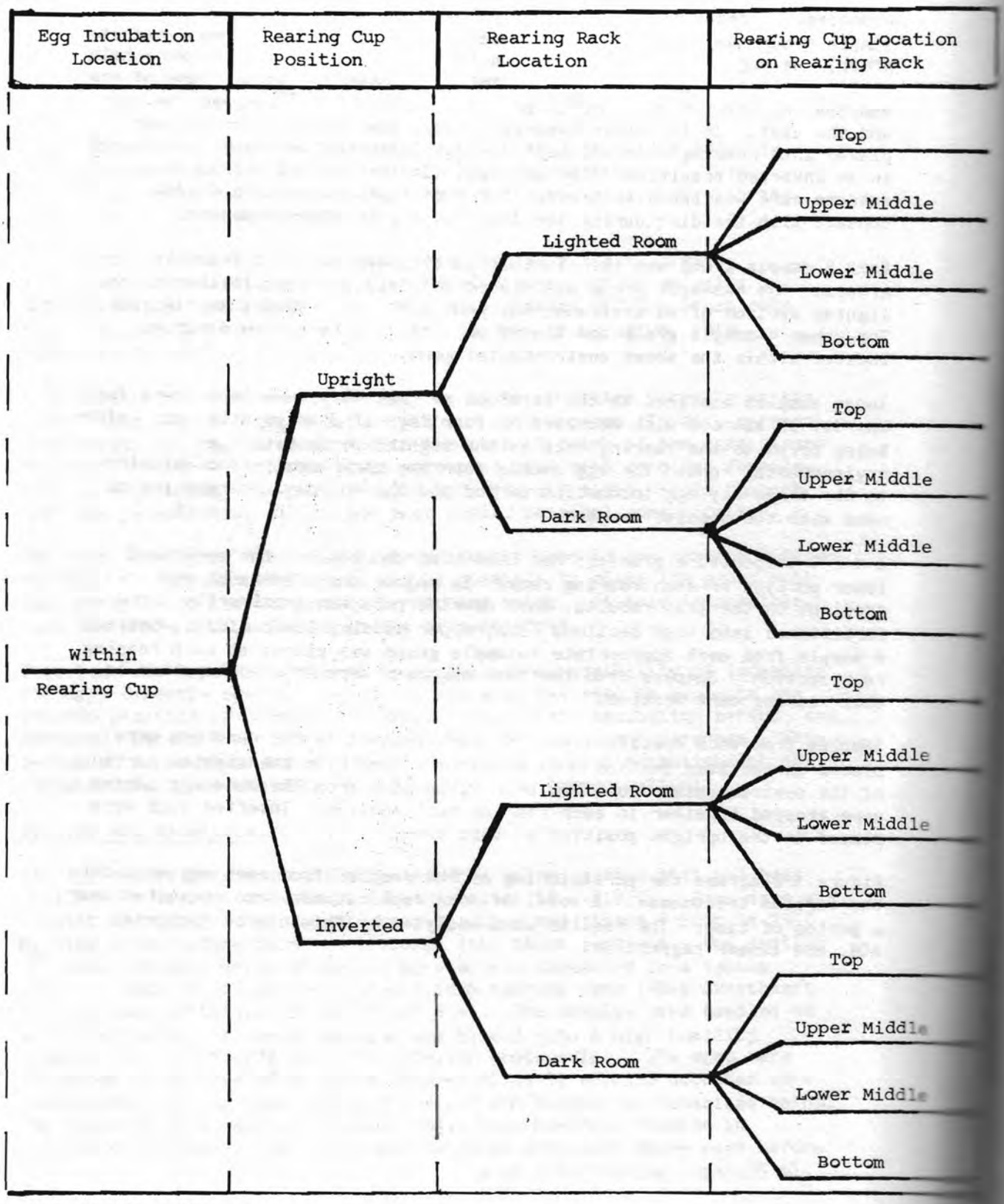
Those samples assigned to the darkroom rearing rack were kept there for one day if started with neonates or four days if started with eggs before being moved to the rearing rack in the lighted portion of the environmental room. The egg sample four day darkrooom period coincided to the three day egg incubation period and the one day darkroom period used with the neonate samples.

A 1-2°C temperature gradient had been observed between the upper and lower portion of each rearing rack. To reduce the effects of the gradient on the test results, each rearing rack was arbitrarily partitioned into four sections (top, upper middle, lower middle, bottom). A sample from each appropriate 4-sample group was placed at each rearing rack section. Samples from the same egg mass were grouped together in each rearing cart section.

Samples from each specified rearing cart section in the darkroom were placed in the same section when moved to the cart in the lighted portion of the environmental room. As before, samples from the same egg masses were grouped together in each rearing cart section. Inverted cups were placed in the upright position at this time.

Figure 1 diagrams the partitioning of the samples from each egg mass into the various treatments. A total of four replications were conducted over a period of time. The results were analyzed by Chi-square, factorial AOV, and linear regression.

Figure 1. Flow diagram of the treatments to which eggs were exposed.



High Humidity Chamber
16L:8D Photo Period

High Humidity Chamber
Continuous Darkness

The flow diagrams for these egg treatments were the same as above.

Results:

Table 1 compares the effect of egg-diet contact on the percent hatch of the eggs. There was a significant reduction in hatch when eggs came into direct contact with the diet. However, the reduction was only 6.1% and under mass rearing conditions, this would be acceptable.

Table 1. The effect of egg-diet contact on the percentage of eggs that hatched.

	Egg-Diet 1/ 2/		
	Contact	Noncontact	Difference
Total Eggs Tested	3183	3165	18
Percent Hatch	75.9 ± 3.3 b	82.0 ± 3.6 a	6.1

1/ Means are significantly different at the .05 level.

2/ ± S.D.

Paired-sample tests were conducted for each egg mass to determine if the above differences in hatch occurred uniformly in all the egg masses. The results are presented in Table 2. Of the 40 egg masses used in this test, 33 or 82.5% showed no difference in hatch when eggs were in contact with the diet compared to eggs not in contact with the diet. One egg mass (2.5%) had greater hatch when the eggs were in contact with the diet. Six egg masses (15%) had reduced hatch when the eggs were in contact with the diet. The number of egg masses containing eggs affected by diet contact was small and it may be possible to reduce this number further by selection.

Table 2. The number of egg masses containing eggs effected by diet contact.

	Diet contact			
	Increase Hatch	Decrease Hatch	No Change	Total
No. of egg masses	1	6	33	40
% of egg masses	2.5	15.0	82.5	100

1/ Paired sample T-Test (.05 level).

The hatch of the gypsy moth eggs was not influenced by the absence or presence of light during the first four days of the test (Table 3) nor was hatch influenced by the location of the cups on each rearing rack (Table 4).

Table 3. The hatch of gypsy moth eggs as influenced by the presence or absence of light.

	Treatment 1/ 2/		
	Light	Dark	Total
No. of eggs	3180	3168	6348
Percent hatch	78.1 ± 11.2	79.2 ± 11.1	

1/ Means were not significantly different at the .05 level.
2/ ± S. D.

Table 4. Percent hatch of eggs as affected by the location of the rearing cup on the rearing rack.

	Cup location 1/ 2/				
	Top	Upper Middle	Lower Middle	Bottom	Total
No. of eggs	1587	1591	1586	1584	6384
% Hatch	80.2 ± 11.6	79.0 ± 11.9	78.2 ± 12.2	78. ± 11.8	

1/ Means were not significantly different at the .05 level.
2/ ± S.D.

Table 5 compares the percent establishment of neonates started from eggs incubated directly in the rearing cups with neonates placed into the rearing cups after hatching from eggs incubated in the high humidity chamber. Significantly fewer neonates established on the diet when the eggs were incubated in the rearing cups. However, the reduction was only 3.5% which would be insignificant under mass rearing condition.

Table 5. Percent establishment and percent survival of neonates hatching from eggs incubated within the rearing cups as compared to neonates placed into the rearing cups after they had hatched from eggs incubated in the high humidity chambers.

	Egg Incubation Location 1/ 2/	
	Rearing Cup	High Humidity Chamber
Percent establishment	93.2 ± 14.2b	96.7 ± 8.3a
Percent survival	94.6 ± 9.7a	94.9 ± 6.9a

1/ Means within a row not followed by the same letter are significantly different at the .05 level.

2/ ± S.D.

The absence or presence of light did not influence the establishment of neonates on diet (Table 6), nor did the cup position (Table 7) or its location on the rearing rack (Table 8). The percent survival of established neonates was similar for all treatments (Tables 5,6,7,and 8).

Table 6. Percent establishment and percent survival of neonates as affected by the presence or absence of light.

	Light 1/ 2/ 3/	
	Absent	Present
Percent establishment	95.4 ± 10.5	94.6 ± 12.8
Percent survival	94.9 ± 7.6	94.6 ± 9.2

1/ Means within a row are not significantly different at the .05 level.
2/ Light present or absent during the first four days of the test.
3/ ± S.D.

Table 7. Percent establishment and percent survival of neonates as affected by rearing cup position.

	Cup position 1/ 2/	
	Upright	Inverted
Percent establishment	95.4 ± 10.8	94.5 ± 12.6
Percent survival	94.4 ± 8.6	95.1 ± 8.2

1/ Means within a row were not significantly different at the .05 level.
2/ ± S. D.

Table 8. Percent establishment and percent survival of neonates as affected by the rearing cup location on the rearing rack.

	Cup Location 1/ 2/			
	Top	Upper Middle	Lower Middle	Bottom
% Establishment	94.6 ± 12.8	95.7 ± 9.8	94.8 ± 11.9	94.8 ± 12.1
% Survival	94.4 ± 8.5	94.1 ± 9.9	95.0 ± 7.5	95.5 ± 7.4

1/ Means within a row were not significantly different at the .05 level.
2/ ± S. D.

An important criterion for determining the acceptability of the egg infestation method for use in the sterile male program is the percentage of male larvae that pupate within four days of each other (Mastro, personal communication). This four day age class is critical when irradiating the pupae (Mastro 1980). A single harvesting of male pupae from each production date is desirable from the standpoint of minimizing labor costs. Currently, male pupae are harvested four days after 10% of the male larvae have pupated. This method normally results in recovery of at least 80% of the males and over 80% of the harvested male pupae are within the critical four day age class (Mastro 1980).

Table 9 shows the effects of the various treatments on the percentage of male pupae within the critical four day age class. There was a significant reduction in yield when reared by the egg infestation method compared to the standard neonate infestation method. The reduction was approximately 8 to 16%. None of the other treatments affected the percentage of male pupae within the critical four day age range (Table 9).

Table 9. The percentage of male pupae which completed pupation within four days after 10% male pupation had occurred as affected by the insect stage that was placed in the diet cups, and the light conditions and diet cup position during the first four days of the test.

Insect Stage Placed on Diet	Eggs				Neonates			
Light Condition During Egg Incubation	Light		Dark		Light		Dark	
Diet Cup Position During Incubation	Upright	Inverted	Upright	Inverted	Upright	Inverted	(Control) Upright	Inverted
Percentage 2/ 1/	$63.0 \pm 13.8_b$	$65.8 \pm 9.1_b$	$64.4 \pm 9.1_b$	$66.2 \pm 11.8_b$	$75.3 \pm 7.6_a$	$74.3 \pm 7.8_a$	$78.6 \pm 6.5_a$	$75.6 \pm 7.8_a$

1/ The percentage of male pupae which completed pupation within four days after 10% male pupation had occurred.

2/ Means within the row not followed by the same letter were significantly different at the .05 level.

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Project Number: AW 1.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasite Redistribution Project
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leaders: D. Simser, P. Kingsley

Introduction:

The valuable forage crop, alfalfa (Medicago sativa), is attacked annually by many species of phytophagous insects; actual or potential crop losses force growers to rely on insecticidal agents for control of these pests. Prophylactic treatments, or timed applications, of insecticides can effectively decrease or eliminate pest populations, and result in qualitative and/or quantitative yield increases. However, these costly materials provide only temporary control, have proven deleterious environmental effects, and can actually magnify the overall pest situation through selective resistance by target species, or elimination of non-target beneficial insects (predators and parasites). Ideally, suppression of insect pests would involve a control technology with no negative side effects, yet perpetually limit economic crop damage by the target pest species.

The alfalfa weevil (AW), Hypera postica (Gyllenhal), is perhaps the single most important alfalfa pest in the United States. These curculionids were first accidentally introduced into Utah (circa 1900), and again into Maryland (1945). Factors such as readily available food sources over wide areas, excellent dispersal prowess, high rate of fecundity, and lack of naturally occurring enemies have enabled this pest to become established in all contiguous states.

Feeding damage by adult and larval AW, and control costs of multiple insecticide applications, create large economic losses for growers annually.

In 1959, federal (USDA) entomologists initiated a classical biological control program against the AW, which would utilize introduced beneficial insects for suppression. Insofar as natural AW suppression by biotic and abiotic factors limited feeding damage in native countries, it was speculated that importation and establishment of AW enemies in the United States would provide similar suppressive measures, with the resultant advantages of a successful classical biological control program.

Foreign countries, where AW is indigeneous, were investigated, alfalfa producing regions were surveyed, and associated beneficial insects (predators and parasites) were discovered. Selected species were screened for possible hyperparasitic activity or other undesirable traits, then utilized in parasite release programs at several eastern sites where AW were abundant. Although many species from several families of hymenopterans were released, seven became established (other species may be established, but have not been confirmed): Microctonus aethiopoides Loan, M. colesi Drea (Braconidae); Bathyplectes curculionis (Thomson), B. anurus (Thomson), and B. stenostigma (Thomson) (Ichneumonidae); Tetrastichus incertus (Ratzeburg) (Eulophidae); and Patasson luna (Girault) (Mymaridae). All seven species are endoparasitic microhymenoptera, attacking certain life stages of the AW.

Establishment of these species in eastern states conicided with greatly reduced yearly AW feeding damage, reduction in the use of insecticides for control, and an estimated savings to growers of 8 million dollars annually. Currently, AW outbreaks in eastern regions are dampened by the controlling pressures of parasites. Most damage is limited, and of a temporary nature, unless insecticide treatments destroy any parasite buildup.

Damage by the AW increases from east to west, due in part, to a slow natural dispersal by the established parasites.

In 1979, federal agencies developed a cooperative, multi state, parasite redistribution program, intended to augment natural parasite dispersal (see 1981 and 1982 Biocontrol action program against the AW). Propagation and redistribution of parasites into western states should yield similar results as have occurred in eastern alfalfa producing states.

Documentation of this redistribution project is important to determine its success in controlling AW. Therefore, a concurrent evaluation study was inaugurated in 1981, designed to establish these objectives:

- determine relationships between AW densities, distributions, and parasite abundance and diversity
- obtain baseline and yearly data of AW densities and distribution prior to and during parasite release, and thereafter
- determine correlations between AW density vs. parasite diversity vs. % parasitism
- record, compare and evaluate changes in density of parasites vs. AW density over a 5 year period.

A portion of the first years results are presented herein. Additional information is detailed in the 1981 and 1982 AW Evaluation Program Work Manuals, and the 1981 AW Evaluation Project Review Session Report.

Materials and Methods:

Survey sites were selected on the basis of a 65 X 65 mi. grid located in four geographic areas (Figure 1). Each area was arbitrarily assigned a code designation I-IV (west to east). Within each site (6/area), five survey fields were selected, based upon these criteria:

- high grower tolerance to AW damage to minimize chemical control
- stand age between two and four year
- as near as possible to site epicenter, but no less then one mile apart
- standard regional cropping practice employed
- average field size for area, but no smaller then five acres.

A training session was conducted prior to sampling, where all sampling aspects, techniques, processing, AW identification, etc. were discussed to standarize handling of samples.

