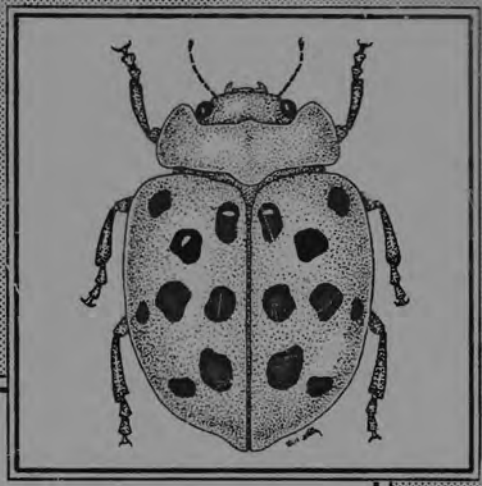

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Summary

The search for suitable ovicides for treating regulated articles infested with gypsy moth egg masses has revealed that a mixture of trichloroethane, castor oil and prim oil is very effective in devitalizing gypsy moth eggs over the period when eggs are present in the field. Extensive tests with the registered product "Spray N Kill" yielded very poor results. The quality of the formulation used in the tests is suspect.

A large number of stickers have been evaluated for improvement of the residual properties of Bacillus thuringiensis (BT) formulations. These tests have shown that RA-1990 is a superior sticker and that more commonly used products, such as Plyac and NuFilm, improve weathering characteristics of BT sprays, but are somewhat less effective than RA-1990. Commercially available formulations of BT were shown to degrade slowly in the presence of natural sunlight, losing approximately 25% of activity after 5 hours exposure in preliminary tests. Several new strains of BT were also tested in connection with their commercial development. Various BT formulations were also tested after periods of storage under different conditions and were shown to withstand severe environmental extremes.

Three oil formulations of BT were tested for phytotoxicity against 15 tree species. All displayed wide ranges of phytotoxicity with poplar, catalpa, birch and maple being the most sensitive species.

Several growth regulators were tested and found effective against gypsy moth larvae at very low dosages. Avermectin was also tested and was shown to give almost 100% control of second instar larvae at .00117 lbs/acre. Rearing tests showed little effect in the F-1 generation of survivors of Avermectin treatments.

Field tests with BT, Pydrin and Alsystin in 1983 are reported. BT was applied against second, third and fourth instar larvae at several dosages. Results indicate that 12 BIU is adequate for controlling second and third instar larvae but increased dosages are required for fourth instar larvae. Pydrin yielded 98% reduction in egg mass density at .06 lbs/acre and the growth regulator, Alsystin, also resulted in 90 - 100% reduction in egg mass density.

Summaries of results from 10 isolated infestations that were treated with BT demonstrate substantial reductions in adult moth densities following multiple applications of BT. Final conclusions regarding effectiveness for eradication are pending 1984 adult survey results.

Several laboratory and field tests were conducted with pyrethroid treated tapes used as barriers for large gypsy moth larvae. Laboratory tests showed short exposures to the insecticide treated tapes resulted in larval mortality. However, trees thus treated were defoliated to the same extent as controls.

A small test was conducted which simulated the conditions gypsy moth egg masses would encounter when brought indoors on Christmas trees during December and January. Eggs held indoors for 3 weeks during that period and returned outside, experienced 80% reduction in hatch. Hatch from those held indoors for 1 or 2 weeks was reduced by approximately 25%.

Abstracted results of a large study conducted since 1980 dealing with inherited sterility induced by partial irradiation of male pupae are reported. The results show that P-1 male pupae administered 6, 8, or 10 krads at 8 to 9 days of age, yield sterility in the F-1 compatible with various management scenarios. It is concluded that 6 krads are appropriate for population suppression, but that 10 krads would be necessary for achieving absolute sterility in the F-1 necessary for eradication use. Survival and development data from all treatments are available. Because of the magnitude of the full body of data, only selected results are reported here. These results form the basis for planning eradication projects with the inherited sterility method.

A demonstration project area in Berrien County, Michigan, which received fully sterile adult male release over 2 consecutive years, was surveyed with negative results, leading to the provisional conclusion that eradication was achieved by release of sterile males.

In 1982 a small isolated gypsy moth infestation was treated in Horry County, South Carolina with male moths that were partially sterilized. In 1983, insects were collected and tested for fertility. Preliminary results indicate that sterile F-1 insects were recovered from the field, along with 3 fertilized egg masses, indicating those male parents were feral. The important result of this test is that sterility was established in a field population and could be detected in the F-1 generation the following year. Similar tests were conducted in Kent County, Maryland in 1983, and evaluation in 1984 will determine the degree to which sterility was introduced into test populations there.

A series of tests was conducted to evaluate the feasibility of releasing F-1 eggs (that will develop into sterile adults) produced in the laboratory from partially irradiated males and normal females. Tests focused on the holding conditions that lead to synchronous and optimal hatch in the field during the spring. Presently a 100 day production "window" has been defined which, if fully used, enables the production of approximately 1,000,000 sterile gypsy moth egg masses for subsequent release. Tests on the development and survival of larvae from eggs thus held indicated that F-1 sterile insects develop more slowly than wild type insects, raising a concern about the competitiveness of these eggs when released into native populations. This feature is currently under thorough study to evaluate its impact on the effectiveness of a sterile egg release.

Comprehensive tests were done on the behavior and ecology of sterile F-1 larvae to assess the competitiveness of these insects in a sterile insect release program. Dispersal of larvae from trees with various defoliation histories was studied and, generally, previous defoliation history did not appear to influence the incidence of dispersal. Late instar larval behavior was evaluated by observing larvae on oak trees surrounded by galvanized steel pipe observation towers. At night larvae were observed under ambient light with night vision goggles. Larvae in outbreaks remained in the canopy and fed on and off during the day, rather than resting in hidden niches near the bases of trees as in endemic populations. Further, larvae in low density populations compared to larvae in outbreaks exhibit relatively short bouts of feeding and change their feeding sites frequently. These and other differences in behavior appear to be tied to defoliation-induced changes in leaf chemistry rather than merely a consequence of insect density.

Laboratory studies were conducted with automatic feeding monitors which showed that the amount of feeding is influenced by the type of foliage insects are offered, and feeding rhythms are dependent upon the type of population from which test insects were collected. When larvae were reared on artificial diets containing tannic acid, they had feeding rhythms similar to those of larvae in outbreak populations; this further suggests that defoliation changes in foliage quality are primarily responsible for density-related changes in the behavior of gypsy moth larvae. It was also shown that body temperature of larvae is dramatically influenced by their location in the forest and that larvae in defoliating populations have higher body temperatures. This, in part, explains the faster developmental rate regularly observed in high density gypsy moth populations.

A series of mass trapping tests was conducted to evaluate the effectiveness of this technique for eradication of isolated infestations. It was shown that trap densities ranging from 2.5 to 25 traps per ha resulted in a reduction of female mating success. It was also shown that males were captured more quickly in plots with higher insect densities. This corroborates previous findings that the important feature in the effectiveness of mass trapping is not the proportion of the male population captured, but also the rapidity with which males are removed from the population. This technique has been evaluated in an isolated infestation in Monona, Wisconsin. Those results show a steady decline of a population concomitant with the use of mass trapping since 1981. The study is continuing into 1984, when it is calculated that eradication should be completely achieved.

Mating disruption, as an eradication technique, is being evaluated in 2 locations, Oconomowoc, Wisconsin and Carteret County, North Carolina. Because of deviations from the original experimental plans, equivocal results are being obtained. However, indications are that mating disruption treatment contributed to the reduction of those populations. Eradication will not, however, be demonstrated by either project.

Egg masses have been shipped to Gainesville, Florida since June 9, 1982 and held in field insectaries for hatch evaluation. Field collected strains from Massachusetts begin hatching in early April in Florida. The New Jersey standard strain reared in the laboratory apparently has a weaker diapause and those eggs delivered to Florida in September begin hatching at low levels as early as February. However, we believe this is atypical, and that most field collected egg masses relocated to Florida any time during the summer and fall will commence hatching in early April.

A number of formulations were tested as dispensers of (+) disparlure for use in gypsy moth survey traps. Those studies have failed to identify a dispenser that performs as favorably as the Hercon laminated design presently in wide scale use. Various trap designs were also tested with emphasis on improving weathering characteristics and reducing cost. A suitable plastic delta trap design performed favorably. Interestingly, traps fashioned out of clear plastic caught significantly fewer moths than those of colored plastic. Other tests with pheromone dispensers indicated that the Hercon dispensers increase in effectiveness after they have been in the field for a period of weeks. Additional tests are underway to clarify this phenomenon.

During the course of the year, nearly 6,000,000 gypsy moth eggs have been infested onto diet in the rearing facility and distributed to at least 15 cooperating scientists in this country and abroad. This facility is the sole source of experimental insects for all of our sterile male projects and innovations have greatly improved the efficiency of operations. Of particular significance was the introduction of a mechanical egg infesting machine to eliminate the tedious process of manually infesting neonate larvae onto diet cups. Quality control measurements of the colonies in production are also reported and compared with similar observations made in 1982.

Studies are underway in Columbia, Missouri to develop an immunological method for identifying the corn pathogens, Drechslera carbonum and Drechslera maydis. Most effort has gone into developing large amounts of conidia for use in obtaining antiserum. The effect of light on conidia production was also noted. This material is now being used for immunizing animals and producing test antiserum. Different types of bean agars were used to show colony differences between the two species; however, it is believed that this method is presently of little practical value because of the high degree of skill required to observe differences in colony characteristics. The direction that the Methods Development projects supporting the Cooperative National Plant Pest Survey Detection Program are discussed.

The alfalfa weevil parasite redistribution project has concluded a third year of a planned 5 year project. New county and state records for several species were reported and Batheplectes anurus is showing a clear westward movement. It appears to be displacing a previously established congener, B. curculionis. Where both species occur sympatrically, parasitism rates eventually favor B. anurus. There also appears to be a decline in the number of alfalfa fields sprayed for alfalfa weevil over the last 3 years and no fields were treated for alfalfa weevil in the 2 eastern areas in 1983. Additionally, 3 years of economic data such as pesticide and control costs, management practices and grower attitudes have been collected and are presently being analyzed by the Economic Research Service.

A diet has been developed for rearing alfalfa weevil larvae and adults during redistribution. Large scale trials are scheduled for 1984 in preparation for general use in future redistribution acitivities. This method will eliminate the use of cut alfalfa for feeding insects during redistribution shipment.

Studies have been conducted in cooperation with the University of Wisconsin to use electrophoretic methods for identifying the distribution of Egyptian alfalfa weevil and eastern and western strains of alfalfa weevil. Early results indicated that strains could be separated based on profiles of lactate dehydrogenase and glucose-6-phosphate dehydrogenase. Upon further investigation, however, it was noted that these differences were due to the physiological state of the adult weevils being tested. Accordingly, this variable (reproductive vs non reproductive) is being taken into account in the search for isozyme differences between the strains.

The dispersal of Pediobius foveolatus from nurse plots infested with Mexican bean beetle was evaluated in Ohio and it was shown that parasites can be recovered as far as 1.5 miles from the release site. High levels of parasitism away from the nurse plot were more common later in the season. These tests were preparatory for a large scale management trial using Pediobius scheduled to commence in 1984.

PESTICIDE TESTING SECTION

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Project Number: GM 6.1.5
Project Title: Regulatory Treatments
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: W. H. McLane and 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.

During the past year, several companies demonstrated interest in developing home-owner treatments such as application of oil-based materials to egg masses, direct spraying of larvae, and placement of treated tree bands and/or natural barriers. Such techniques could be useful in the regulatory program directed toward recreational vehicles, mobile homes and outdoor furniture.

S. C. Johnson and Son, Inc. has been developing insecticides for use from hose end sprayers and on tree bands.

TABLE 1. Mortality of 2nd instar gypsy moth larvae 4 days after placement on oak foliage treated and aged with 5302D86-1, 5302D86-2 and Sevin.

	Days of aging following treatment	Percent mortality
5302D86-1	2	100
5302D86-1	6	90
5302D86-2	2	66
5302D86-2	6	59
Sevin	2	79
Sevin	6	30
Check	-	-

TABLE 2. Mortality of 4th instar gypsy moth larvae 6, 24 and 48 hours after a 30-minute direct exposure to treated tree bands that were aged for various periods.

	Days aged	Percent mortality 6 hours	24 hours	48 hours
5682D54-1	6	27	27	27
5682D54-1	4	7	13	13
5682D54-1	2	27	47	47
5682D54-1	0	7	27	27
5682D54-2	6	7	7	13
5682D54-2	4	27	40	47
5682D54-2	2	20	40	40
5682D54-2	0	7	40	40
Check	-	0	0	0

TABLE 3. Mortality of 4th instar gypsy moth larvae 24 to 96 hours after placement on oak foliage treated with a hose and spray can and aged 3 days.

	Percent mortality 24 hours	Percent foliage consumed	Percent mortality 96 hours	Percent foliage consumed
White	0	27	0	47
Blue	0	7	25	24
Yellow	0	30	0	56
Red	0	46	0	70
No food or treatment	0		0	

The following formulations were tested as ovicides against egg masses in the field:

TCOPO - Trichloroethane + Caster oil + Prim oil
KCOPO - Kerosene + Caster oil + Prim oil
40-1 - 1 oz/gal Spray-N-Kill
40-1 - 2 oz/gal Spray-N-Kill
40-2 - 1 oz gal Exp.
40-3 - 1 oz/gal Exp.
40-4 - 5 oz gal Exp.

Starting in early September, 1982, 10 egg masses were treated with each material on a monthly basis until April 12, 1983. Each egg mas was saturated with ca. 10 ml of formulation. Treated eggs were were collected in mid-April and returned to the laboratory for hatchability tests. Egg masses were placed individually in petri dishes and incubated for hatch. Results were poor with all treatments except TCOPO. Even "Spray-N-Kill", a registered product, gave poor results.

TABLE 4. Percent hatch of gypsy moth egg masses treated with 7 formulations over an 8-month period.

	DATES TREATED								
Material	9/9	10/14	11/19	12/7	1/12	2/1	2/25	3/23	4/12
TCOPO	0	0	0	0	0	0	0.1	0	0.2
KCOPO	86	99	100	80	100	100	100	100	50
40-1 (1 oz)		100	100	100	100	100	70	80	100
40-1 (2 oz)		100	100	100	100	80	100	100	100
40-2 (1 oz)		100	100	100	100	90	100	100	90
40-3 (1 oz)		100	100	100	100	90	100	100	80
40-4 (5 oz)		100	100	100	100	70	100	100	100
Check	100	100	100	100	100	100	100	100	100

Health-Chem personnel discovered after the test was completed that the sample of Dylox used to prepare 40-1 was nearly 4 years old. Shelf life of the material is normally not more than 18 months. This may account for the poor results with "Spray-N-Kill".

TCOPO, a J. T. Baker mix gave excellent control of hatch over the complete treatment time frame. However, J. T. Baker has expressed little interest in registering the formulation because of cost of the trichloroethane in the mix. There is also question of the safety of this material for such use.

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period: October 1, 1982 - September 30, 1983
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 and to increase the 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.

During 1983 a number of State and Federal agencies used Bacillus thuringiensis to treat gypsy moth infestations. In all, more B. thuringiensis was used in 1983 than any previous year. In most cases, single applications of 12 BIU/acre were very effective in controlling defoliation.

Although results were generally good with B. thuringiensis during 1982, there were many questions about stickers: which one to use, will it affect car paint, will it clog screens, how costly is it, what's in it, is it cleared for use and will it help stick the material to foliage? While there are a number of stickers available for use, further testing was conducted with formulations of B. thuringiensis and various sticking agents. The following tables summarize that work.

TABLE 1. Mortality of 2nd instar gypsy moth larvae 4 days after placement on oak foliage treated with 8 BIU of Dipel 4L and 2% sticker and exposed to various amounts of rain.