Because of fluctuations in temperature, etc. at the onset of the survey, the first and second samples were based on plant height (six inches to start). All samples thereafter were conducted at weekly intervals for a total of eight weeks.

Alfalfa weevil field densities were estimated weekly by identifying and counting all adults and larvae collected by the first 100 sweeps. Sweeping continued thereafter to collect an additional 100 adults and 300 larvae, when possible, for dissection and rearing. Personnel were required to record weekly data concerning weather conditions, plant phenology, sprayed materials and other variables to facilitate data analysis. Sampling was discontinued in fields where insecticides were applied.

Specimens were provided with ample foliage, then packaged and shipped to processing laboratories [1/]. A portion of each sample was frozen (up to 100 adults and larvae when possible), while the remaining weevils were provided alfalfa and reared for parasite emergence. Frozen samples were later dissected and parasitized hosts were noted. Identification of immature parasites was based upon morphological and phenological differences between species; cases of superparasitism were noted and recorded. A total of 960 samples were collected during the entire project.

Results and Discussion:

Alfalfa weevil density and parasitism data from two sites in area IV were not included in the final analysis. Sampling in site A was terminated after the second week due to a personnel problem, and site C produced too few weevils for adequate parasitism information.

In some cases the number of weevils shipped did not match the number received at the processing lab, due either to misidentification or miscounting. If the total number of sweeps taken to obtain the number shipped was given, the densities (number of weevils collected in the first 100 sweeps) were corrected.

The first and last sampling dates for each site are given in Table 1. In general, sampling began and ended a week earlier in the two western areas (I and II).

[1/] Samples from Mo, IA and IL were sent to: USDA, APHIS, PPQ; Biological Control Satellite Facility at Niles, Michigan; while those from OH, PA and NJ went to: USDA, SEA-AR; Beneficial Insects Research Laboratory at Newark, Delaware.

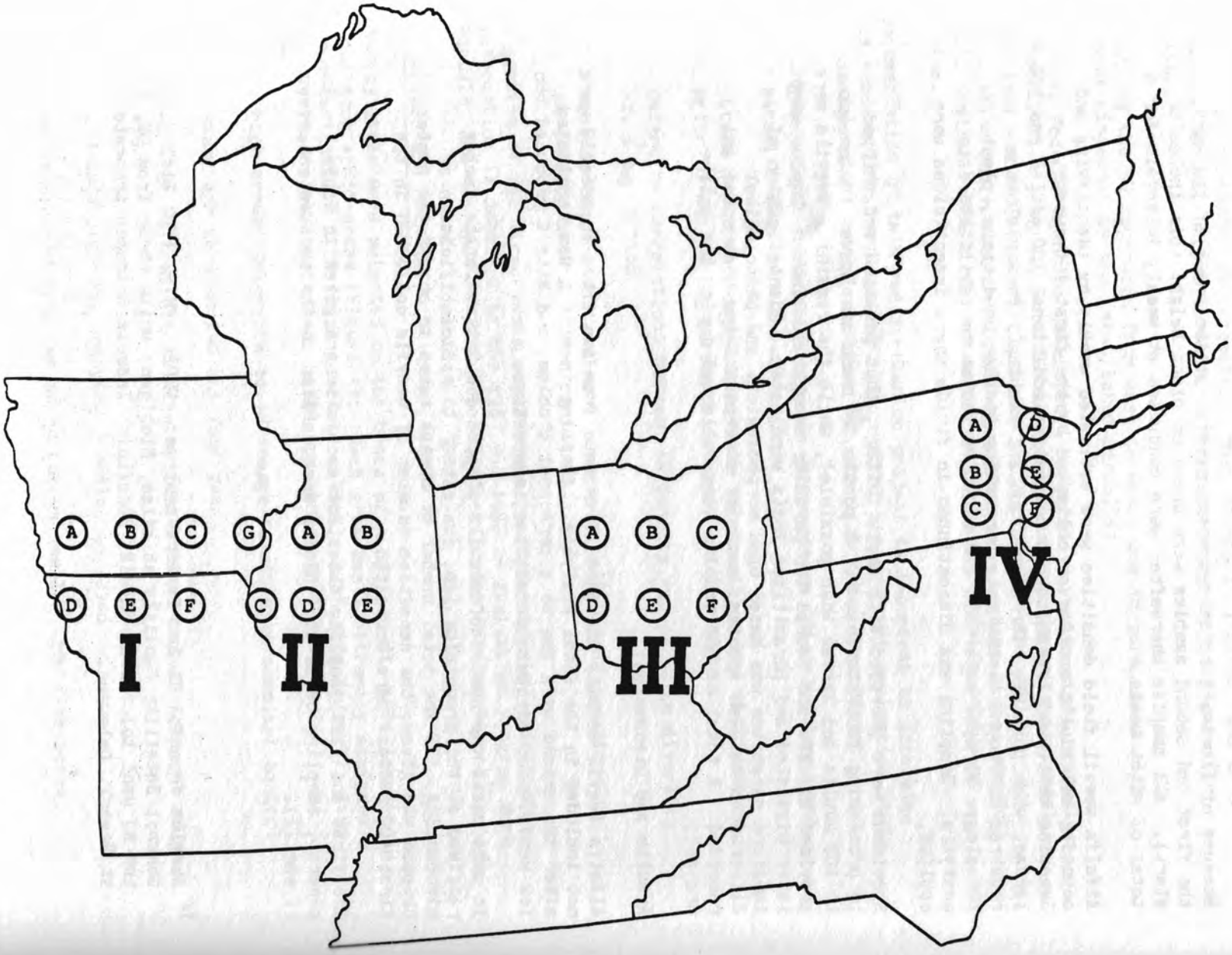


FIGURE 1. SERVICE SERVICE EVALUATION SURVEY SITES

Table 1. Initial and final sampling dates for all alfalfa weevil evaluation sites (1981).

Area	Site	Date started	Date completed
I			
MO - IA	A	4/13	6/9
	B	4/4	6/6
	C	4/5	6/8
	D	4/6	5/15
	E	4/9	5/27
	F	4/7	5/26
II			
IA - IL	G	4/4	6/4
	A	4/9	5/28
	B	4/14	5/28
	C	4/7	5/26
	D	4/10	5/27
	E	4/17	5/29
III			
OH	A	4/15	6/9
	B	4/14	6/9
	C	4/16	6/4
	D	4/17	6/11
	E	4/13	6/11
	F	4/15	6/2
IV			
NJ - PA	A[1/]	5/1	5/9
	B	4/30	6/16
	C	4/21	6/12
	D	4/27	6/17
	E	4/16	6/11
	F	4/13	6/4

1/ Sampling was terminated prematurely at this site.

Adult and larval AW densities over the eight week sampling period are summarized for each area in Figures 2 and 3. Parasitism by all identified species is compared with Area densities of adult and larval AW (Figures 4-7), where the number of hosts parasitized was calculated by multiplying the percentage of adult or larval parasitism (determined by dissection) times the appropriate AW density.

Of the five AW parasites recovered during the evaluation (Table 2), Microctonus colesi and Tetrastichus incertus were far less common then the two Bathyplectes species and M. aethiopoides. Microctonus colesi was found in 50% (15/30) of the area III fields but only accounted for 2.6% (26/1013) of the total parasitism (Figure 8). Only four T. incertus individuals were recovered (three from dissection and one by rearing), each from a different Area IV field (sites D, E and F).

Weevil densities were consistantly higher in the two western areas, even though the variation between individual fields was large. When peak larval densities from each field are considered, their averages are significantly higher in areas I and II than those in the two eastern areas (Table 3). During our eight week survey, the top density occurred in an Illinois (site E) field, where 6248 larvae were collected in 100 sweeps.

This east/west increase in AW numbers is consistant with a significant decrease in both parasite species diversity and overall AW parasitism (Table 3). Of the 120 fields in our evaluation, the number sprayed for weevil control also showed an east/west trend (Figure 9). In Area I 13 out of 30 fields were sprayed during the eight week period, while in the Area IV sites none were treated.

The initial objectives of the evaluation survey have been achieved through documentation of AW densities and parasite diversity prior to the redistribution of parasites into western areas. Succeeding evaluation surveys will demonstrate relationships between parasite establishment and AW densities. Control influences by recolonized parasites will be reflected by reductions in annual insecticide applications, and crop feeding damage in all study sites.

Additionally, modifications and improvements in sampling techniques and efficiency, as described in the 1982 AW Evaluation Manual, will serve to strengthen data collection procedures, but will not compromise earlier sample data collection and comparison.

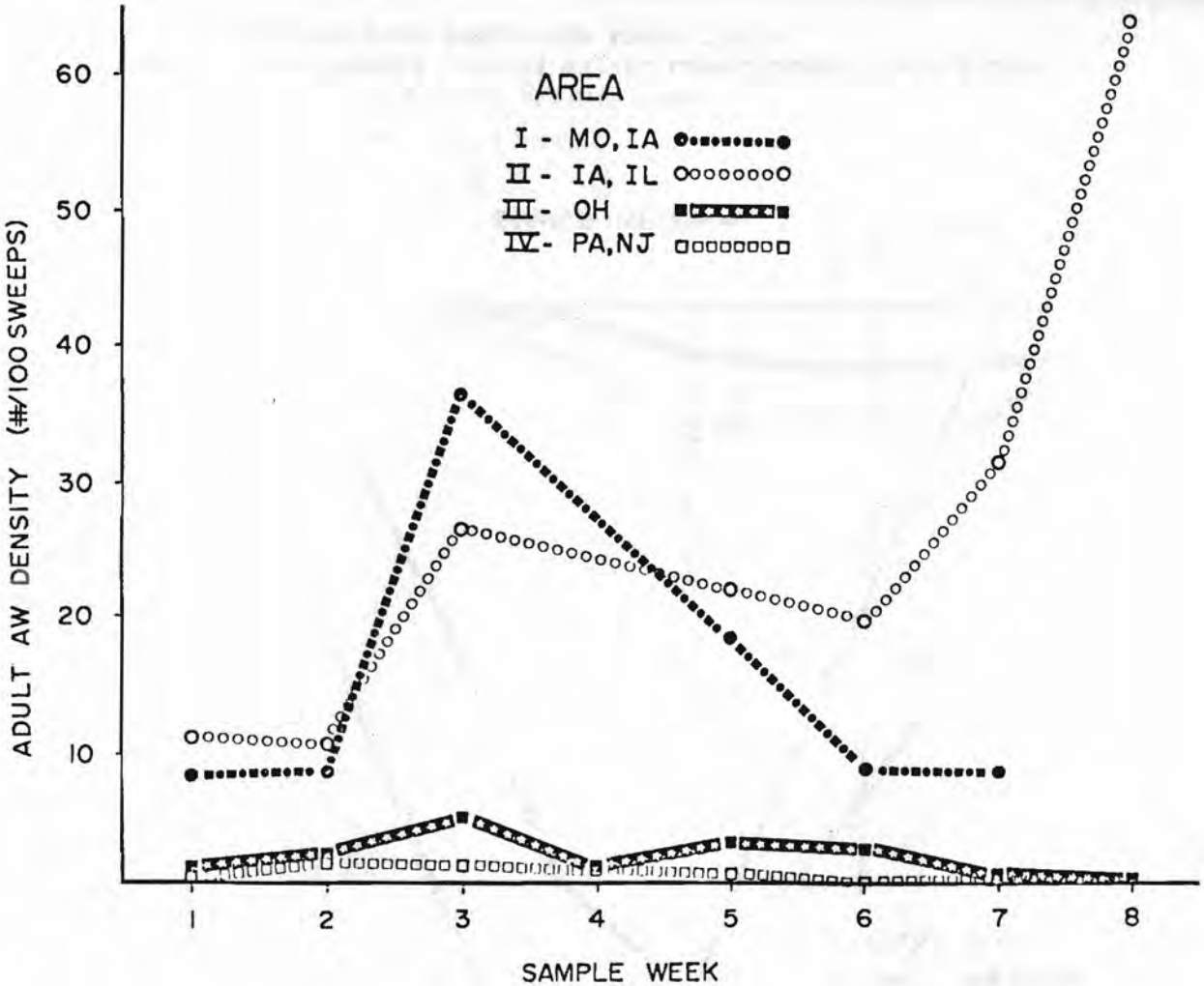


FIGURE 2. MEAN WEEKLY ALFALFA WEEVIL ADULT DENSITIES FOR EACH OF THE FOUR EVALUATION AREAS (1981).

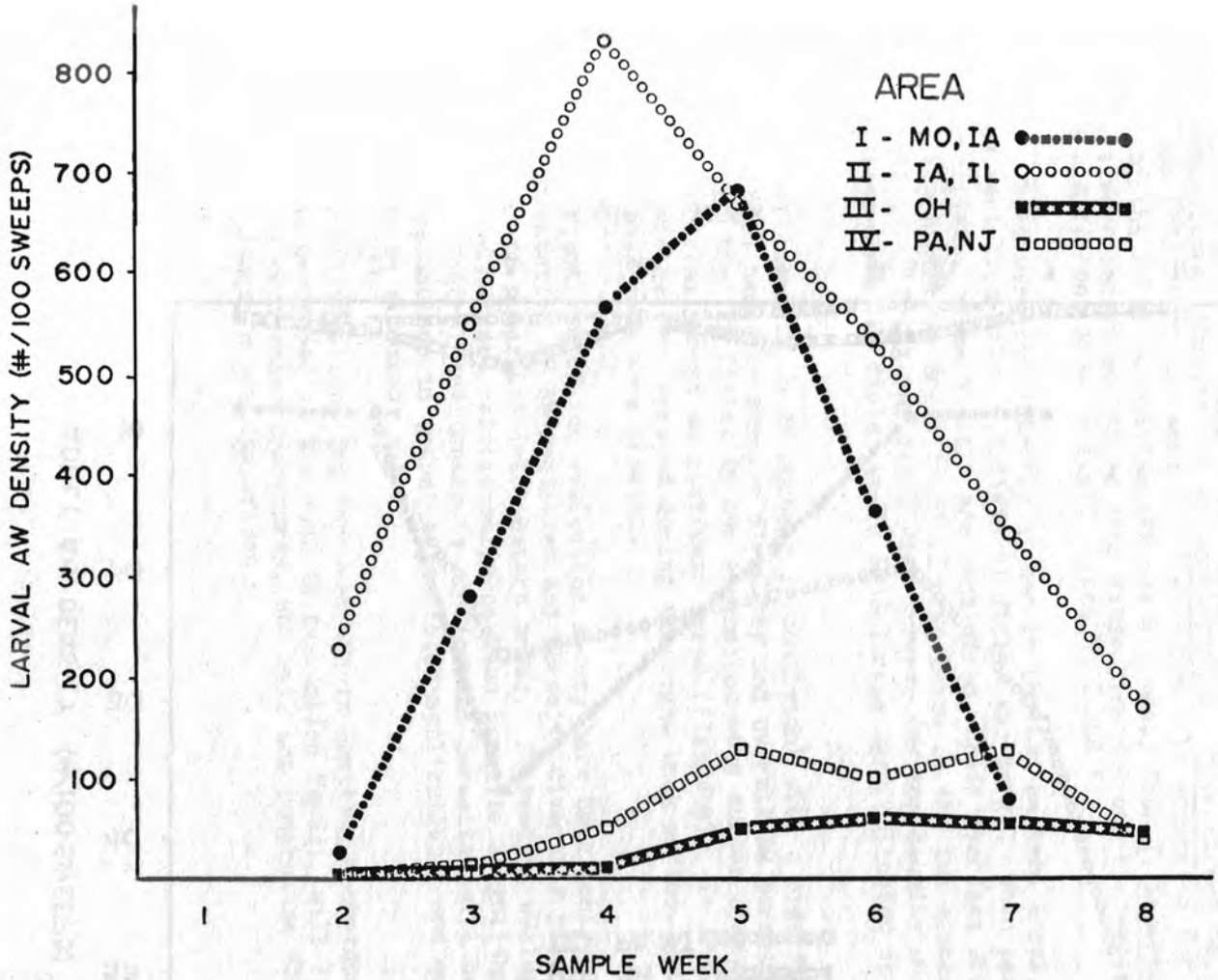


FIGURE 3. MEAN WEEKLY ALFALFA WEEVIL LARVAL DENSITIES FOR EACH OF THE FOUR EVALUATION AREAS (1981).