	Percent mortality				Percent defoliation after 3 days			
Sticker	0.05"	0.1"	0.2"	.5"	0.05"	0.1"	0.2"	0.5"
No sticker-no rain	60	42	64	60	10	16	6	16
No sticker	38	9	2	0	29	79	79	90
Polyco - 2142	11	12	15	8	54	63	46	66
Polyco - 522	24	2	6	2	39	72	71	79
Polyco - 2149	37	9	18	7	31	57	46	58
Rhoplex - AC33NP	24	2	24	30	29	70	46	68
TS-36	20	3	6	2	37	62	66	84
TS-83	22	7	8	4	40	65	50	77
TS-100	7	6	7	4	63	68	51	74
RA-1990			53				22	
Check	0	0	1	1	97	100	100	94

TABLE 2. Mortality of 2nd instar gypsy moth larvae 4 days after placement on oak foliage treated with 8 BIU of Dipel 4L containing .5 or 3 percent sticker and exposed to .1 or 5" of rain.

Sticker	Percent mortality 3%/0.5"	Percent mortality 0.5%/0.1"	Percent defoliation 3%/0.5"	Percent defoliation 0.5%/0.1"
No sticker - no rain	69	85	7	13
No sticker	1	18	93	78
Polyco 2142	6	19	65	80
Polyco 522	3	34	77	51
Polyco 2149	12	53	63	48
Rhoplex AC33NP	8	49	72	48
TS-30	2	19	75	58
TS-85	5	36	66	45
TS-100	1	55	78	42
RA-1990		66		34
Check	1		97	98

TABLE 3. Average defoliation of oak seedlings in 14 tests using Dipel 4L and various stickers against 2nd instar gypsy moth larvae.

Sticker	Percent defoliation after 3 days
No sticker - No rain	12
No sticker - Rain	78
Polyco - 2142	62
Polyco - 522	69
Polyco - 2149	52
Rhoplex - AC33NP	59
TS-30	68
TS-85	62
TS-100	67
RA-1990	26
Check	100

TABLE 4. Mortality of 2nd instar gypsy moth larvae 3 days after placement on oak foliage treated with 8 BIU of Dipel 4L and various formulations of Target at 2% and exposed to 0.2 inches of rain.

Sticker	Percent mortality	Percent defoliation
No sticker - No rain	61	6
No sticker - Rain	3	66
Target 148212	6	50
Target 148211	8	65
Target 148206	9	51
Target 148205	9	58
Target 148204	28	33
Check	1	100

TABLE 5. Mortality of 2nd instar gypsy moth larvae 3-4 days after placement on oak foliage treated with 12 BIU of Thuricide 48LV and various amounts of Sup-R-Stk.

Percent sticker	Inches rain	Percent mortality 3-day	Percent mortality 4-day	Percent defoliation 3-day
-	-	34	71	11
-	0.25	16		38
-	0.50	12	31	23
-	1.0	14	46	24
1.0	0.25	19		39
1.0	0.50	10	28	34
1.0	1.0	17	49	14
2.0	0.25	13	20	31
2.0	0.50	11	19	36
2.0	1.0	13	41	10
3.0	1.0	19	43	9
Check	-	0	0	100

TABLE 6. Mortality of 2nd instar gypsy moth larvae 3-4 days after placement on oak foliage treated with 12 BIU of Dipel 6L and various amounts of Sup-R-Stk.

Percent sticker	Inches rain	Percent mortality 3-day	Percent mortality 4-day	Percent defoliation 3-day
-	-	31	57	24
-	0.25	2		90
-	0.50	2	3	94
-	1.0	0	3	84
1.0	0.25	3		76
1.0	0.50	0		60
1.0	1.0	1	1	90
2.0	0.25	3		72
2.0	0.50	2	5	74
2.0	1.0	0	1	83
3.0	1.0	1	2	78
Check	-	0	0	100

TABLE 7. Mortality of 2nd instar gypsy moth larvae 3-6 days after placement on oak foliage treated with 12 BIU of Dipel and 2 percent of various stickers with exposure to natural sunlight.

Sticker	Inches rain	Minutes sunlight	Mortality 3 days	Mortality 6 days	Defoliation 3 days	Defoliation 6 days
-	-	-	57	100	5	10
-	0.25	-	7	72	33	68
-	0.25	60	6	98	28	62
Nufilm 17	0.25	-	7	88	38	56
Nufilm 17	0.25	30	16	84	25	57
Nufilm 17	0.25	60	8	95	19	46
RA 1990	0.25	-	24	99	25	43
RA 1990	0.25	30	35	100	12	20
RA 1990	0.25	60	54	100	8	8
Plyac	0.25	-	11	84	45	59
Plyac	0.25	30	38	99	14	28
Plyac	0.25	60	25	98	13	24
Check	-	60	0	0	100	100
Check	-	-	0	0	100	100

TABLE 8. Mortality of 2nd instar gypsy moth larvae 3-6 days after placement on oak foliage treated with 12 BIU of Dipel 6L and Thuricide 48LV with 3 percent Nufilm 17 and RA-1990 and exposed to natural sunlight.

Material	Sticker	Inches rain	Minutes sunlight	Mortality 3 day	Mortality 6 day	Defoliation 3 day	Defoliation 6 day
Thuricide	-	-	-	49	100	5	8
Thuricide	-	0.25	-	20	99	14	21
Thuricide	Nufilm	0.25	30	37	95	8	14
Thuricide	Nufilm	0.25	120	49	99	7	13
Thuricide	RA-1990	0.25	30	64	100	10	11
Thuricide	RA-1990	0.25	120	54	99	8	10
Dipel	-	-	-	26	98	12	31
Dipel	-	0.25	-	2	48	66	82
Dipel	Nufilm 17	0.25	30	6	67	40	62
Dipel	Nufilm 17	0.25	120	15	92	24	44
Dipel	RA-1990	0.25	30	15	96	23	23
Dipel	RA-1990	0.25	120	27	100	13	26
Check	-	-	120	0	0	90	

TABLE 9. Mortality of 2nd instar gypsy moth larvae 3 days after placement on oak foliage treated with 12 BIU of Dipel 6L and 2 percent Nufilm 17, and exposed to rainfall and natural sunlight.

Sticker	Inches rain	Minutes sunlight	Mortality 3 day	Defoliation 3 day
	-	-	43	8
-	0.25	-	0	93
-	1.0	-	0	88
-	2.0	-	0	88
Nufilm 1	0.25	120	1	79
Nufilm 17	1.0	120	0	82
Nufilm 17	2.0	120	1	80
Check	-	-	0	85

TABLE 10. Mortality of 2nd instar gypsy moth larvae 4 days after placement on oak foliage treated with 12 BIU of Dipel 6L and 3 percent RA-1990, and exposed to natural sunlight.

Hours sunlight	RA-1990	Percent mortality	Percent defoliation
-	-	70	12
2.0	-	52	19
4.0	-	46	20
-	yes	55	25
2.0	yes	80	13
4.0	yes	66	21
-	-	3	94

TABLE 11. Mortality of 2nd instar gypsy moth larvae 4 days after placement on oak foliage treated with 12 BIU of Thuricide 48LV, Dipel 6L and Bactospeine, and exposed to various amounts of natural sunlight.

	Hours sunlight				Percent defoliation			
Material	0.0	1.0	3.0	5.0	0.0	1.0	3.0	5.0
Dipel	59	68	40	49	14	13	21	21
Thuricide	65	70	53	36	10	7	10	15
Bactospeine	61	72	57	46	13	10	21	15
Check	1			0	100			100

Two new strains of B. thuringiensis were tested on a limited basis. Both NRD-10 and NRD-12 appeared to be no more effective than the presently used HD-1 strain when tested without exposure to rainfall. When exposed to 0.5 inches of rainfall, the NRD-12 strain appeared to be less affected.

TABLE 12. Mortality of 2nd instar gypsy moth larvae 4 days after placement on oak foliage treated with 3 strains of B. thuringiensis.

Bt Strain	BIU acre	Percent mortality	Percent defoliation
HD-1	4	49	7
	2	17	19
	1	8	33
NRD-10	4	40	35
	2	24	52
	1	12	60
NRD-12	4	26	27
	2	14	50
	1	3	64
Check	-	0	100

TABLE 13. Mortality of 2nd instar gypsy moth larvae 4 days after placement on oak foliage treated with 12 BIU of 3 B. thuringiensis strains and exposed to 0.5 inches of rain.

Strain	Rain	Sticker	Percent mortality	Percent defoliation
HD-1	-	-	91	4
	x	-	43	23
	-	x	94	3
	x	x	18	35
NRD-10	-	-	83	7
	x	-	24	40
	-	x	88	12
	x	x	15	43
NRD-12	-	-	79	7
	x	-	88	7
	-	x	93	5
	x	x	76	11
Check	-	-	0	90

A new Agway formulation of B. thuringiensis (AGY-038308) was screened and found to be less effective than commercially available formulations.

TABLE 14. Mortality of 2nd instar gypsy moth larvae 4 days after placement on oak foliage treated with 4 B. thuringiensis formulations.

Material	BIU acre	Percent mortality	Percent defoliation
AGY-038308	8	13	84
	12	29	58
	16	14	67
Thuricide 48LV	8	60	26
	12	83	15
	16	77	14
Dipel 6L	8	87	11
	12	95	6
	16	91	8
Bactospeine	8	71	22
	12	72	22
	16	92	10
Check	-	0	92

Three separate tests were conducted to compare the 3 main B. thuringiensis formulations used for gypsy moth control.

TABLE 15. Average mortality (3 replicates) of 2nd instar gypsy moth larvae four days after placement on oak foliage treated with B. thuringiensis formulations.

Material	BIU acre	Percent mortality	Percent defoliation
Thuricide 32LV	8	93	7
Dipel 4L	8	89	9
Bactospeine	8	79	12
Futura	8	81	10
Thuricide 32LV	4	69	15
Dipel 4L	4	69	15
Bactospeine	4	61	24
Futura	4	66	12
Thuricide 32LV	2	55	20
Bactospeine	2	36	46
Futura	12	88	5
Check	-	0	100

All materials used in 1983 experimental field trials were tested on oak seedlings with 3rd and 4th instar larvae as test insects.

TABLE 16. Mortality of 3rd and 4th instar gypsy moth larvae 4 days after placement on oak foliage treated with 12 BIU of B. thuringiensis samples from 1983 experimental field trials.

Material	Instar	Percent mortality	Percent defoliation
Thuricide 48LV	III	34	18
Thuricide 48LV	IV	45	41
Thuricide 64BX	III	45	12
Thuricide 64BX	IV	42	34
Dipel 6L	III	35	18
Dipel 6L	IV	21	100
Dipel 8L	III	28	32
Dipel 8L	IV	32	64
Bactospeine	III	39	21
Bactospeine	IV	26	74
Futura	III	33	21
Futura	IV	37	42
Check	III	0	100

Thuricide 32 LV, Dipel 4L and Bactospeine were mixed at 8 BIU/gallon and allowed to set over a period of time in 4 environmentally different locations. Small samples (500 ml) were held in a greenhouse, a laboratory, in a freezer, and out of doors. Materials were bioassayed at time of preparation and 8 and 23 days following storage.

TABLE 17. Mortality of 2nd instar gypsy moth larvae 5 days after placement on oak foliage treated with 8 BIU of 3 B. thuringiensis formulations held under various climatic conditions for various periods of time.

Material	Environmental condition	Percent mortality days in storage			Percent defoliation days in storage		
		0	8	23	0	8	23
Bactospeine	Outdoors	96	98	80	10	12	17
Bactospeine	Greenhouse	92	89	85	11	12	19
Bactospeine	Freezer	90	88	80	10	19	22
Bactospeine	Freezer (con)	92	100	71	10	10	21
Bactospeine	Laboratory	90	96	95	10	11	10
Thuricide 32LV	Outside	100	100	97	5	6	14
Thuricide 32LV	Greenhouse	98	98	90	5	5	12
Thuricide 32LV	Freezer	98	98	91	10	10	20
Thuricide 32LV	Freezer	98	99	82	10	14	22
Thuricide 32LV	Laboratory	95	95	80	10	8	14
Dipel 4L	Outside	98	98	87	5	6	9
Dipel 4L	Greenhouse	98	96	94	10	10	5
Dipel 4L	Freezer	95	97	97	10	11	19
Dipel 4L	Freezer (con)	96	84	51	10	21	28
Dipel 4L	Laboratory	95	95	82	10	11	12
Check	-	0	0	0	100	100	100

As a result of this year's spraying for gypsy moth, there have been a number of reports concerning phytotoxicity. Fifteen tree species were checked for phytotoxicity. Foliage was sprayed with a hand pump sprayer using a coverage greater than that normally acquired from aircraft treatment. Foliage was removed from the treated trees at 24-hour intervals, and checked for phytotoxicity. Untreated foliage was used as a check. A series of dilutions were used with each material.

TABLE 18. Degree of phytotoxicity on foliage of 15 tree species treated with Dipel 6L, Dipel 8L and Thuricide 64BX, 48 hours prior to visual inspection.

Tree Species	Dipel 6L				Dipel 8L				Thuricide 64BX			
	N	L	M	H	N	L	M	H	N	L	M	H
Poplar				X				X				X
Red oak		X				X				X		
White oak			X				X			X		
Pine		X				X				X		
Plum	X				X				X			
Lilac			X				X				X	
Catalpa				X				X				X
Blueberry			X					X				X
Pear		X					X					X
Birch				X				X				X
Maple				X				X				X
Cucumber	X				X				X			
Peach	X				X				X			
Locust	X				X				X			
Ash	X				X				X			

Above test results based on a dilution of 1.0 part Bt. and 1.0 part water.

N - no evidence of burning
L - light burning and droplets vague
M - burning easy to see and very obvious
H - extensive burning and leaf curl

Four experimental insect growth regulators were tested. BTS-48011, a BFC product, and 141305, a BASF Wyandotte material, gave excellent results. Sumitomo products 4496 and 4624 gave very poor results when compared to Dimilin. Poor results were no doubt due to formulation and volume used.

TABLE 19. Mortality of 2nd instar gypsy moth larvae 6 days after placement on oak foliage treated with S-4496, S-4624 and Dimilin.

Lbs/acre	Percent mortality S-4624	S-4496	Dimilin
0.125	0	1	100
0.0625	0	1	99
0.015	3	0	91
0.0075	1	1	92
0.0037	1	0	98
Check	0	0	0

TABLE 20. Mortality of 2nd instar gypsy moth larvae 6 days after placement on oak foliage treated with 141305I and Dimilin.

Lbs/acre	Percent mortality 141305I	Dimilin
0.25	94	98
0.125	97	98
0.0625	74	99
0.0312	75	97
0.0156	72	96
0.0078	51	98
0.0039	22	96
Check	0	0

TABLE 21. Mortality of 2nd instar gypsy moth larvae 6 days after placement on oak foliage treated with BTS-48011.

Lbs/acre	Percent mortality
0.003	100
0.0015	97
0.00075	75
0.000375	36
0.000187	15
0.0000935	0
Check	0

TABLE 22. Mortality of 2nd instar gypsy moth larvae 6 days after placement on oak foliage treated with BTS-48011 and exposed to various amounts of rain.

Lbs/acre	Percent mortality No Rain	0.5"	1.0"	2.0"
0.4	100	100	100	100
0.2	100	100	100	100
0.1	100	100	100	100
0.05	100	100	100	100
0.025	100	99	100	100
0.0125	100	100	100	100
0.006	100	100	100	100
0.003	100	100	100	99

Two oil formulations of Orthene were tested using cottonseed oil as a solvent. We encountered some difficulty in mixing as the material became very thick.

TABLE 23. Mortality of 2nd instar gypsy moth larvae 3 days after exposure to oak foliage treated with 2 Orthene formulations and exposed to rainfall.

Material	Lbs/acre	Percent mortality	
		No Rain	1.0 Inches Rain
12400	1.0	100	83
12400	0.5	100	34
12400	0.25	96	41
12402	1.0	100	29
12402	0.5	100	36
12402	0.25	100	17
Check	-	0	0

TABLE 24. Mortality of 2nd instar gypsy moth larvae 6 days after exposure to oak foliage treated with BAS-263I and Sevin XLR using acetone and isopropyl alcohol as solvents for BAS-263I.

Lbs/acre	Percent mortality		
		BAS-2631	
	Sevin	Acetone	Isopropyl Alcohol
1.0	100	97	72
0.5	97	82	45
0.25	96	48	42
0.125	98	31	48
0.062	82	20	25
Check	0	0	0

Rhodamine B dye was used as a spray marker in 2 field tests of Alsystin. A small test was conducted to determine if the addition of Rhodamine B would effect the activity of Alsystin.