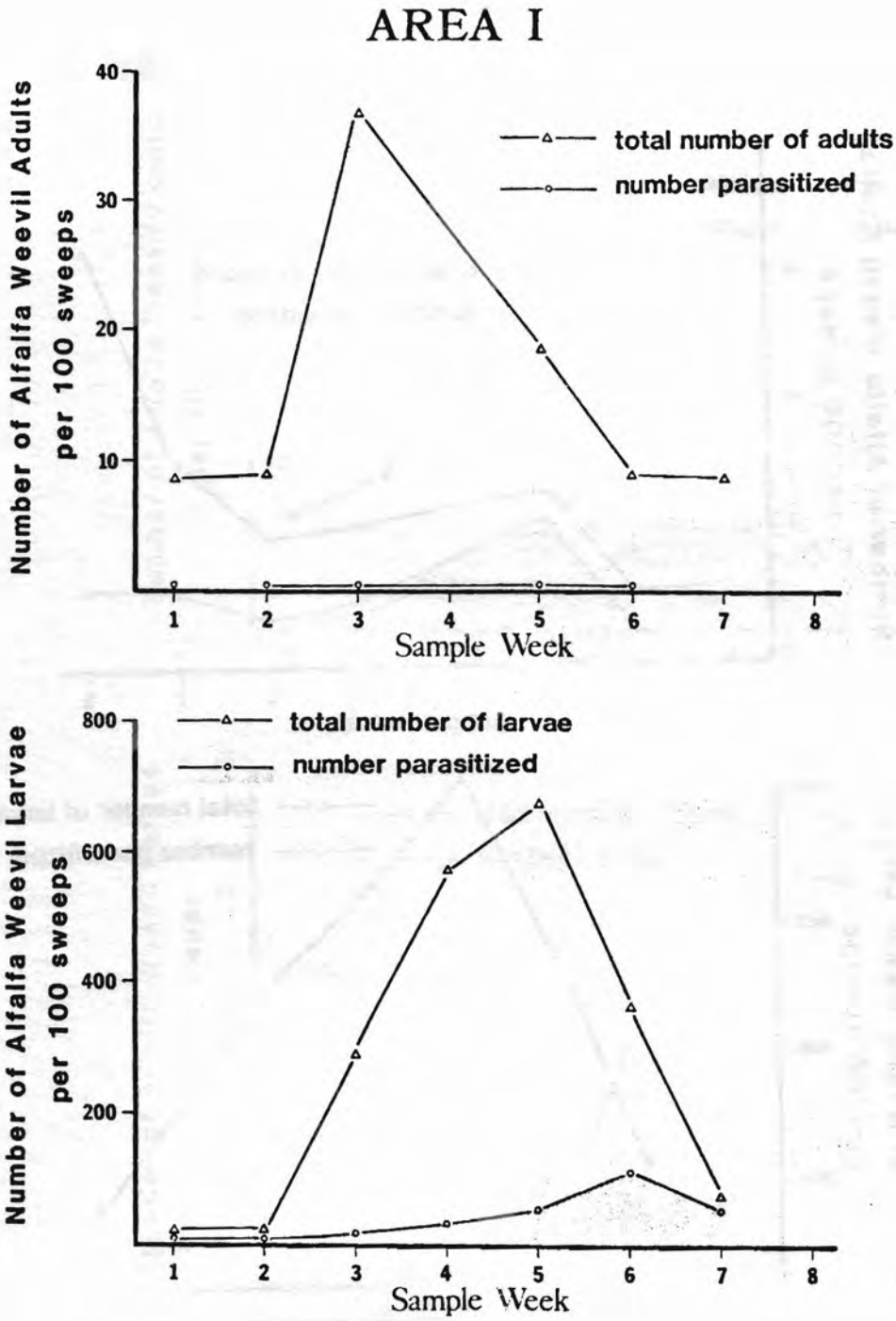


FIGURE 4. NUMBER OF HOSTS IN AREA I PARASITIZED DURING THE EIGHT WEEKS OF THE AW EVALUATION SURVEY (1981).

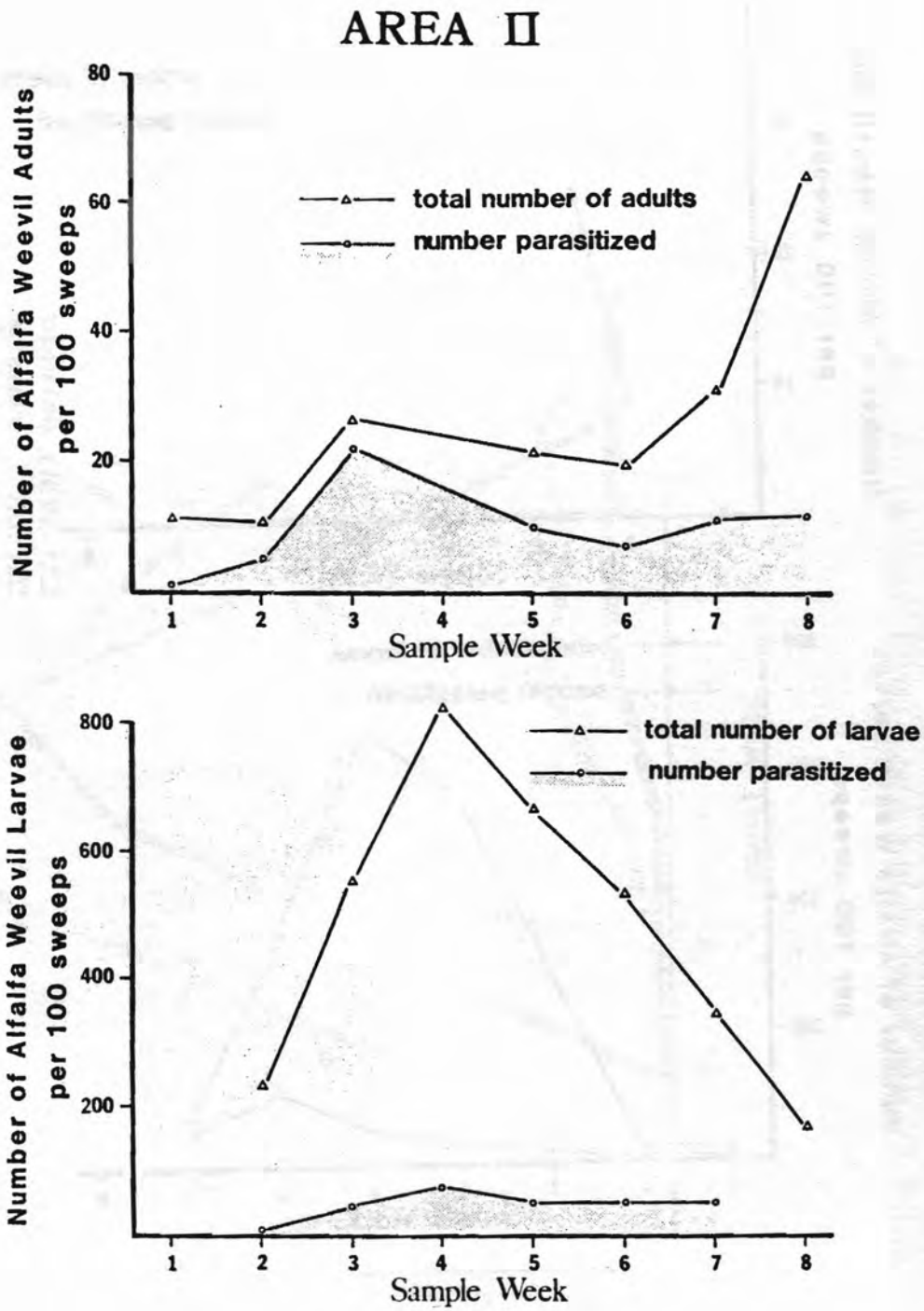
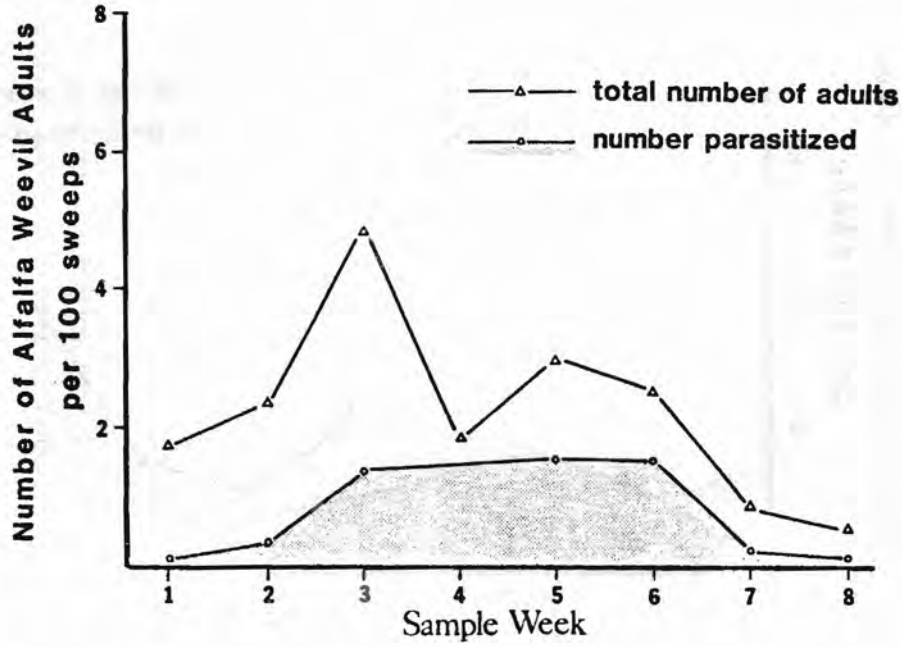


FIGURE 5. NUMBER OF HOSTS IN AREA II PARASITIZED.

AREA III



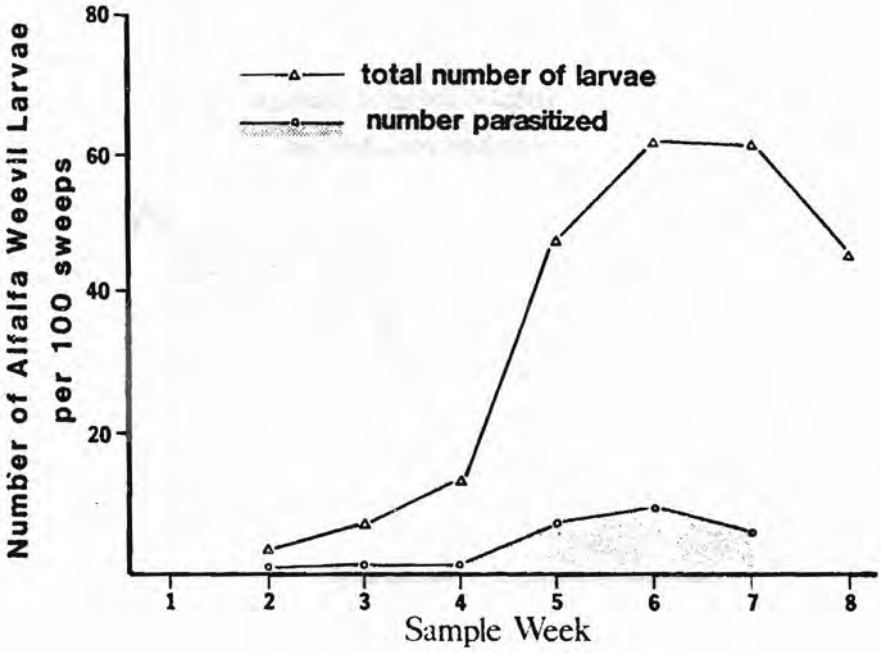
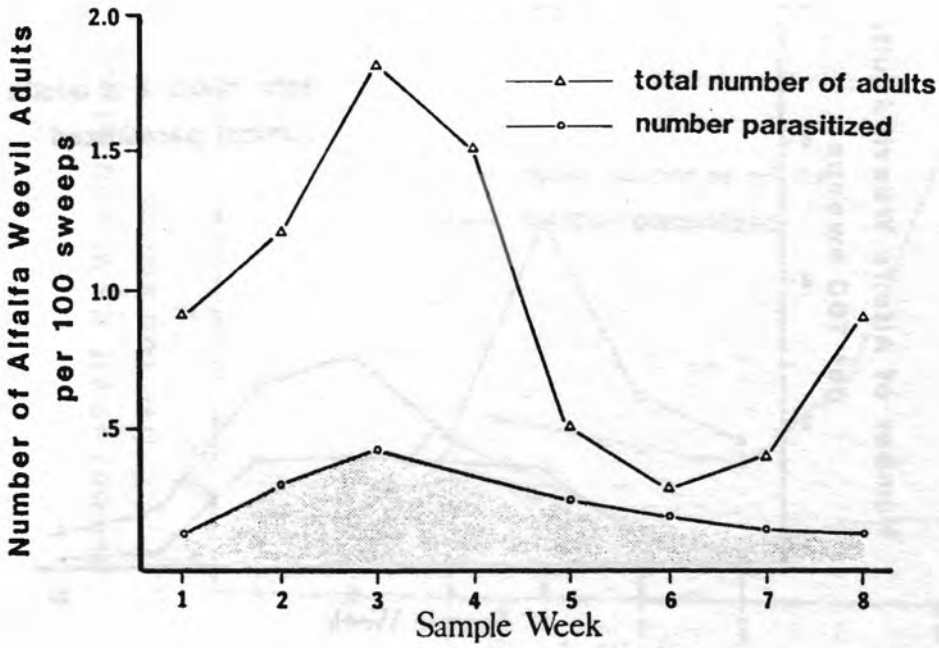


FIGURE 6. THE NUMBER OF HOSTS IN AREA III PARASITIZED.

AREA IV



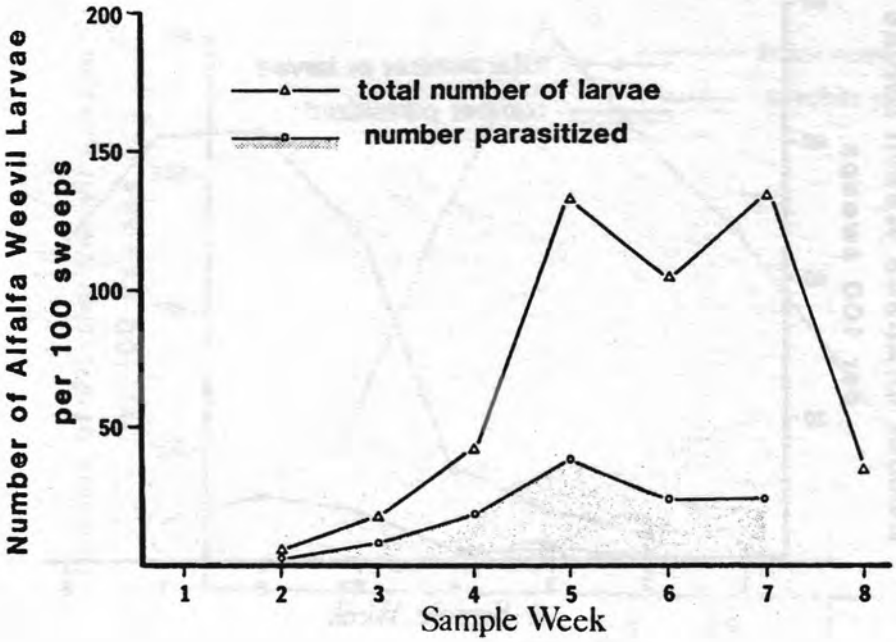


FIGURE 7. THE NUMBER OF HOSTS IN AREA IV PARASITIZED.