TABLE 25. Mortality of 2nd instar gypsy moth larvae 7 days after placement on oak foliage treated with 0.06 lbs. of Alsystin and Rhodamine B and with Rhodamine B only.

Material	Percent dye	Percent mortality
Alsystin and Rhodamine B	-	100
Alsystin and Rhodamine B	0.5	80
Alsystin and Rhodamine B	1.0	90
Rhodamine B only	0.5	3
Rhodamine B only	1.0	3
Check	-	0

A new emulsifiable concentrate formulation of Avermectin (MK-936) was tested.

TABLE 26. Mortality of 2nd instar gypsy moth larvae 3 days after placement on oak foliage treated with various amounts of Avermectin (MK-936).

Lbs/acre	Percent mortality
0.01875	100
0.009375	100
0.00468	97
0.00234	100
0.001171	99
0.000585	76
0.000292	49
0.000146	44
0.000073	15
Check	0

TABLE 27. Mortality of 2nd instar gypsy moth larvae 3 days after placement on oak foliage treated with Avermectin (MK-936) and exposed to 0.25 and 0.5 inches of rain.

Lbs/acre	Percent mortality			Percent defoliation	
	no rain	0.25	0.05	no rain	0.5
0.0187	100	86	97	0	36
0.0046	100	79	70	10	95
0.00117	99	32	86	1	47
Check				100	

Avermectin (MK-936) was tested on oak seedlings using sub-lethal dosages of 63, 15, 3.7 and .91 mg/acre. Twenty seedlings were treated with each dosage and 20 newly moulted 2nd instar larvae were placed on each seedling. Twenty untreated plants were used as a check. Larvae were allowed to completely eat the treated foliage over a 4 day period and live larvae were then transferred to artificial diet. Insects were allowed to develop on diet until all had pupated.

Pupae were then sexed and placed into gallon containers for mating. Resulting eggs were held for 30 days at 26°C and were then placed in cold storage (2°C) for 175 days to complete diapause. Eggs were then removed and incubated for hatch tests. Hatch was 80-90 percent in all treatments as well as check.

TABLE 28. Results of sub-lethal dosages of Avermectin (MK-936) on life stages of gypsy moth.

Mg/acre	No. larvae at start	Normal pupae	Deformed pupae	Dead larvae	Number E/M	Percent hatch
63	400	17	3	380	0	0
15	400	175	17	208	67	80
3.07	400	233	51	116	52	80
.91	400	263	35	102	61	80
Check	400	245	28	127	59	80

During 1982 three small field plots were treated with 400 mg/acre of Avermectin (MK-936) using a mist-blower. Control was poor and a number of new egg masses were deposited by adult gypsy moths that had obviously come in contact with the material as larvae. A number of eggs were collected from the treated plots in March of 1983 and checked for hatch. Better than 80 percent hatch occurred from all collected egg masses.

Based on data collected from the field and laboratory tests, Avermectin (MK-936) has little effect on the F1 generation of gypsy moth. Using sub-lethal dosages may result in 5-15 percent of resulting pupae being deformed.

Project Number: GM 3.1.1
Project Title: Field Studies with Bacillus thuringiensis, Pydrin and Alsystin
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leaders: W. H. McLane, J. A. Finney and T. Roland

Alsystin (SIR-8514) has been field-tested for 4 years on 50 to 1,000 acre plots in New Jersey, Rhode Island and Pennsylvania with outstanding results. However, before being submitted for registration, a study of its effect on aquatic and fish life was necessary. With the cooperation of the Mobay Chemical Company and the Pennsylvania State University, three sites, comprised of 2010 acres, were treated with Alsystin 4-flowable. Treatment areas were located on Pennsylvania State Forest and Pennsylvania State Park lands near Mid-State Airport, Philipsburg, Pennsylvania. Dr. E. A. Cameron and his associates at Pennsylvania State University conducted all fish and aquatic studies; those results will not be discussed in this report. USDA involvement was to make aerial application to test sites.

Bacillus thuringiensis formulations were tested on Pennsylvania State Forest lands near Wellsboro, Pennsylvania. Studies were directed at determining the effects of B. thuringiensis applied at various dosages against 3 instars of gypsy moth larvae. Forty-eight 50-acre woodland plots were treated.

Pydrin, under Experimental Use Permit, was applied to 3 50-acre woodland plots. Treatment sites were located in such a manner that the spray would not come in contact with any running water.

Pennsylvania Alsystin Studies

The main treatment location of 960 acres was located in an area where a trout stream crossed through the spray plot, but such that headwaters would not be treated. The stream had a suitable native population of brook trout and aquatic life. A nearby area with a comparable stream was used as a check. The treatment area also had beaver ponds so that studies in standing water could be made. Two plots of smaller size were also treated with lower dosages.

TABLE 1. Alsystin test parameters with pre-spray egg mass counts.

Plot number	lbs/AI acre	Number of acres	Pre-spray egg masses per acre
1	.06	960	558
2	.03	650	215
3	.015	400	206
Check	---	---	282

Treatment areas were located so that nearly all corners were on or near roadways. Corners were marked with orange surveyor tape and tags containing plot information were attached to each corner tree. Pre-spray egg mass counts were made in each treatment area and check plot during early April. Ten prism points were established at 3 locations within each plot to determine egg mass density. Egg masses were counted on all prism trees and on a fixed radius plot at each point. The true DBH was recorded for each tree included in the survey.

On April 6, 1983, Mobay Chemical Corporation was granted a Federal Experimental Use Permit for Alsystin to be used in Pennsylvania (312-EUP-183). Approval for the study was also granted by the Pennsylvania Department of Environmental Resources, Pennsylvania Fish Commission, Federal Aeronautics Administration and Pennsylvania Aeronautics Commission.

Treatments were applied June 9 and 10, 1983. Gypsy moth larvae were 80 percent 2nd instar and foliage development was near 50 percent, except on white oak where it was somewhat less.

Mixing and aircraft operations were conducted out of Mid State Airport, Philipsburg, Pennsylvania. The 4-flowable material was mixed in a nurse tank before being transferred to the aircraft. No sticker was used; however, Rhodamine-B dye was used so that spray deposit would be more visible on white kromekote cards.

Application was made with a Turbo-Thrush aircraft and conventional spray equipment. Twenty-two 8006 flat fan nozzles were pointed 90° to the aircraft slipstream. Speed of the aircraft was 125 mph, using a boom pressure of 40 psi and a swath width of 100 feet. The aircraft flew 20 to 30 feet above treetop level. Plot corners were marked with kytoons and radios were used to communicate between ground crews and aircraft.

A defoliation survey was conducted in each treated and check area during mid-July. All treated areas had defoliation in the range of 0 to 5 percent. Defoliation in the untreated area was 25 to 30 percent.

Post-spray egg mass counts were made in all plots during November. Sampling techniques were the same as those used for pre-spray counts.

TABLE 2. Egg mass data from Alsystin experimental spray plots.

Plot Number	Pre-treatment egg masses/acre	Post-treatment egg masses/acre	Percent egg mass change
1	558	42	- 92
2	215	0	-100
3	206	21	- 90
Check	282	726	+ 61

Alsystin again was demonstrated effective against gypsy moth larvae. Registration of this material should be forthcoming in 3 years or less.

The 4-flowable formulation was a superior mix compared to 25 w/p. It resulted in easy handling and mixing and did not settle out, as 25 w/p was observed to do when allowed to stand for a short period of time.

Pennsylvania Bacillus thuringiensis and Pydrin Studies

Forty-eight 50-acre plots to be treated with 6 B. thuringiensis formulations were established in the Tioga Forest District. Three 50-acre plots were also established for the application of Pydrin. Ten untreated plots were used as check blocks.

Using a compass, topographical maps, rope and surveyor tape, plots were established and allocated to the treatments listed in Table 3. Boundary lines were surveyed and marked in fluorescent orange tape and each corner tree was marked with double fluorescent orange tape and a tag identifying corner and plot number. Minimum distance between plots was 400 feet. Plots were located so that there would be the maximum number of corners on or near roadways.

TABLE 3. Materials tested against gypsy moth larvae in 1983 experimental spray program in Pennsylvania, using 96 oz/acre.

	Dosage BIU/acre	Instar II	III	IV
Thuricide 48 LV	12	x	x	x
Thuricide 48 LV	16	x	x	x
Thuricide 48 LV	20	x	x	x
Thuricide 64 BX	12 1/	x		
Dipel 6L	12	x	x	x
Dipel 8L	12 1/	x		
Bactospeine	12	x		
Futura	12 1/	x		
Pydrin	.06 lbs AI/ac	x		
Check				

1/ Volume applied per acre was 64 ounces.

Within the center 10 acres of each plot, 20 prism points were established, 5 points on 4 parallel lines. During March and early April, pre-spray egg mass counts were made at each prism point. New egg masses were counted and recorded on each prism tree and within each fixed radius plot. Prism tree DBH was also recorded. A limited number of egg masses were collected from the field and returned to the laboratory for hatchability tests. Hatch was uniform at 80-90 percent.

Shell Development Company and Sandoz, Inc. received Experimental Use Permits to apply Pydrin and Thuricide 48 LV (20 BIU/acre), respectively. Approval for the study was granted by the Pennsylvania Department of Environmental Resources, Federal Aeronautics Administration and Pennsylvania Aeronautics Commission. On March 28, the Pennsylvania Fish Commission was notified about our planned field work. Dr. A. Cameron, Dr. R. Light and W. McLane met with

H. Hesser and M. Marino of the Fish Commission on May 12, 1983 to discuss our various studies. We went over materials to be used and plot selection. At the conclusion of the meeting, all were in agreement that the studies should be conducted as outlined and that planned activities were seen to be of little, if any, threat to fish life.

Just prior to spraying, State and local police were notified. Spray applications were started on June 1 and ended June 22.

TABLE 4. Gypsy moth larval size at time of treatment.

Material	BIU and lbs/acre	Planned larval size	Percent of larvae in various instars at time of treatment				
			I	II	III	IV	V
Thuricide 48	12	II	70	30	-	-	-
Thuricide 48	12	III	-	20	65	15	-
Thuricide 48	12	IV	-	-	20	60	20
Thuricide 48	16	II	70	30	-	-	-
Thuricide 48	16	III	-	20	65	15	-
Thuricide 48	16	IV	-	-	20	60	20
Thuricide 48	20	II	70	30	-	-	-
Thuricide 48	20	III	-	20	65	15	-
Thuricide 48	20	IV	-	-	20	60	20
Thuricide 64	12	II	5	85	10	-	-
Dipel 6L	12	II	60	40	-	-	-
Dipel 6L	12	III	-	20	65	15	-
Dipel 6L	12	IV	-	-	20	60	20
Dipel 8L	12	II	70	30	-	-	-
Bactospeine	12	II	60	40	-	-	-
Futura	12	II	20	75	5	-	-
Pydrin	.06	II	15	80	5	-	-

Mixing and aircraft operations were conducted out of Canyon Airport, Wellsboro, Pennsylvania. All materials, excluding Pydrin, were mixed in a nurse tank before being transferred to the aircraft. All *Bacillus thuringiensis* mixes contained 2 percent RA-1990 by volume. The sticker was screened through a 50-mesh screen as it entered the nurse tank. Water was first added to the nurse tank, then *B. thuringiensis*, and sticker. All mixes were blended by agitation for at least 10 minutes before loading into aircraft. The nurse tank was equipped with a 50-mesh in-line screen. Pydrin was mixed with soybean oil in the aircraft hopper.

The aircraft spray system and nurse tank were thoroughly cleaned between formulation changes. There were no mixing and/or handling problems encountered with Thuricide, Dipel or Pydrin. We had no buildup of sticker on in-line or nozzle screens. Only one clogged nozzle was encountered during the entire program.

Bactospeine-35 pumped well from a 55-gallon drum and appeared to mix well. However, when the final mix was transferred to the aircraft, the main in-line 50-mesh filter clogged completely after a transfer of only 25 gallons. In order to complete the transfer of 135 gallons, the main screen had to be cleaned 4 times. When Futura was used, the same thing occurred and the screen had to be cleaned a number of times before it could all be transferred to the aircraft. The foreign matter causing the problem appeared to be undissolved fish meal and/or other similar material used in the fermentation process. Although there was a small amount of paint flakes from within the recycled drums, it appeared that this was not the main cause of screen clogging. The Futura formulation was extremely thick and would be very difficult to work with on an operational program.

A Turbo-Thrush aircraft equipped with conventional spray equipment was used to apply all B. thuringiensis and Pydrin. All applications were made with 8006 flat-fan nozzles using 50-mesh nozzle screens. The aircraft was equipped with a 50-mesh in-line screen and quick drain valves.

TABLE 5. Aircraft and boom characteristics.

Material	Dosage BIU/acre	Rate (oz/ac)	Number nozzles	Pressure (PSI)
Thuricide 48 LV	12-16-20	96	34	38
Thuricide 64 BX	12	64	23	38
Dipel 6L	12-16-20	96	34	36
Dipel 8L	12	64	22	38
Bactospeine	12	96	34	38
Futura	12	64	22	38
Pydrin	.06 1/	64	23	38

1/ lb/acre.

The aircraft was calibrated for a 100-foot swath and travelled at 135 mph, 20-30 feet above tree-top level.

Three days following treatment, 100 larvae were collected from each plot and placed into plastic cups containing 90 ml of artificial diet. Ten larvae were placed into each cup when 2nd and 3rd instar were collected. When 4th instars were collected, 5 were placed into each cup. Larvae were also collected from untreated plots. All collections were made well within the plot boundaries (500'). Because of a lack of oak foliage within reach of the collectors, a large percentage of larvae were collected from low-growing vegetation such as witch hazel. As a result, larvae collected may not have been exposed to the B. thuringiensis to the extent that those in the top part of the canopy were. Personnel had some difficulty in finding the necessary number of larvae in some treatment plots. Once collected and placed in containers, they were held under field conditions in a camper-trailer. Mortality readings were made and

recorded at various times. Larvae that were collected from plots treated at the 4th instar stage evidently did not adjust to artificial diet and starved to death; all 4th instar larvae collected died within a week from the time collected. Younger larvae adjusted to the change from foliage to artificial diet.

TABLE 6. Percent mortality of gypsy moth larvae collected from the treated and untreated plots 3 days after treatment.

Material	BIU/ acre	Larval instar	Percent mortality on days following treatment 7	10	14	21
Thuricide 48 LV	12	II	33	37	40	57
Thuricide 48 LV	16	II	28	33	40	56
Thuricide 48 LV	20	II	49	51	61	69
Thuricide 48 LV	12	III	42	51	62	80
Thuricide 48 LV	16	III	67	78	90	96
Thuricide 48 LV	20	III	34	51	74	89
Dipel 6L	12	II	42	48	52	64
Dipel 6L	12	III	45	59	79	86
Dipel 8L	12	II	41	48	53	63
Bactospeine	12	II	48	53	56	67
Check	--	II	8	8	15	34
		III	4	6	20	48

Post-spray defoliation surveys were made in all spray plots and checks at the time of peak defoliation in mid-July. Treated areas and large acreage of untreated forest land had far less defoliation than expected. Within all treated plots, defoliation was so light (0 - 5 percent) that it was impossible to distinguish differences between plots. Check plots averaged slightly higher defoliation (15 percent). All plots were also observed and photographed by air. Some spray plots were fairly visible from the air, but did not stand out as they would have, if there had been more extensive defoliation in the untreated areas.

Following fall foliage drop, post-spray egg mass counts were made in each plot. Twenty points were sampled in each plot using the same technique as was used for pre-spray calculations. All ribbon and tags were removed from each plot.

TABLE 7. Percent egg mass change in B. thuringiensis and Pydrin spray plots, based on an average of 3 replications.