Table 2. Major Alfalfa weevil parasites recovered during the 1981 AW evaluation project.

Parasite Species	Family	Weevil Stage Attacked	Percentage of fields per area where parasite species occurred			
			I MO,IA	II IA,IL	III OH	IV PA,NJ
Bathyplectes anurus (Thomson)	Ichneumonidae	early instars	0	0	37	95
B. curculionis (Thomson)	"	early instars	100	80	83	85
Tetrastichus incertus (Ratzeburg)	Eulophidae	larvae	0	0	0	20
Microctonus colesi Drea	Braconidae	late instars	7	3	50	20
M. aethiopoides Loan	"	old adults	27	100	100	90

Table 3. Area summary for peak larval densities of the alfalfa weevil, overall percent parasitism (adults and larvae) and parasite species diversity.

Area	Peak larval density (#/100 sweeps)	(SE)	Overall parasitism	Number of parasite species	(SE)
			%		
I - MO,IA	815.5b[1]	(110.7)	9.4a[1]	1.67a[1]	(0.33)
II - IA,IL	1166.5b	(243.6)	19.9c	2.17a	(0.17)
III - OH	100.6a	(24.0)	18.3b	3.50b	(0.22)
IV - NJ,PA	196.2a	(38.2)	30.5d	4.50c	(0.29)

[1] Values followed by the same letter are not significantly different at the 95% level of confidence. Peak larval densities and the number of parasite species were tested with a Newman-Keuls multiple comparison test while parasitism frequencies were tested using a Chi-square analysis.

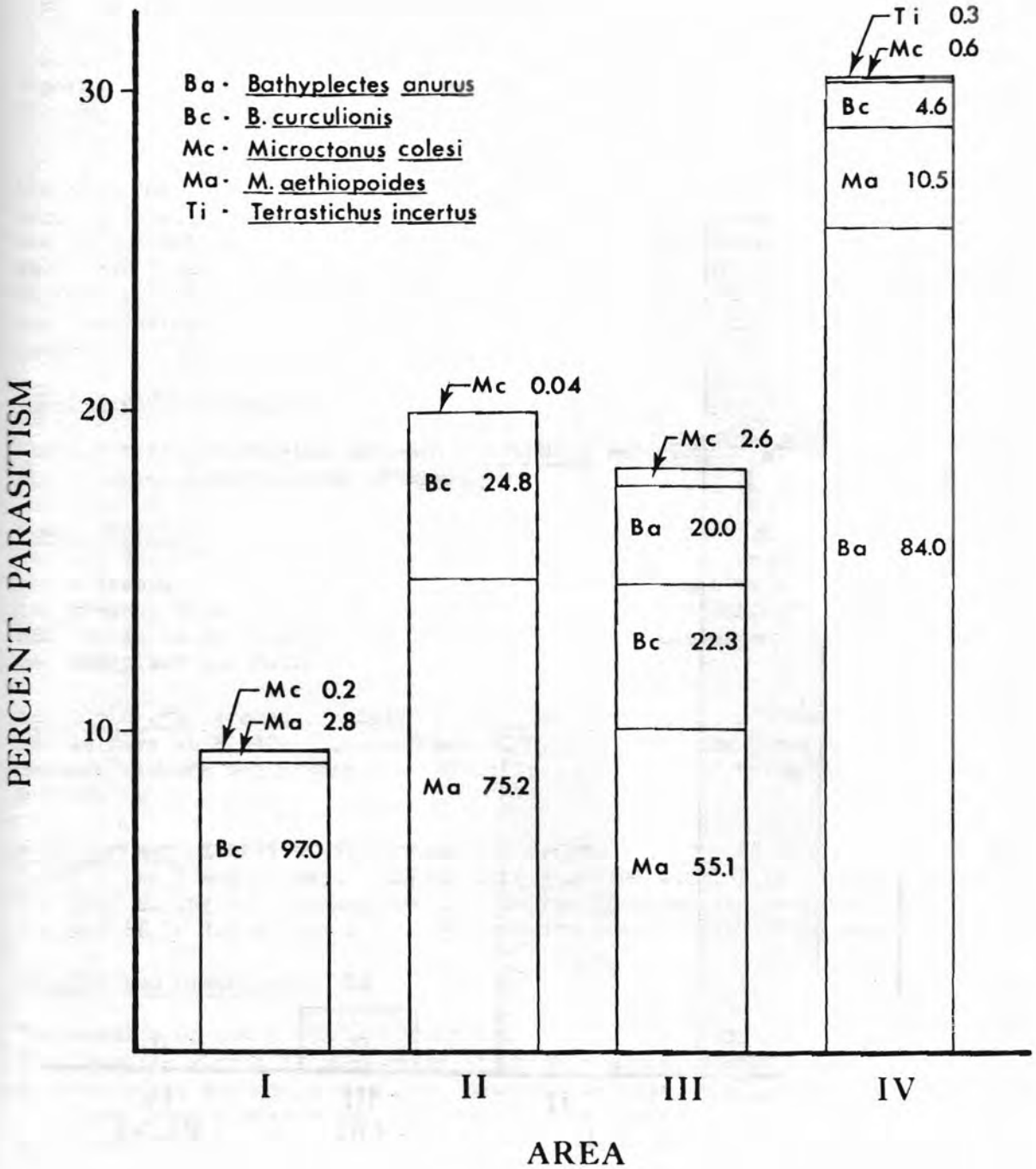


FIGURE 8. OVERALL PERCENT PARASITISM OF ADULT AND LARVAL ALFALFA WEEVILS (TOTAL # OF HOSTS PARASITIZED/TOTAL # OF HOSTS DISSECTED), AND THE PERCENTAGE OF THAT TOTAL BY PARASITE SPECIES, FOR EACH OF THE FOUR 1981 EVALUATION SURVEY AREAS.

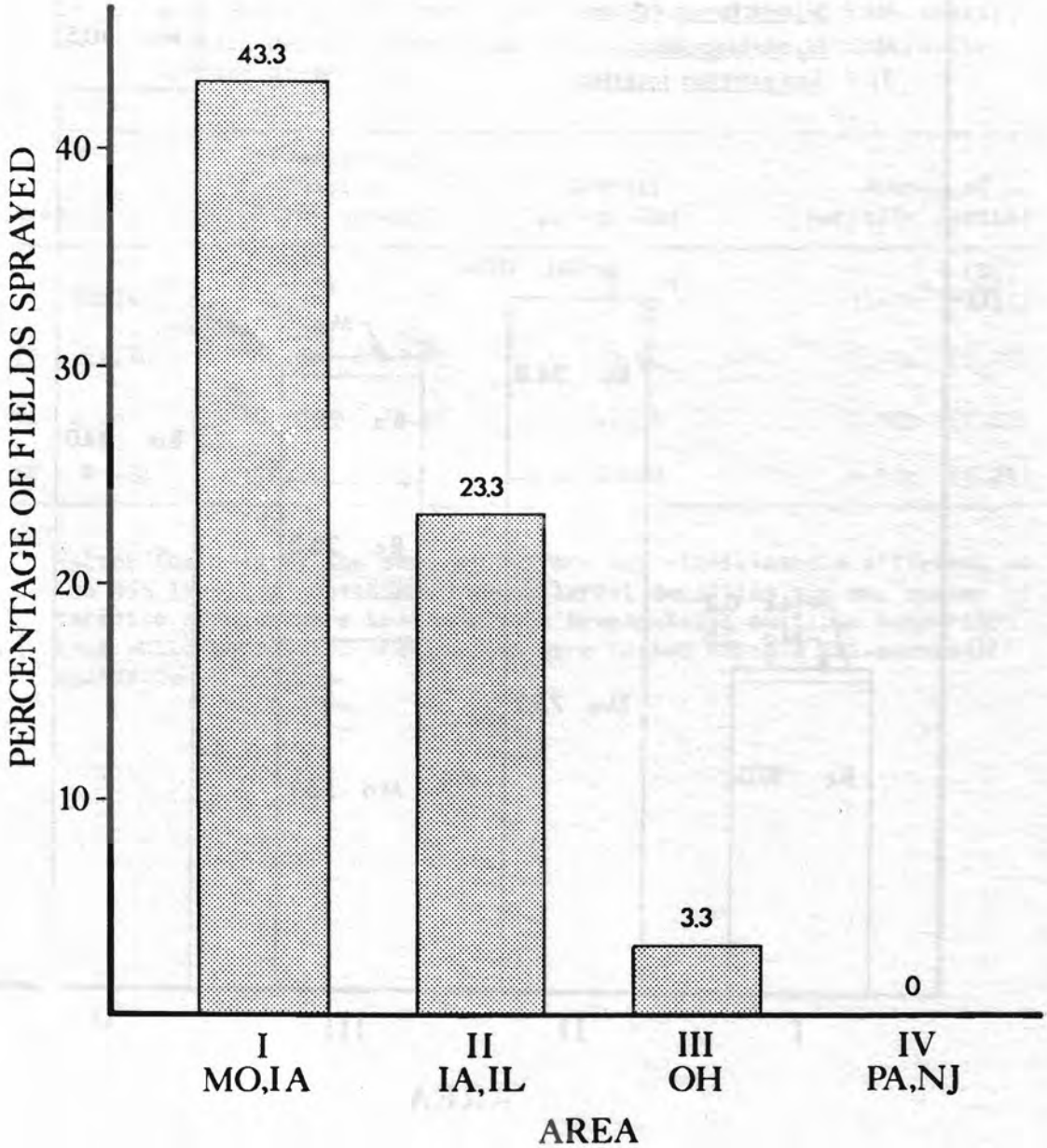


FIGURE 9. PERCENTAGE OF FIELDS, IN THE 1981 ALFALFA WEEVIL EVALUATION SURVEY, SPRAYED FOR WEEVIL CONTROL.

Project Number: MBB 1.1.1
Project Title: Development of Efficient Methods of Mass Rearing *Pediobius foveolatus*
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leader: O. T. Forrester

The scale of *Pediobius* production is directly related to the availability of host material. The Mexican bean beetle biological control demonstration project is not, at present, self sufficient and other sources are needed to meet peak demand. If host material is limited, then it is desirable to improve the yield from the available host material or to develop procedures for increasing the "shelf life" of Pf to permit stock piling to meet peak needs.

Methods and Materials

Storage tests of mummies and adult *Pediobius* were conducted in Percival I-30 BLL reach-in environmental chambers.

Mummy storage; groups of 50, 4th instar MBB were exposed to 25 Pf and held for 10, 12, and 13 days at 25°C, 50% RH and 12:12 L:D. Mummies from the above treatments were stored for 14 days at 8, 10, and 12°C. Fecundity of the progeny from the stored mummies was checked by exposing 50, 4th instar MBB larvae to 25 Pf and analyzing the number of mummies produced, Pf yield per mummy and sex ratio.

Adult storage; groups of adult Pf were fed for 1 day and stored for 14, 20, and 26 days at 8, 10, 12, 10-25 and 25°C. Pf were fed honey streaked on the organdy closure and misted with distilled water daily during the storage period.

Host storage; groups of 4th instar MBB larvae were stored at 8, 10 and 10-25°C for 7 and 14 days. Larvae were supplied with split green bean pods for food during the storage period. Stored host material was stung with 1-3 day old Pf in 1/2 gallon ice cream containers lined with brown paper towels.

Results and Discussion

The results of mummy storage tests are given in Table 1. Sex ratio of Pf from mummies stored 10 days after stinging produced normal appearing progeny. Fecundity was adversely affected, however, and parasites from stored mummies produced fewer mummies than the progeny from mummies stored after 12 and 13 days development. The sex ratio of progeny produced from the 10 day mummies was also adversely affected.

Table 1. Fecundity of progeny from variously aged mummies stored 14 days at several temperatures.

Age of Mummy (days)	Temp (°C)	Days in Storage	No. mummies Produced/ 50 larvae	No Eclosed	% Eclosed	Pf per Mummy	Sex Ratio
10	8	14	32	18	56	14.9	0.9:1
10	10	14	37	27	73	16.5	1.7:1
10	12	14	38	22	58	12.9	1.6:1
Control	25	0	44	42	95	16.9	4.0:1
12	8	14	50	33	66	13.4	4.6:1
12	10	14	49	46	94	22.5	1.9:1
12	12	14	49	39	80	10.4	4.1:1
13	8	14	49	42	86	15.4	2.8:1
13	10	14	41	32	83	13.6	2.7:1
13	12	14	46	38	83	16.1	5.4:1
Control 1/	25	0	49	45	92	20.7	2.7:1

1/ Controls for 12 and 13 day storage.

The results from adult storage tests are given in Table 2. The fecundity of Pf stored as adults was reduced more at the lower temperatures tested. The sex ratio did not appear to be altered at the lower temperature as in the mummy storage tests. Adult Pf have been stored for 26 days with a temperature regime of 10°C for 22 hours and 25°C for 2 hours, and displayed normal fecundity. Progress will be reported in more detail in the next report.

Table 2. Fecundity of stored adult Pf.

Days Stored	Temp (°C)	No. Mummies Produced (per 50 MBB)	% Eclosed	No. Pf per Mummy	Sex Ratio
14	6	28	56	16.4	3.9:1
14	8	45	89	17.1	4.0:1
14	10	40	80	16.4	3.3:1
14	12	47	94	20.7	2.7:1
14	15	40	80	13.9	3.8:1
14	10-25	49	98	18.5	4.2:1
14	25	43	86	17.6	1.5:1
20	8	20	40	3.4	3.1:1
20	10	36	72	15.1	2.1:1
20	12	39	78	17.5	1.9:1
20	10-25	43	86	20.0	3.9:1
20	25	44	88	16.9	4.5:1
Control	25	48	95	21.2	4.2:1

The results from host storage tests are given in Table 3. Host storage does not show much promise as yet. MBB larvae survive storage for 7-14 days with little mortality and were readily accepted as hosts by Pf. They did not produce a high yield in mummies, however. The problem appears to be a disease induced by Pf oviposition. Host development continues in storage and some material developed beyond stages acceptable to Pf.

Table 3. Parasite production from hosts stored under various conditions.

Temp (°C)	Days Stored	No. MBB Stored	Mummies Produced	% Eclosion	Pf per Mummy	Sex Ratio
8	7	95	82	68	20.1	5.0:1
8	14	50	47	34	5.4	5.8:1
8	14	214	28	53	8.9	5.8:1
10	7	110	59	45	13.1	6.3:1
10	14	40	18	50	5.0	0.8:1
10	14	100	19	79	15.5	3.1:1
10	14	100	37	100	17.7	5.9:1
10	14	94	45	77	14.9	2.0:1
10	14	100	32	100	19.8	3.8:1
10-25	7	95	93	82	12.1	6.4:1
10-25	14	50	36	91	13.3	2.7:1

Conclusion

Mummy and adult Pf storage are viable methods of increasing the "shelf life" of Pf. The optimum temperature and conditions of storage need more investigation. Host storage does not offer a solution to increasing Pf production at this time.

Project Number: MBB 1.1.2B
Project Title: Influence of Host Nutrition on Development and Emergence of *Pediobius foveolatus*
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leader: Dave Simser, Pam Moran, O. T. Forrester

Introduction:

Production of the eulophid parasite, *Pediobius foveolatus*, for release against the economically important coccinelid, *Epilachna verivestis*, requires efficient rearing techniques, for both the parasite and the host. Suboptimal host material results in a decrease in parasite production.

A simple requirement of developing *E. verivestis* larvae is continuous supply of a food source, usually snap bean foliage or bean pods. Mass production of larvae, however, is restricted by space and cost limitations of maintaining a thriving bean plant population.

The current study was conducted to determine the effects of lack of food, or provision of three alternative nutritive sources (cut foliage, honey water, or cranapple-vitamin mix) on host and subsequent parasite development, measured by these criteria:

- parasite yield/host invested
- sex ratio
- fecundity of progeny
- longevity of progeny
- developmental rate of parasites
- number of unemerged mummies

Materials and Methods:

Fourth instar *E. verivestis* were selected from the stock colony on the basis of large relative size and dark coloration (a darkly colored ventral crop is indicative of a recently completed feeding period). Larvae nearing pupation continue feeding sporadically; feeding behavior is terminated by caudal attachment to the substrate. Selected larvae had not attached, and were placed with a supply of fresh snap bean pods and distilled water on cotton dental wicks for 24 h prior to testing. At the onset of the experiment, any caudally attached larvae were discarded.

During testing, 320 E. verivestis were selected at random, divided into groups of 20, and each group placed within an 8 oz paper container containing a dental wick saturated with honey water. Eight female P. foveolatus (0.4:1 parasite-host ratio) were introduced into each container via a pasteur pipette sealed at one end. Containers were held at constant conditions (26 1°C, 50% RH, 16:8 L:D) for 24 h, at which time parasites were removed by gentle aspiration. All groups of E. verivestis were pooled into a larger container. Parasitized larvae were then divided into groups of 20 and placed within 8 oz containers and held under described conditions.

The treatments evaluated were:

GROUP A larvae were provided only with 2 clean, dry cotton dental wicks, (control group).

GROUP B larvae were provided with a supply of freshly cut foliage, which was replaced on a daily basis.

GROUP C larvae were provided with a continuous supply of 10% honey water saturating 2 clean cotton dental wicks.

GROUP D larvae were provided with a continuous solution of cranapple (Veryfine Products Juice) (75%) and vitamin mix (25%) saturating 2 cotton dental wicks.

Four replicates (20 E. verivestis/replicate) of each treatment were compared. Larvae continued feeding until caudal attachment or parasite induced mortality ensued. Following mummification, pupation, or mortality of all larvae, each mummy was placed within a gelatin capsule (#00) and held under described conditions until parasite emergence.

RESULTS:

Nutritional deficiencies resulting from inadequate intake, or provision, of a food source are apparent in host insects as a reduction in final larval biomass, premature mortality in larval or pupal stages, or eclosion failure. Similarly, host nutritional deficiencies lead to reduced development and emergence of P. foveolatus. Such deficiencies resulted in differences among treatment groups.

Developmental rate

Parasite developmental rate was compared by recording the period between parasitization and mummy (or pupal) formation within an entire treatment replicate and by recording the eclosion dates of parasites in all groups (Table 1). Hosts were considered still feeding if movement or curling behavior occured in response to a probe prod.

Group B larvae ceased feeding and attached caudally 4 days after parasitism, whereas all other groups required at least an additional day to cease movement and attach.

Eclosion dates of parasites from all groups were consistent; however, in groups C and D emergence occurred gradually over days 14-17, whereas groups A and B emerged more rapidly, within days 14-15; a small percentage emerged on days 16 and 17.

Table 1. Developmental rate of P. foveolatus within E. verivestis fed upon various food sources.

GROUP	% Mobile 1/ days post-parasitism 2	3	4	5	% Eclosion 2/ days post-parasitism 14	15	16	17
A	70	65	13	0	57.2	35.7	7.1	0
B	61	32	0	0	50.7	39.4	7.0	2.8
C	61	39	13	0	22.7	34.1	27.3	15.9
D	58	49	10	8	23.5	43.1	15.7	17.6

1/ % still moving when prodded, or still feeding, of entire treatment group

2/ % eclosion based on total eclosion from mummies within entire treatment group

Percent parasitism, unemerged mummies, and corrected parasitism

Nutritional effects on parasite development are apparent (Table 2), and demonstrate the importance of host development towards successful parasite eclosion. The low incidence of % parasitism of group A larvae (55.8%) was reduced further by a relatively high percentage (20.3%) of unemerged parasites, thus yielding a lower percentage of parasite-producing mummies (35.5%). Premature host mortality probably resulted in a termination of parasite development and parasite emergence was apparently hampered by host nutrition as well, as fewer parasites successfully chewed through the host cuticle (possibly hardened by premature host mortality) in groups A and D.

Table 2. Percent parasitism of treatment groups.

GROUP	% parasitism [1/]	% unemerged [2/]	% corrected parasitism [3/]
A	55.8	20.3	35.5 c
B	95.0	5.1	89.9 a
C	67.3	11.4	56.3 bc
D	86.3	21.5	64.8 ab

1/ All % values are averages of four replicates per treatment

2/ Values are significantly different (P .05) by Kruskal-Wallis rank test (df=3; H=10.54).

3/ Values followed by different letters are significantly different (P .01). Percentages were transformed to arcsine and submitted to analysis of variance and mean separation with Duncan's Multiple Range Test.

Parasite yield/host invested

Although the result of host submission to parasites is a discrete number of emergent parasites/mummy, dependent upon many variable conditions, a useful expression of parasitism is the total number of emergent, viable parasites from the total number of hosts submitted, an important consideration in mass rearing programs. Differences between these criteria (Table 3) reflect losses due to host mortality, pupation, or lack of parasite emergence.

Table 3. Parasite yield per viable mummy vs parasite yield per host submitted.[1/]

GROUP	Pf per mummy	Pf per submitted host	difference	% reduction
A	12.1	5.3	6.8	56.2
B	17.3	12.8	4.5	26.0
C	15.6	8.5	7.1	45.5
D	14.3	9.6	4.7	32.9

1/ Values are mean averages of 4 replicates per treatment

Sex Ratio

This criterion was measured by examining parasites ca 24 hours following emergence. Gelatin capsules with parasites were placed in a refrigerated room (4 °C) for ca 1/2 h, removed and examined microscopically (30 x). Parasites within capsules moved slowly, and males were identified by a retracted abdomen, due to the 24 h starvation period. Females remained normal in appearance, probably due to developing ovaries filling the abdominal cavity. When provided with a honey water source, male abdomens regained normal appearance.

The results (Table 4) of these examinations indicate that sex ratio was not affected by host nutrition; however, an interesting phenomenon, occurring in all treatment groups, was the production of male offspring only from certain mummies. Because male numbers in these cases were typically high, the sex ratio of all treatment groups was skewed towards males. This apparent parthenogenesis was surprising, because superparasitism of hosts is common with P. foveolatus, and thus it is likely that several females oviposited within all hosts, per container. Possibly, progeny gender was influenced by ovipositing females.

Table 4. Female to male sex ratio of P. foveolatus

GROUP	Female to male sex ratio 1/
A	3.84
B	3.65
C	4.95
D	3.73

1/ Values were averaged from 4 replicates. Numbers of emerged and unemerged parasites were pooled for each treatment group and recorded.

Longevity and fecundity of progeny

Treatment group wasps were pooled, and each group was held separate within 1/2 gal ice cream containers. Containers were held under described conditions, and continuous honey water was provided. Weekly mortality counts were recorded, and all treatment progeny survived for 4 weeks, at which time the experiment was terminated.

Six-day old females were removed from each group and placed with 4th instar E. verivestis at 0.4:1 parasite:host ratio under described parasitism conditions. Two replicates of each group were sampled. Results (Table 5) demonstrate no apparent differences between progeny fecundity; however the criterion of fecundity, defined as total number of offspring/female, may confuse interpretation of results. Large numbers of P. foveolatus, e.g. 40-50/mummy, are typically small and inactive, compared to parasites developing at lower numbers per host (10-20/mummy). Thus, as defined, female fecundity is not necessarily a positive criterion in assessing developmental parameters of P. foveolatus.

Table 5. Number of P. foveolatus emerged from each treatment group

GROUP	% parasitism	# of P. foveolatus/mummy 1/
A	90	19.9
B	88	15.3
C	80	21.3
D	97	16.1

1/ replicates were pooled and averaged. Sex ratio of progeny was not recorded.

Parasite development and emergence are influenced by host nutrition following parasitization. Provision of alternate (liquid) nutrients resulted in increased rearing efficiency as compared to lack of nutrients, but not when compared to a natural diet of bean foliage. However, the materials tested did not represent the most effective alternate food available, and additional nutritive sources should be tested, including artificial diets, bean pods, or bean sprouts, etc.

Project Number: MBB 1.1.3
Project Title: Determination of the Most Effective Release Formula for Pediobius in Nurse Plots
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leader: O. T. Forrester

Introduction

Pediobius foveolatus (Pf) is not known to over winter in the United States. The success of utilizing this parasite as a biological control agent for Mexican bean beetle is dependent on its reestablishment each year.

The technique of planting nurse plots with mixtures of snapbeans and soybeans is an effective method of attracting over-wintered adult Mexican bean beetles to a desired area near soybean fields. This concentration of host material increases the probability of a successful establishment of the parasite.

The number of Pf released into a nurse plot is determined by the MBB larval density of the last 3 instars in 15 row feet multiplied by 20. The factor of 20 times the larval count is a workable release ratio, but an optimum release ratio has not been determined. It may be higher or lower than the current factor.

The objective of this test is to observe the effect of host:parasite ratio on the production of Pf produced per Pf released in nurse plots and use these data to determine the most effective release ratio.

Methods and Materials

This test was executed in the MBB Biocontrol Demonstration Project area in Maryland. Pediobius release ratios used were 10, 20, 40, 80 and 160 times the highest number of 2nd thru 4th stage larvae counted in 15 rowfeet. Releases were made in populations of 25-75, 100-150, 200-250 and 500-600 2nd thru 4th stages in 15 row feet.

Nurse plot monitoring, data collection and parasite release for this study were conducted (to the extent possible) as described in the MBB Biological Control Project Manual. Each study nurse plot was sampled according to the following schedule. When plants had emerged, visits were made weekly for general observations. When overwintering adult beetles were observed and each week thereafter, five survey units, (each consistently of a section of 3 rowfeet) were randomly selected and sampled. The number of adults and egg clusters were counted and recorded. After larvae were found the nurse plots were sampled twice each week. The number of adults, egg clusters and larvae by instar were counted and recorded for each survey unit. When the larval counts in the sample units indicated that 50% of more of the larvae in the nurse plot were 2nd instar or older, the "trigger date" was met and a release ratio was assigned. Only one Pediobius release was made in study plots.

Following parasite release, the plots were sampled twice weekly. The number of adults, egg clusters, larvae (by instar), mummies (by instar), prepupae, pupae, and percent defoliated for each survey unit was counted and recorded. A sample of the first 10 mummies encountered was taken from the plots 10 days after the parasite release. The collected mummies were placed in individual containers and allowed to eclose; yield and sex ratio were recorded. The mummies that did not eclose were dissected and counted. A total of 617,500 Pediobius were used in this test.

Results and Discussion

Pediobius production was affected by release ratio and larval population density. In the overall analysis, the yield ratio (Pf produced - Pf released/Pf released) decreased as the release ratios were increased. The net Pediobius production (Pf produced - Pf released) increased as the release ratios were increased (Figure 1). As host density increased, Pf yield ratios decreased slightly and net production increased (Figure 2).

Table 1 compares yield ratio, net Pf production, actual release ratio (Total Pf released/total host count per nurse plot) and formula release ratio. The highest yield ratio was obtained at 20x and the lowest at 40x. The highest overall net Pf production was at 80x and the lowest was at 10x. Comparing yield ratios, formula release ratios and release host count (Figure 3) the optimum release ratio appears to be 10x or 20x. Comparing net Pf production with release host counts, the optimum release ratio changed with host density (Figure 4). Comparing net Pf production with release ratio (Figure 5) the optimum release ratio also changes with host density. The optimum release ratio for the host densities tested is the highest yield ratio and highest net production.

Conclusion

The optimum release ratio varied according to the population densities tested. The optimum release ratio for 25-75, 100-150, 200-250 and 500-600 host per 15 row feet was 10x, 20x, 80x and 80x respectively.

The increase of Pf production is not necessarily increased by increasing the formula release ratio, unless the host population is high. Less than 5 percent of the Maryland nurse plots would benefit from high formula release ratios (Figure 6).

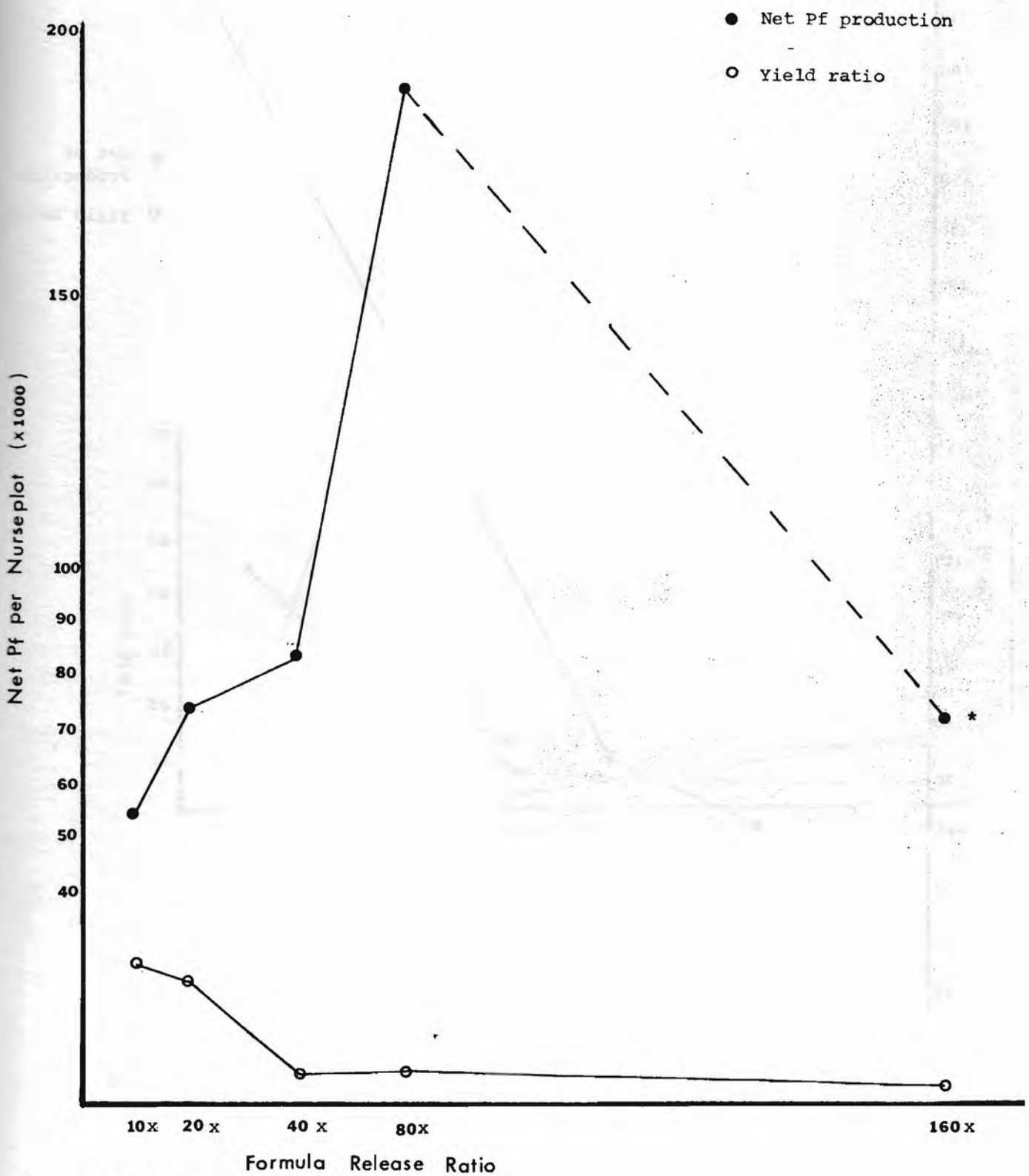
The yield ratio curve can be used to estimate the net Pf production of nurse plots at various host densities at "trigger" date.