Material	BIU/ acre	Larval instar	Pre- em/ac	Post- em/ac	Percent change
Thuricide 48 LV	12	II	237	39	- 84
Thuricide 48 LV	12	III	351	74	- 79
Thuricide 48 LV	12	IV	218	393	+ 45
Thuricide 48 LV	16	II	118	50	- 58
Thuricide 48 LV	16	III	286	66	- 77
Thuricide 48 LV	16	IV	169	93	- 45
Thuricide 48 LV	20	II	107	23	- 78
Thuricide 48 LV	20	III	133	53	- 60
Thuricide 48 LV	20	IV	307	273	- 11
Thuricide 64 BX	12	II	17	7	- 59
Dipel 6L	12	II	125	28	- 78
Dipel 6L	12	III	150	133	- 11
Dipel 6L	12	IV	189	194	+ 3
Dipel 8L	12	II	243	31	- 87
Bactospeine	12	II	71	23	- 68
Futura	12	II	179	75	- 58
Pydrin	.06[1/]	II	276	6	- 98
Check	--	--	237	601	+ 61

B. THURINGIENSIS RESULTS

Pre-spray egg mass density was much lower than had been expected and, as a result, defoliation was light in the study area. Based on defoliation data, no differences were observed between the various treatments, regardless of dosage and/or larval instar. Untreated check plots did suffer approximately 10 percent more defoliation than any treated area. Therefore, all treatments were effective in reducing defoliation.

Based on mortality of collected larvae, data show mortality to be in the 35 percent range when 2nd instar larvae are exposed to 12 BIU/acre. All B. thuringiensis formulations gave similar results regardless of whether they were applied at 96 or 64 oz/acre. When 3rd instar larvae were treated with the same dosage, mortality tended to increase. This may have been due to larger larvae consuming more foliage and therefore more B. thuringiensis. Treatments of 16 BIU/acre to 2nd instar resulted in no more mortality than applications of 12 BIU/acre. However, when applied to 3rd instar, larvae mortality increased by ca. 50 percent. Application of 20 BIU/acre to 2nd

instar increased mortality by 20 percent over dosages of 16 BIU/acre; however, mortality was somewhat lower when applied to 3rd instar larvae.

Egg mass density changes show little difference among all formulations used on 2nd instars at 12 BIU/acre. Thuricide 48 LV and Dipel 8L appeared to be more active, and Futura and Thuricide 64 BX less active. This may be due to a difference between 96 and 64 oz/acre. However, Dipel 8L was applied at 64 oz/acre. Thuricide 48 LV against 2nd and 3rd instar larvae at 12-16-20 BIU/acre demonstrated little difference in activity using any combination. Dipel 6L used at 12 BIU/acre against 3rd instar larvae was much less effective than Thuricide. Dipel 6L and Thuricide 48 LV were not effective against 4th instar larvae used at 12 BIU/acre. A reduction of 45 percent was recorded when Thuricide 48 LV was used at 16 BIU/acre against 4th instar larvae. Twenty BIU/acre of Thuricide 48 LV only gave an 11 percent reduction of 4th instar larvae. However, if only 2 of the replications were used, we would have had a reduction of 91 percent. There was a healthy increase of 61 percent in 10 check plots.

SUMMARY:

Alsystin was very effective in these tests as evidenced by dramatic reductions in egg mass density. This material will not be registered for use before 1985 at the earliest.

Pydrin used with soybean oil was very effective (98 percent reduction) against 2nd instar larvae when used at .06 lbs AI/acre. There is concern about its toxicity to fish and aquatic life and this will limit its use on forest land, if it is registered; it will likely be 3 years or more before registration. Pydrin can be a very useful material for homeowner treatments applied by a commercial applicator using mist-blower and/or hydraulic equipment.

Applications of B. thuringiensis gave similar results against 2nd and 3rd instar larvae at 12-16-20 BIU/acre. When treating 4th instar and larger, a minimum of 16 BIU/acre should be used. Thuricide 48 LV and Dipel 8L gave egg mass reduction in excess of 80 percent at 12 BIU/acre against 2nd instar larvae. Dipel 8L gave excellent population reduction at 12 BIU/64 oz/acre. Based on this year's work, in a light, building population, little is gained by using dosages greater than 12 BIU/acre against 2nd and 3rd instar larvae.

All materials mixed and handled well, with the exception of Bactospeine and Futura. Because of foreign material in the formulations, excessive clogging of in-line screens occurred with Bactospeine and Futura. Unless the formulations are cleaned up, they should not be used in an operational program. Futura is also extremely thick and may cause handling problems under cold field conditions.

Each B. thuringiensis formulation contained 2 percent RA-1990 (Monsanto) sticker by volume. We encountered no problems at all with the use of RA-1990 and only clogged one nozzle during the entire operation.

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Project Number: GM 3.1.2
Project Title: 1983 Eradication Projects with Bacillus thuringiensis
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: W. H. McLane, G. Moorehead, K. Kruse and D. Keim

Improved trap design, superior pheromone formulations and improved survey techniques have resulted in the detection of male gypsy moths in a number of states located outside of the generally infested Northeast. Over the past 5 years, infestations have turned up in Wisconsin, Illinois, California, Oregon, Washington, South Carolina, Tennessee, Florida and Arkansas. Most isolated infestations have been detected in residential areas and can be traced to the movement of households from the northeastern states.

When detected and delimited, isolated infestations are treated with the latest control techniques. Dimilin and Sevin have often been used to eliminate small spot infestations, but recently, Bacillus thuringiensis (Bt) has been identified as an attractive potential alternative. Historically BT had given erratic control of gypsy moth larvae in the northeastern states. Recently, new formulations, effective stickers, increased dosages and lower cost have renewed interest in this biological material. During 1983, over 300,000 acres were treated with BT to control gypsy moth larvae. Single treatments of 12 BIU/acre normally prevent most defoliation and lower egg mass density by 70 percent. Small plot tests during 1981 demonstrated the advantage of applying multiple applications of BT to suppress gypsy moth populations.

For the past 2 years, APHIS has cooperated with State agencies in applying multiple application of BT to isolated infestations of gypsy moth. Some treatments have been coupled with mass trapping. During 1982, six applications of Dipel 4L were applied to a 15 square mile area at Santa Barbara, California. Where egg masses were found, 3 applications of carbaryl were applied with ground equipment (276 acres). A mass trapping program was utilized in addition to the normal detection traps.

Table 1. History and treatment of Santa Barbara, California gypsy moth infestation.

Year	Males trapped	Eggs found	Acres treated Bt	Acres treated Carbaryl
1979	2	0	-	-
1980	4	0	-	-
1981	41	4	-	-
1982	0	0	9,600 2/	300 3/
1983	3 1/	0	-	-

1/ Two finds are 0.5 miles from the 1982 spray boundary and the third was one mile away.
2/ Applied by helicopter at 12 BIU/acre. There were 6 applications of 120 oz.
3/ Applied with hydraulic spray equipment using approximately 1.0 lbs AI/acre around sites of egg mass finds. Three applications were made.

In the spring of 1983, Plant Protection and Quarantine began efforts to eradicate six outlying gypsy moth infestations with BT or combinations of BT and mass trapping. Eradication activities at these infestations located in Illinois and Wisconsin are being conducted in cooperation with the following State and local governmental entities:

Illinois Department of Agriculture
Wisconsin Department of Agriculture
City of Bensenville, Illinois
City of Downers Grove, Illinois
City of Naperville, Illinois
City of Wheaton, Illinois
City of Wood Dale, Illinois
City of Elm Grove, Wisconsin
Addison Township in DuPage County, Illinois
DuPage County Forest Preserve, Illinois
St. Charles Township in Kane County, Illinois

The technologies applied were:

1. Two aerial applications of 2 quarts 35 BIU BT plus 2.5 ounces of Plyac sticker and 2 quarts of water to equal 1 gallon per acre, followed by mass trapping at 3 traps per acre.

2. Three aerial applications of 2 quarts 35 BIU BT plus 2.5 ounces of Plyac sticker and 2 quarts of water to equal 1 gallon per acre, followed by 32 traps per square mile (for evaluation and delimitation).