Table 1. Pediobius production from Maryland release ratio test.

	Trigger Date Host Counts				No. Nurse Plots
	25-75	100-150	200-250	500-600	
		Formula Release Ratio 10X			
Net Prod	26,715	39,383	32,053	109,935	13
Yield Ratio	44.06	28.64	12.2	19.98	
Release Ratio	0.064	0.096	0.081	0.083	
		Formula Release Ratio 20X			
Net Prod	16,833	193,154	34,746	48,859	12
Yield Ratio	15.32	68.17	6.95	4.16	
Release Ratio	0.137	0.171	0.182	0.145	
		Formula Release Ratio 40X			
Net Prod	18,457	4,384	15,902	290,437	13
Yield Ratio	8.2	0.89	1.84	12.53	
Release Ratio	0.243	0.297	0.263	0.412	
		Formula Release Ratio 80X			
Net Prod	24,049	42,989	142,910	492,785	11
Yield Ratio	2.62	4.30	7.33	12.02	
Release Ratio	0.655	0.485	0.981	0.80	
		Formula Release Ratio 160X			
Net Prod	42,981	28,698	139,197	*	11
Yield Ratio	5.73	1.42	2.41	*	
Release Ratio	0.97	1.56	1.09	*	
No. Nurse Plots	17	19	15	9	

Net Prod = No Pf produced - No. Pf released
Yield Ratio = Net Pf produced/Pf released
Release Ratio = Total No. Pf released/Total hosts per nurse plot

Figure 1. Comparison of net Pf production and yield ratio with formula release ratio.



* No release at 500 per 15 row feet

Figure 2. Comparison of Net Pf Production and Yield Ratio with Host Density

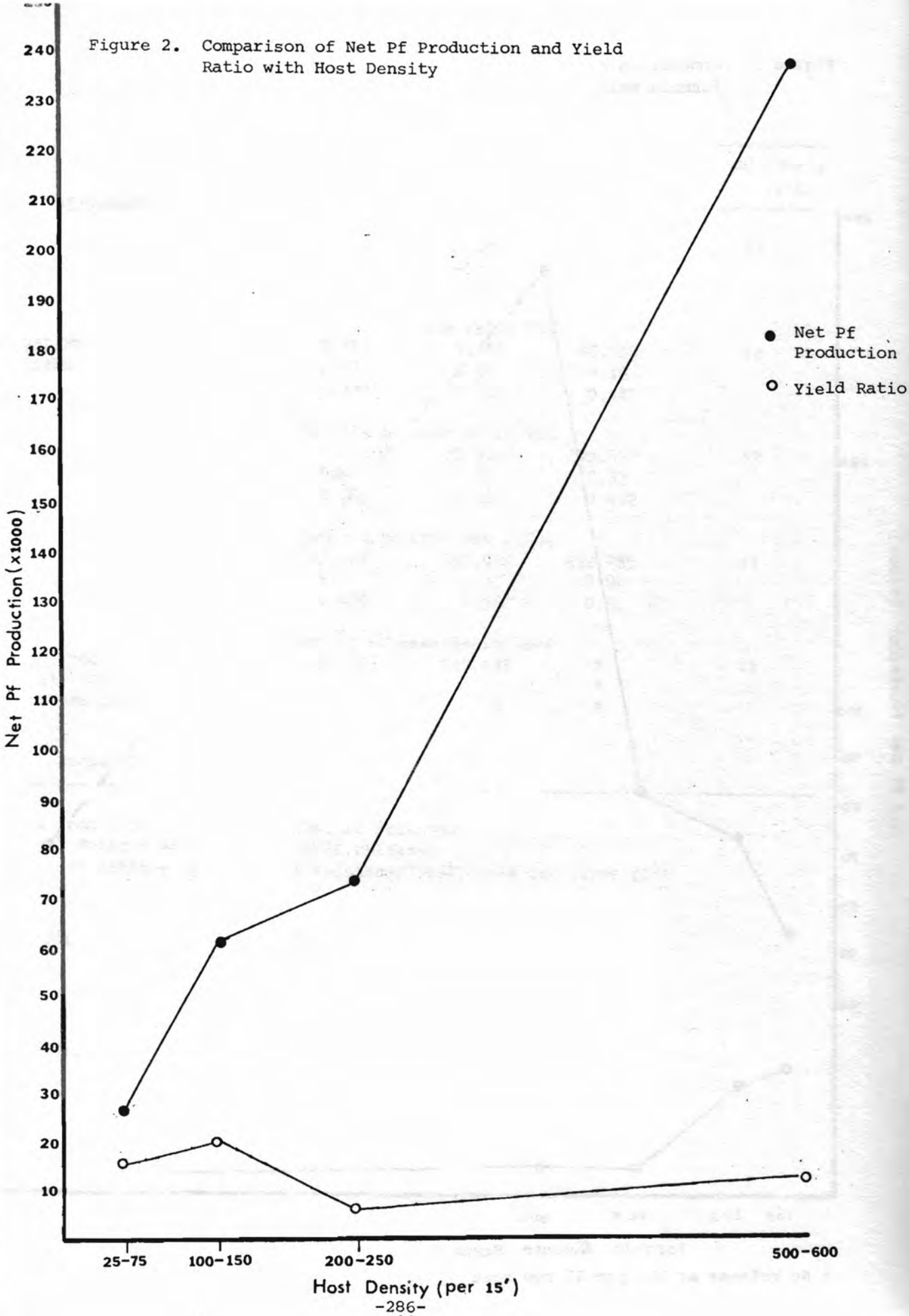


Figure 3. Comparison of Yield Ratio with Host Density

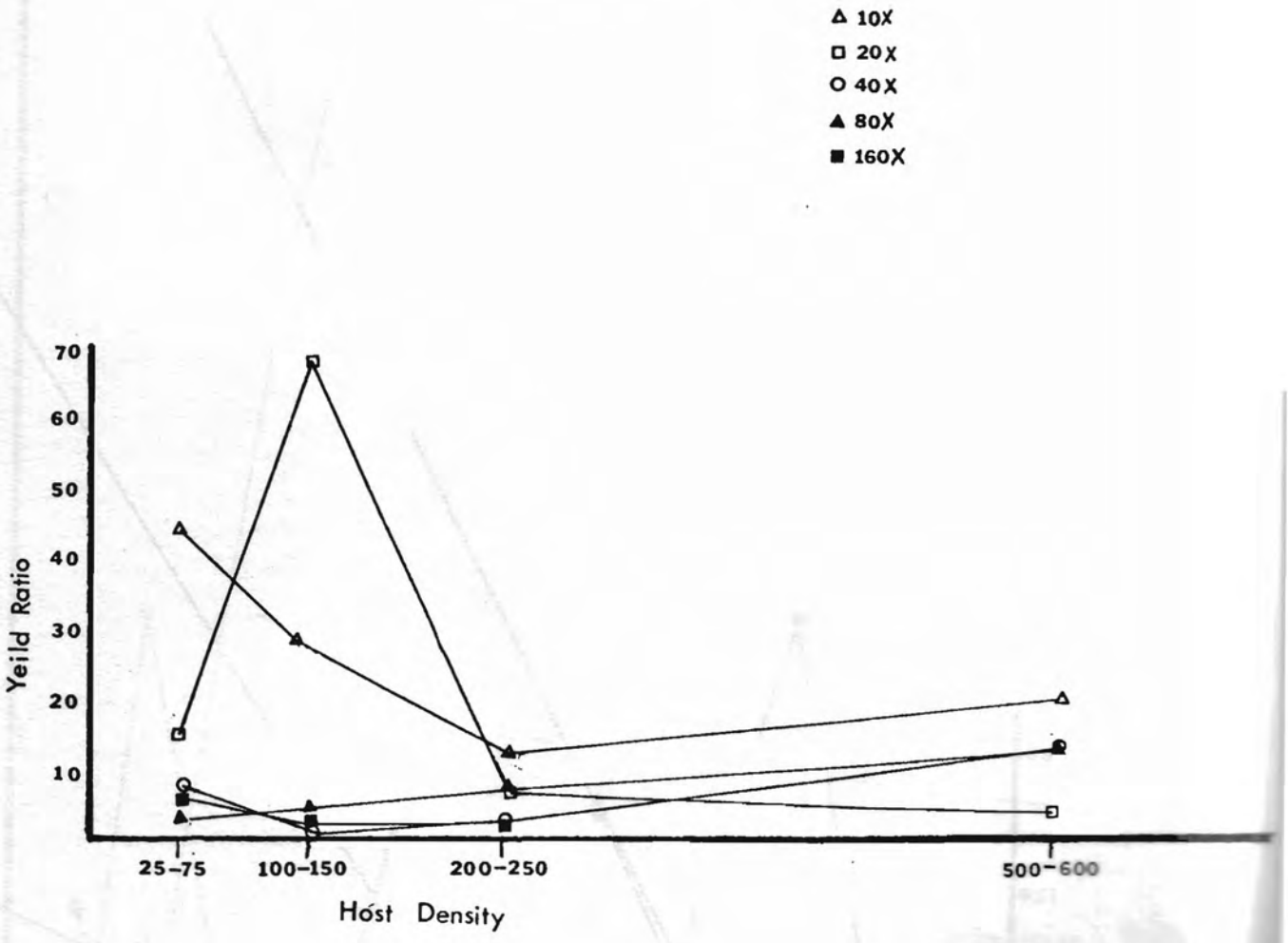


Figure 4. Comparison of Net Pf Production with Host Density

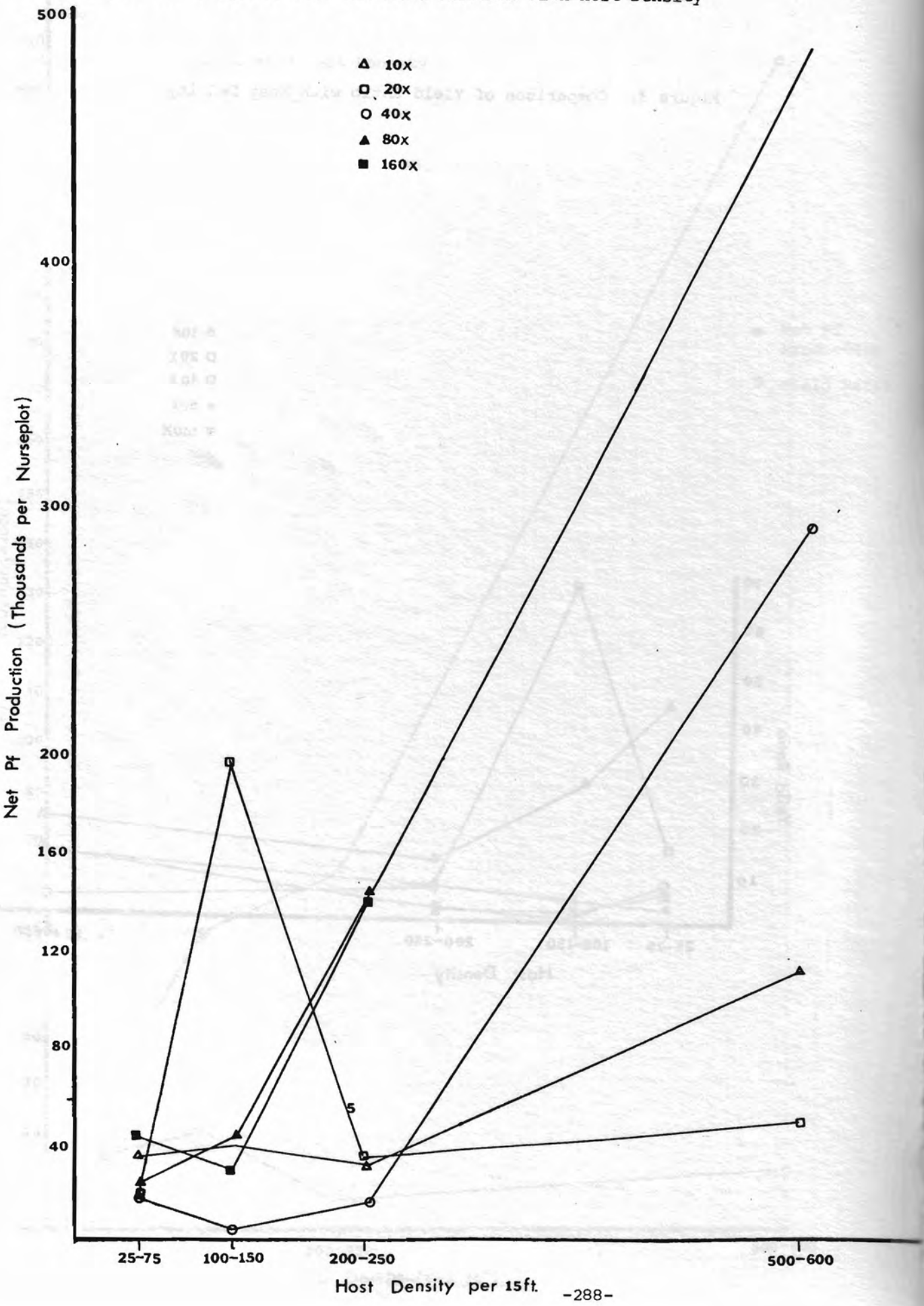


Figure 5. Comparison of Net Pf Production with Formula Release Ratio

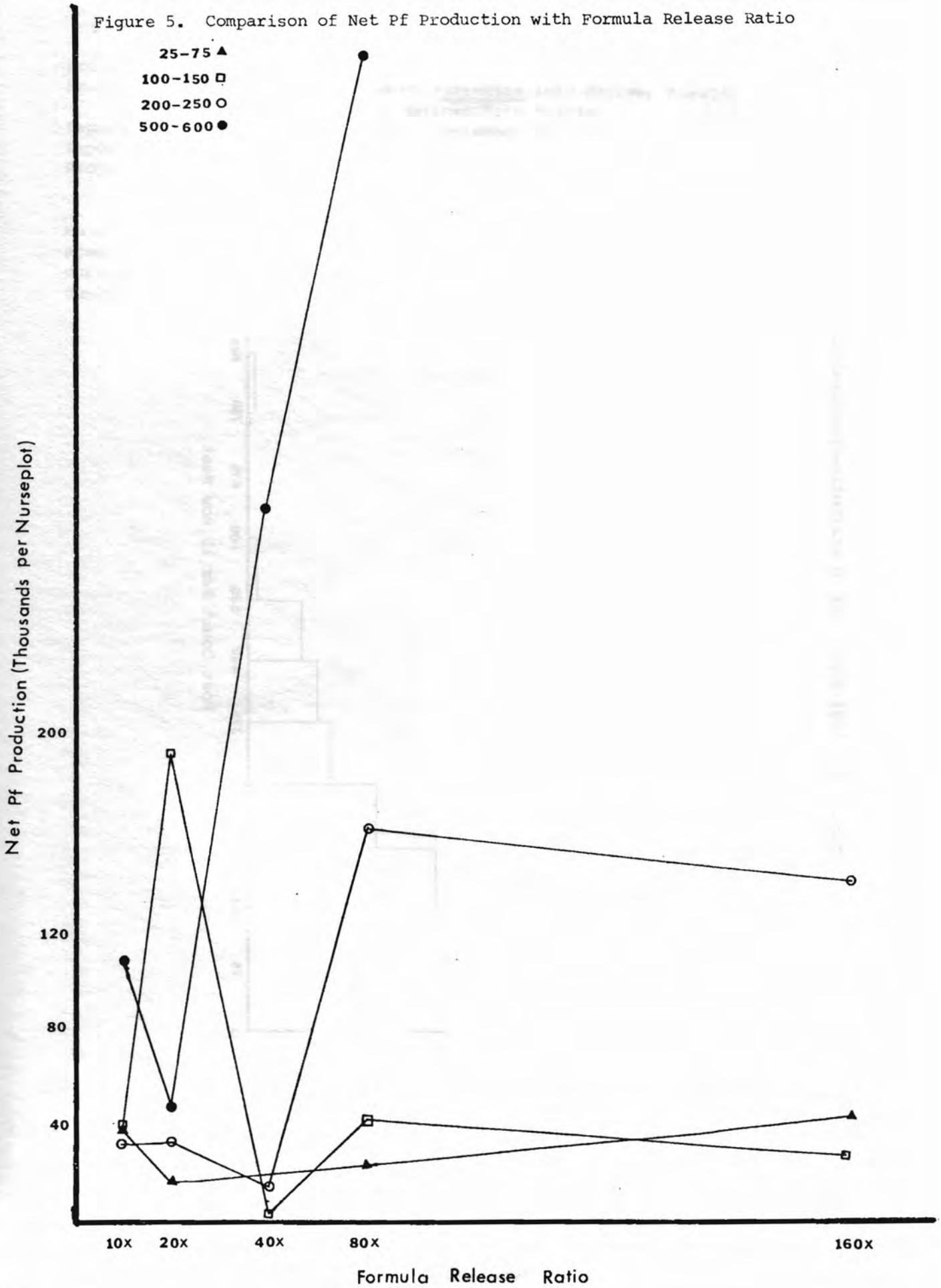
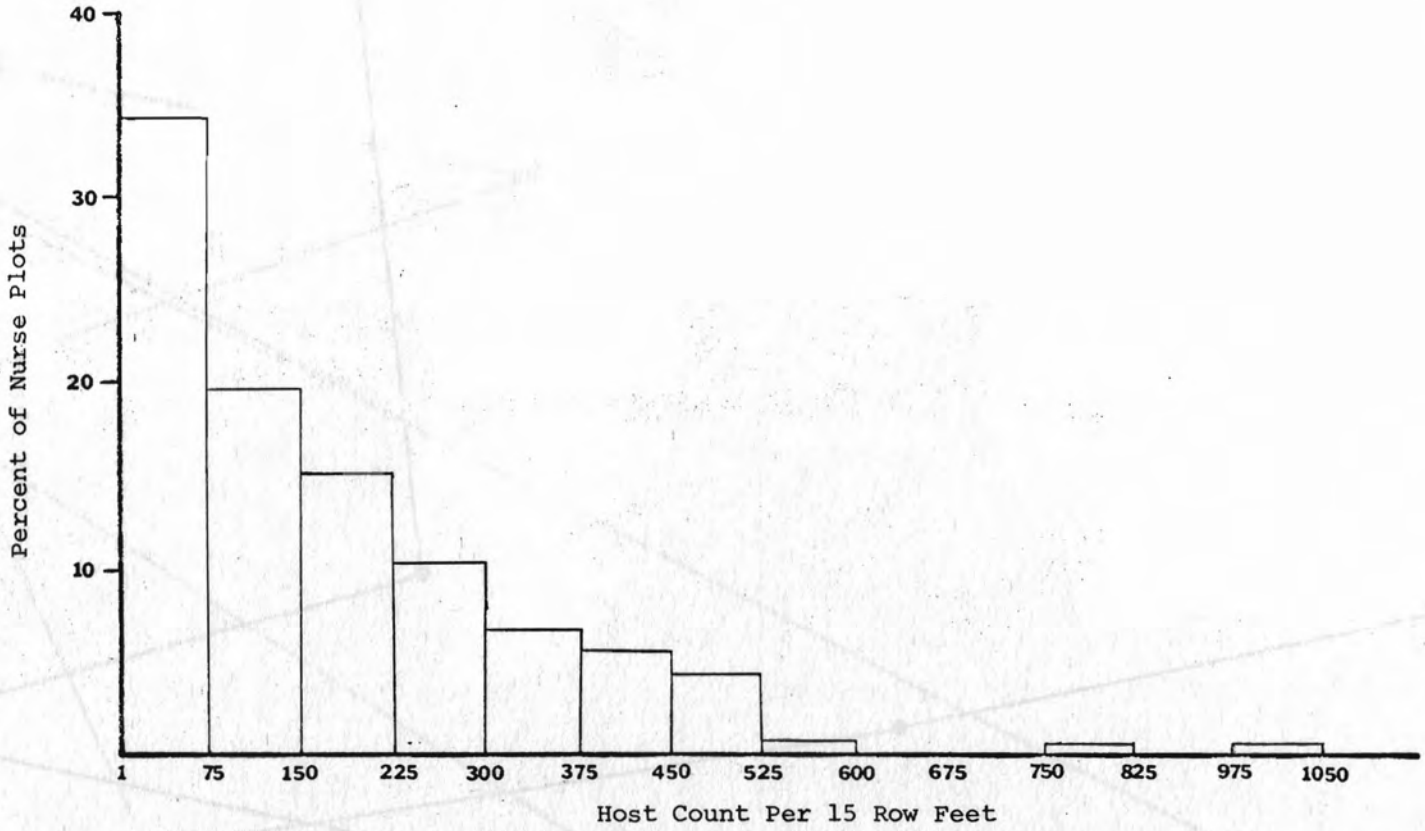


Figure 6. 1981 Population Distribution Maryland Nurse Plots



Project Number: MBB 1.1.4
Project Title: Direct Release of Pediobius into Soybean Fields for Control of Mexican Bean Beetle
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leader: O. T. Forrester

No work was accomplished on this project because of a lack of study areas well removed from the demonstration project area. We are currently evaluating the prospects of completing this project in the coming season.

Project Number: MBB 1.1.5
Project Title: Control of Mexican Bean Beetle in Truck Farm and Small Plantings of Green Beans with Pediobius foveolatus
Report Period: October 1, 1980 - September 30, 1981
Report Type: Interim
Project Leader: O. T. Forrester

Introduction

The control of Mexican bean beetle with Pediobius foveolatus is being demonstrated on a large scale in the middle Atlantic soybean producing area. There is an increasing interest in extending this technology into other areas of the bean producing industry (Truck farms, seed production and home gardens). The nurse plot technique being used to establish Pediobuis in soybean production areas would not, in general, be practical in small plantings of snap beans. Therefore, a direct field release would be necessary.

The objective of this test is to develop and demonstrate a control strategy for Mexican bean beetle using Pediobuis foveolatus (Pf).

Methods and Materials

This test was executed on a truck farm in East Sandwich, MA. Data collections were conducted as follows: When plants were up, general observations were made weekly. When adult Mexican bean beetles were observed and each week thereafter, 5 survey units (consisting of 3 row feet each) were selected at random and sampled. The number of adults and egg clusters were counted and recorded. After larvae were found, the plantings were sampled twice each week.

When larvae reached 2nd instar, the "trigger" date was met and parasites were released. A single release was made when parasite availability permitted. If adequate parasites were not available on the trigger date, the specified number was released over 2 consecutive days.

Following parasite release, the plantings were sampled twice weekly. The number of adults, egg clusters, larve by instar, mummies, prepupae and pupae for each survey unit were counted and recorded. The plantings were sampled until they senesced.

Results and Discussion

Data collection began on June 10 on adjacant plantings of 2 rows of pole lima beans, (780 row feet) and 7 rows of bush beans (2,380 row feet). Adult beetles and egg clusters were observed on the first visit. Release criteria were met on June 25. Parasite release was made on June 26 and 27. Host:parasite ratio at time of release was 3:1, and the mean larval instar was 1.5. Mummies were reported 12 days after the first release.

A release of 16,000 Pf was not sufficient to provide foliage protection for 3,160 row feet of beans. A larval count of 599 per 15 row feet was recorded on July 8 and Malathion was applied on July 10. The grower was not satisfied with the control and Thiodan was applied on July 11. A sample of mummies and larvae was collected on July 13. All of the larvae collected died and 77.8% of the mummies produced parasites (Table 1). The chemical treatment reduced MBB population from 599 larvae per 15 row feet to 8 per 15 row feet. The mummy count increased from 31 per 15 row feet on July 8 just before the chemical treatment to 73 per 15 row feet on July 16, the first sampling after chemical treatment. The chemical treatment did not adversly effect Pf in the mummy stage as evidenced by the successful eclosion of parasites from the collected mummies. The bush beans senesced and were not checked after July 8. The MBB population was apparently controlled by Pf following the chemical control (Figure 1). Monitoring of the second planting of pole beans began on July 16.

The second field studied in this test was upwind from the first and separated by 200 yards, interspersed with trees and buildings. This planting contained 15 rows of beans 25" apart and 250 feet long. Monitoring began on July 6, and 6,000 Pf were released on July 8. MBB larval count at release was 118 per 15 row feet, the release ratio was 4.9:1 hosts:parasite and the mean host instar was 2.7. Chemical controls were not required. Pedobius appeared to control the MBB population in this planting (Figure 2).

Field 3 in this test was 60' higher in elevation than other plantings and separated from field 1 and 2 by 1/4 mile. Monitoring of field 3 began July 21 and was terminated on September 17. The highest larval count was 14 per 15 row feet. Pf were not released. Mummies were observed in this planting on August 18 (Figure 3). Chemical controls were not required in this plot.

A fourth planting which was studied was in a low area 30 feet downwind of the first plantings. Monitoring began September 9 and ended October 1. The highest Mexican bean beetle count recorded was 28 per 15 row of feet. The highest mummy count was 19 per 15 row of feet. Controls were not applied (Figure 4).

Larval and mummy samples were taken from the pole beans and the fourth planting on September 17, 25, 29 and October 1. Table 1 compares Pf yields, sex ratio and percent eclosion with the July 13 collection (first Pf generation). Sex ratio difference between the first generation and those of later collections show shift from 5 :1 to nearly 1:1. This ♀♂ shift may be of little importance because the mean yield of female Pf per mummy remained fairly uniform, 9.7 at 5:1 and 7:4 at 1:1. Larval samples from the September 17 and 25 were 95.5 and 100% parasitized, respectively.

According to the owner of the farm on which this test was conducted, in a normal season all plantings of beans require a minimum of one application of insecticide to control Mexican bean beetle. The introduction of Pf this season saved checmical treatments on the second planting of pole beans, fields 2, 3, 4 and a probable second treatment of the early pole beans. The introduction of Pediobius this season saved 4 or 5 treatments.

Mexican bean beetle can be controlled in truck farm situations by using direct field releases of Pediobius foveolatus. The early plantings of beans should be managed as nurse plots. Pediobius release should be made in the mid second larval stage. It may be necessary to apply chemical control to keep the first generation of MBB below economic levels, but chemicals should not be applied before mummies are observed in the field.

The second season of Pediobius use on this truck farm may not require insecticide for control of Mexican bean beetle. The highest count of overwintered adults during June was 30 per 15 row feet, the mean adult count for June was 16.8. The highest adult count during August was 8 per 15 row feet and the mean was 4.5. The highest September adult count and mean per 15 row feet was 3 and 0.9 respectively. The percent parasitism based on larval collections in September was 95.5 and 100%. One could estimate that adults available for overwintering was 3-8 per 15 row feet. An overwintered population of 3-8 adults per 15 row feet could be controlled by a Pediobius release.

Table 1. Pediobuis yeild from larvae and mummies collected from pole beans and field 4 following innoculative release.

Date sampled	Yield ♀	Yield ♂	Total Mean Per Mummy	Sex Ratio ♀ ♂	Percent Eclosion
July 13	9.7 ±3.6	1.9 ± 1.3	11.6 ± 4.1	5.1:1.0	77.8
Sept 17	8.5 ±6.4	11.1 ± 1.0	19.6 ±15.0	1.0:1.3	89.3
Sept 25	8.6 ±6.7	10.1 ±13.8	19.2 ±19.6	1.0:1.2	86.9
Sept 29	5.2 ±3.1	6.7 ± 7.8	12.0 ±10.0	1.0:1.3	66.7
Oct. 1	7.1 ±5.8	6.0 ± 8.3	13.8 ±12.1	1.2:1.0	91.7

October 1, collection one mummy not Pediobius.

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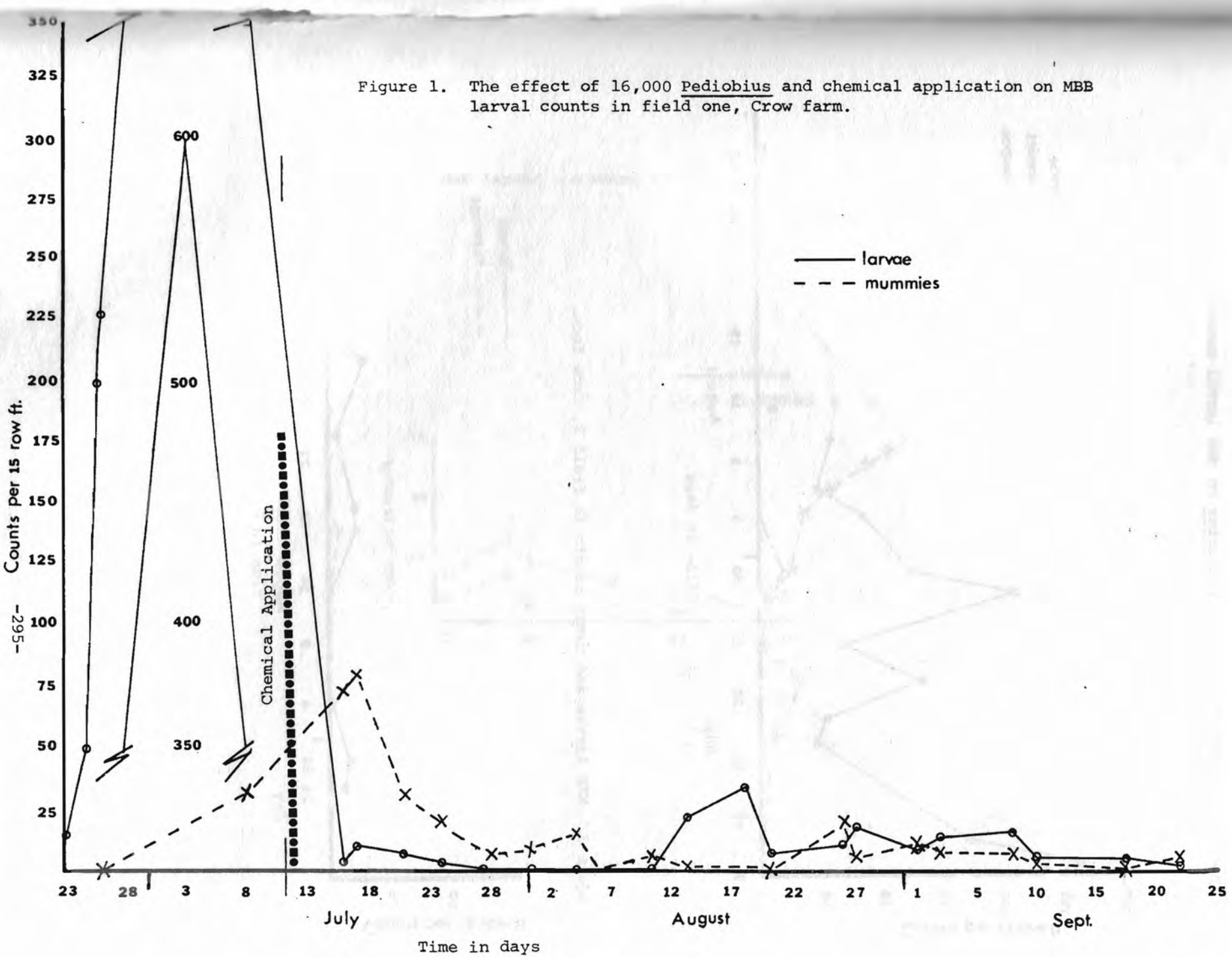


Figure 1. The effect of 16,000 Pediobius and chemical application on MBB larval counts in field one, Crow farm.

Figure 2. The effect of 6,000 Pediobius on MBB larval counts in field 2, Crow farm.

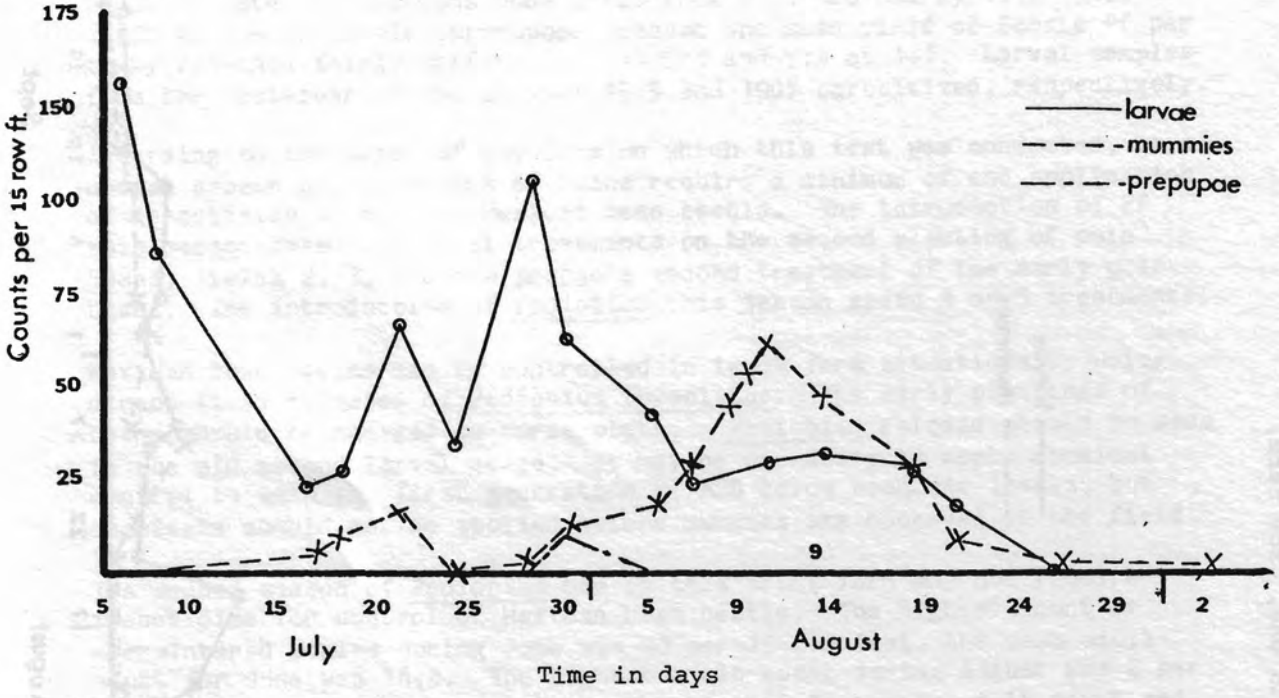


Figure 3. MBB larvae and mummy counts in field 3, Crow farm.

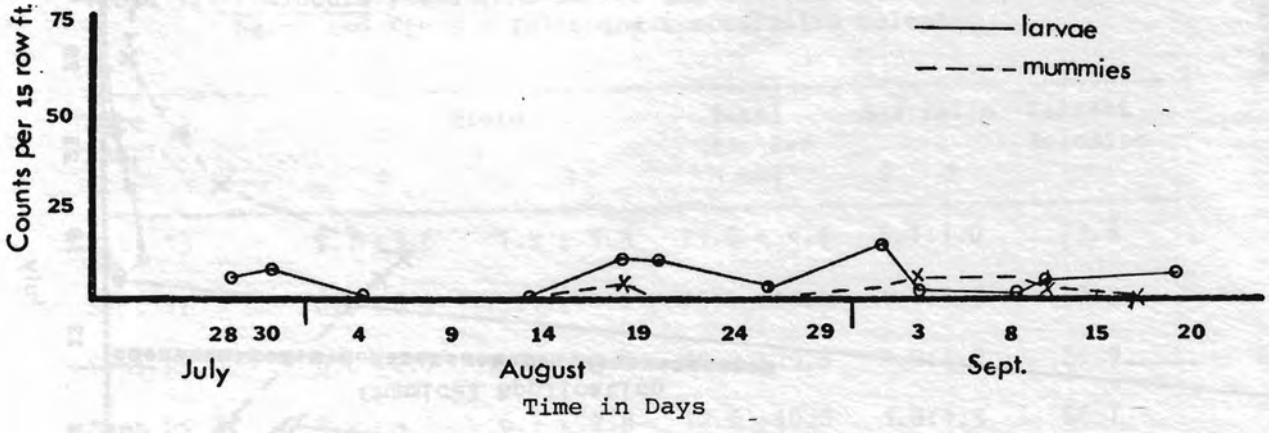
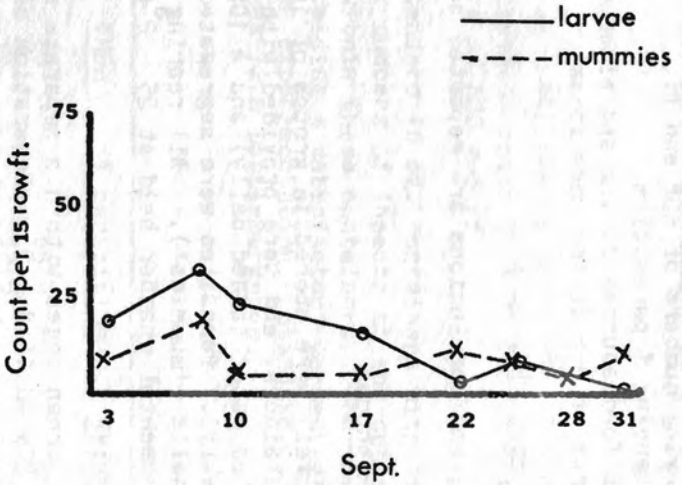


Figure 4. MBB larvae and mummy counts in Field 4, Crow farm.



Time in days

Project Number: MBB 1.1.6
Project Title: Interactions between the Mexican Bean Beetle, Epilachna verivestis, and a eulophid parasite, Pediobius foveolatus.
Report Period: October 1, 1980 - September 30, 1981
Report Type: Final
Project Leader: Dave Simser, Pam Moran

A series of experiments was conducted to investigate interactions between Epilachna verivestis the Mexican Bean Beetle (MBB), and the parasite, Pediobius foveolatus (Pf). Although lack of treatment replication of some experiments precluded statistical analysis of data, these preliminary observations are reported to document results which may be useful in planning and conducting future studies of interactions between Pf and MBB.

Experimental series:

A. Parasite - host ratio (0.4 to 1) (within a 16 oz container) adjusted by varying numbers of MBB and Pf.
B. Age of Pf influencing % parasitism.
C. Alternative MBB food sources during and after parasitism.
D. Food requirements of MBB during parasitism.
E. Food requirements of MBB after parasitism.
F. Storage of 4th stage MBB at 6°C.

Objective, results, and conclusions are reported separately for each experiment.

Standard Handling Methods:

Parasites (mixed sexes) were stored in groups of 100-500 within 16 or 32 oz cardboard containers, and were provided with cotton dental wicks soaked with distilled water (added daily) and a 10% honey water solution (added weekly). Parasites were segregated by emergence dates from host larval shells ('mummies'). All rearing containers were held in a walk-in environmental chamber held at 25 2°C, 50% RH and 16:8 L:D.

MBB were reared in screen cages within a separate walk in environmental chamber (25 2°C, 80% RH 16:8 L:D). Separation of parasites and hosts was necessary to guard against accidental parasitism of the MBB colony. Larval and adult MBB were provided with a continuous supply of snap bean plants (L-4 stage), which were replaced as necessary.

Parasitism of MBB:

Female Pf were removed from a rearing container via gentle aspiration into a Pasteur pipette. Pipettes were then securely taped at both ends.

Fourth instar MBB were selected from the larger group and placed within a 16 oz container with bean foliage (when applicable). Containers were then transported into the parasite chamber.

Hosts were submitted to Pf by opening the large end of the pipette and inserting it into the MBB container. Due to positive geotropic and phototropic response, Pf crawled into the container, contacted hosts, and initiated oviposition probes and subsequent parasitism. Standard parasite:host ratio was 0.4:1 unless otherwise indicated, and a cotton wick saturated with distilled water was added to each container during parasitism.

Following a 24 h parasitization period, Pf were removed via aspiration and held separate from the stock colony. Parasitized MBB were supplied with cut snap bean foliage daily until mummification or pupation ensued. Mummies were collected and placed individually within #00 gel capsules and held at described conditions until parasite emergence.

Parasite eclosion was not hampered by containment within the gel capsules; containment facilitated counting parasites and determining sex ratios.

EXPERIMENTAL SERIES

Experiment A. Parasite:host ratio (0.4:1) constant with varying numbers of MBB and Pf.

Objective: Use of standard 16 oz. containers with Pf and MBB required comparison of varying numbers of insects at the optimum 0.4:1 parasite:host ratio within these containers. The lowest number of parasites per host yielding a satisfactory percentage of parasitism was chosen for further experimentation. Standard conditions were employed and Pf age was 72 h post-emergence.

Results

Treatment	#Pf	#MBB	% parasitism	#Pf/mummy	# replicates
A	8	20	65.0	17.5	2
B	16	40	52.5	18.3	2
C	24	60	53.5	21.2	2

Because treatment A gave satisfactory results with regards to % parasitism and #Pf/mummy, twenty hosts and eight Pf were chosen as standard numbers for subsequent tests.

Experiment B. Influence of Pf on % parasitism.

Objective: Previous studies have demonstrated that Pf fecundity is affected by parasite age. This experiment concerned identifying a standard Pf age which yielded the highest % parasitism. Three replicates were conducted.

Results

Treatment	Parasite Age (hr)	% parasitism
A	24-48	87.5
B	72	70.0
C	96	68.3

Age of Pf for subsequent experimentation was between 24-48 h post emergence, on the basis of the highest percentage parasitism among treatment groups.

Experiment C. Alternative MBB food sources during and after parasitism.

Objective: Because parasitized MBB require a continuous supply of snap bean foliage to complete development to the mummy stage, an alternate inexpensive food source is desirable. Ideally, the food source could be easily obtainable and economical, and would not affect development of MBB or resultant Pf.

The objective of this study was to compare several food sources with standard snap bean foliage, provided during and post parasitism.

Treatments:

Groups of 30 MBB were submitted for parasitism as follows:

A. Mung Bean Sprouts provided during + after parasitism.
B. Alfalfa Sprouts provided during + after parasitism.
C. Snap Bean Foliage provided during + after parasitism.
D. Honey water (10%) provided during + after parasitism.
E. Mung Bean Sprouts provided after parasitism.
F. Alfalfa Sprouts provided after parasitism.
G. Snap Bean Foliage provided after parasitism.

All handling techniques were standardized. Provision of food sources in applicable treatment groups was continued until pupation or mummification occurred throughout that group. Ample food was provided as required, with any dried or otherwise unpalatable material discarded.

Results

Treatment	% to MBB adults	% mortality of MBB	% parasitism
A	3.3	10.0	86.7
B	3.8	53.8	42.3
C	17.2	0.0	82.8
D	43.3	40.0	16.7
E	36.7	20.0	43.3
F	30.0	16.7	53.3
G	37.9	16.7	44.8

Treatments A (Mung Bean Sprouts) and C (Snap Bean Foliage) resulted in low mortality and high parasitism percentages, with varying results among other treatment groups. Lack of food source during the parasitism period resulted in high mortality of Pf females, which may account for lower % parasitism of groups E-G. Although MBB larvae were parasitized, nutritional requirements continued to be important for their survival. Groups B and D thus represented poor nutritional sources for MBB, resulting in high host mortality and, subsequently, low Pf survival and emergence.

Experiment D. Food requirements of MBB during parasitization.

Objective: Provision of snap bean foliage during the parasitization period may not be required for adequate Pf yield; this assumption was tested in separate experiments. Five or six developed bean leaves were added at daily periods, as required.

Test 1 conditions: Fifty MBB per 1/2 gallon container; group A with foliage during 24h parasitization period; group B without.

Test 2 conditions: Thirty MBB per 8 oz container; group A with foliage during the 24h period; group B without.

Results

	Test 1		Test 2	
Group	% parasitism	Pf per mummy	% parasitism	Pf per mummy
A	50.0	15.2	50.0	12.4
B	60.0	14.7	53.3	11.7

No differences were noted between groups in either experiment, and foliage was discontinued during parasitization in subsequent trials. However, a moistened dental wick was placed in containers during parasitism to provide a water source for Pf.

Experiment E. Food requirements of MBB after parasitism.

Objective: Documentation of the requirements of parasitized MBB to complete development to the mummy stage was necessary to conduct additional and concurrent studies.

Treatments:

A. Provision of snap bean pods during and after parasitism.
B. Provision of distilled water on cotton wicks during and after parasitism.
C. Provision of snap bean foliage during and after parasitism.

Results:

Treatment	% parasitism	Pf/mummy
A	80.0	11.0
B	40.0	11.4
C	89.0	13.0

Parasitized hosts require continual food to complete development to the mummy stage. Lack of a food source decreases MBB survival and subsequent Pf emergence.

Experiment F. Storage of 4th stage MBB at $6^{o}C$.

Objective: Storage of nonparasitized host larvae for varying periods at low temperatures could increase parasite yield by increasing the number of hosts available per stinging date, standardizing parasitism rates, and generating a buildup of host material for crucial release dates. In this study, groups of MBB were held at $6^{o}C$ for 6-10 days, then removed, and fed bean foliage under constant conditions until mortality or adult emergence ensued. Mortality totals were tabulated.

Treatments:

Five groups of 20 MBB were held at $6^{o}C$ 16:8 L:D, 90% RH. After 6 days, one group was removed and provided with bean foliage; the remainder were removed at successive 24 h periods and reared under standard conditions.

Results:

Days at $6^{o}C$	% survival to adult	% mortality
6	85.0	15.0
7	80.0	20.0
8	50.0	50.0
9	30.0	70.0
10	10.0	90.0

Survival of MBB larvae decreased with successive storage days at 6 ^{o}C. A 6-7 day storage period is apparently the maximum period of MBB survival at the tested temperature.