Table 2. The 1981-1983 history of moth catches and control or eradication actions plus recommended 1984 eradication actions are as follows:

Location	Year	Acres Treated	Applications and Dosages (BIU)	Type Trapping[1]	Moths Caught
Naperville, IL	1981			Del	36
	1982[2]	60	2 x 8	Del	17
	1983	40	2 x 17.5	MT (200)	0
	1984[4]			Del	
Wood Dale/ Bensenville, IL	1981			Del	1479
	1982[2]	500	2 x 8	Del	166
	1983	989	2 x 17.5	MT (1532)	13
	1984			MT (400)	
Wheaton, IL	1981			Del	286
	1982[2]	300	2 x 8	MT (300)	103
	1983	230	2 x 17.5	MT (800)	5
	1984[4]			MT (80)	
Downers Grove, IL	1981			Del	73
	1982[3]	800	2 x 8	MT (800)	28
	1983	50	2 x 17.5	MT (367)	1
	1984[4]			MT (40)	
St. Charles, IL	1981			Det	5
	1982			Del	26
	1983	90	3 x 17.5	Del	1
	1984[4]			MT (40)	
Elm Grove, WI	1981			Det	2
	1982			Del	70
	1983	60	2 x 17.5	MT (110)	5
	1984[4]			MT (300)	

1/ MT (acres) - number of acres mass trapped at 3 traps per acre.
Del - delimited at 32 traps per square mile
Det - detection survey at 1 trap per square mile

2/ Two aerial applications of Sevin-4 oil were aborted. Infestations were partially treated with BT followed by mass trapping.

3/ Two aerial applications of Sevin-4 oil were aborted. BT was applied over the identical area followed by mass trapping.

4/ The 1984 plans are recommendations.

In 1983, our efforts to eradicate six isolated infestations in Illinois and Wisconsin with application of BT or combination of BT and mass trapping equalled or surpassed our expectations. Our conclusions are that (1) eradication has been accomplished at Naperville, (2) eradication has been accomplished or has progressed toward that objective at the other five infestation locations, and (3) unusually high environmental stresses (extreme winter cold) could have been placed upon the life stages of the gypsy moth during 1982-1983 which would have biased these results towards eradication. Subsequent work will determine whether these results in general can be duplicated.

The 1984 program of continued mass trapping will verify eradication or continue the eradication pressure on these infestations.

During 1983, four locations within the State of Washington were treated with multiple applications of BT to control gypsy moth. Three 1 gallon applications were applied by helicopter at 16 BIU/acre.

Table 3. History and treatment of 4 gypsy moth infestations in Washington.

Location	Males trapped 1982	Males trapped 1983	Acres treated 1983	Acres mass trapped
Ravenna Park 1/	400	37	1,040	1,040
Tacoma - East	117	5	800	-
Tacoma - West 2/	253	33	320	-
Vancouver	10	0	360	-

1/ 1983 trap patterns indicate two possible foci of reproduction are still present near the core of the treatment block site where 1981 treatments were applied (17th Avenue) and where egg masses were found in 1982 (12th Avenue).

2/ All 1983 moths were caught in the edge of the treatment block and it is likely they originated from separate areas of infestation to the west.

Summary

The treatment conducted at Santa Barbara indicates that very sparse isolated gypsy moth infestations can be reduced to undetectable levels in a one year period using multiple (6) applications of BT by aircraft and ground applications of Carbaryl. However, the effectiveness of BT should not entirely be based on these results. Carbaryl was applied directly to the areas where egg masses were found and may have been mainly responsible for reducing the population to undetectable levels during 1982.

Treatments in Washington, Illinois and Wisconsin using 2 and 3 applications of BT reduced male moth detection substantially. Areas in Illinois where BT was applied in 1982 and 1983 had fewer moths than areas where treatment was made only in 1983. Although multiple applications (2-3) are being made in any given year, infestations may need to be treated for 2 or more years consecutively before moth detection will be eliminated.

Over the past 2 years we have learned that BT can be used successfully to treat isolated infestations. As more is understood about dosages, rates, stickers, number of applications and timing, our success rate will improve.

Project Number: GM 3.1.3
Project Title: Laboratory Screening and Field Testing of Pyrethroids and Natural Pyrethrins Against the Gypsy Moth
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leaders: E. C. Paszek, R. Kelliher, W. H. McLane

The objective of this project was to collect and evaluate mortality data on a natural pyrethrin and 2 synthetic pyrethroids. These are powerful, degradable contact insecticides that give a rapid initial knockdown of treated insects and have a low order of toxicity to warm-blooded animals. By combining them with latex to increase their residual action, they could effectively be used against gypsy moth larvae in their later instars.

Fenvalerate (Evercide®), a synthetic pyrethroid, and pyrenone, a natural pyrethrin, were screened against 4th and 5th instar gypsy moth larvae. Cotton fabric tapes[1/] were treated with 3 formulations of these 2 insecticides at dosages of 1, 5, and 10 percent of the active ingredients. Resmethrin[2/], a synthetic pyrethroid, in combination with d-trans allethrin, with an active ingredient of 2.62 percent, were also screened. The larvae were exposed at time intervals of 1, 2, 5, 10, 15, 30, 60 and 120 minutes.[3/] They were transferred into rearing dishes and placed in a temperature-humidity controlled chamber. In the pyrenone screenings of 2, 5, and 10 minute intervals in all of the treatments A, B, and C, no mortality or symptoms of poisoning were observed. However, in the 2 hour observations of the 60 and 120 minute screening intervals for treatments A and B, dosage rates of 5 and 10 percent, there was a high percentage of larvae knocked down with symptoms of poisoning. At the 48 hour observation, all of the larvae recovered and were feeding (Table 1). In the Resmethrin-d-trans allethrin combination, there were no symptoms of poisoning or mortality in any of the 6 screening intervals. The Fenvalerate tapes treatments A and B had a high percentage of mortality in all of the screening intervals at the 10 percent dosage rate. In treatment C, where the tapes were saturated with latex and then overcoated with Fenvalerate in an acetone solution, there was a low rate of mortality. Fenvalerate, when not incorporated into latex, is not an effective insecticide on gypsy moth larvae.

1/ Tapes 3" in width, prepared by Tim Nestor and Mark Chase, 19 Highland Avenue, Randolph, Massachusetts 02368 by the following treatments: A-tape saturated with insecticide in latex solution; B-tape saturated with insecticide in latex and overcoated with insecticide in acetone solution; C-tape saturated with latex and overcoated with insecticide in acetone.

2/ This insecticide was formulated into a 2" wide styrofoam-type band and is marketed under the name of Drop Dead Tree Band by the Tuxis Corporation, Ltd., Madison, Connecticut 06443.

3/ 10.9" of the insecticide-treated tapes were placed in the bottoms of open metal petri dishes, 3.75" in diameter. Repl'M II®, a sticky, gypsy moth caterpillar tape 2" in width, was wrapped around the open dishes forming an open testing container with a 1.5" high escape barrier.

Screenings were continued with Fenvalerate at the dosage rates of 5 and 10 percent. Since latex is supposed to prolong the residual effect of this insecticide, a procedure was devised to attempt to break down the Fenvalerate latex combination by means of sunlight.4/ Tapes were exposed to UV light 11 times from February 14 to March 17, 1983, for a total cumulative exposure time of 17 hours (Tables 2 and 3). After each exposure to UV light, the tapes were screened, along with "not exposed" (control) tapes, to larvae at intervals of 1/4, 1, 2, 5, 10, 30 and 60 minutes. The mortality Tables 2 and 3 indicate that the 2 sets, exposed and not exposed at the 5 and 10 percent dosages, killed a high percentage of the larvae in the 7 screening intervals. Only at the 1/4 minute interval was the mortality 25 percent or less. The UV light-exposed tapes in both treatments A and B had a higher percentage of mortality than the not exposed controls (Graphs 4 and 5). A production run Fenvalerate tape treatment A, 10 percent A.I. was exposed to 4 hours of UV light. It compared favorably to treatment A of the original tapes that were exposed to 17 hours of UV light (Table 6).

The UV light breaks down the latex in the Fenvalerate-latex combination, releasing the insecticide. When the UV light cabinet door was opened, there was a slight odor of burning rubber. From the above data and observations, it is concluded that latex gives Fenvalerate a longer residual life, and sunlight slowly breaks down the combination, releasing the insecticide.

4/ Tapes exposed in a 54 X 36 X 18" cabinet 3.75" below a 40w sunlamp and a 40w blacklight blue lamp. The tapes rested upon a stand 7" above the cabinet floor. A #2 filter was suspended 0.75" beneath the lamps to screen out harmful rays below 280 nanometers, which are not normally encountered in the field because they are filtered out by the ozone layer in the atmosphere.

TABLE 1. Percent mortality of 4th and 5th instar larvae placed on the following unaged insecticide tapes.1/

Active ingredient	Fenvalerate (5 reps)									Pyrenone (1 rep.)								
	10 percent			5 percent			1 percent			10 percent			5 percent			1 percent		
	A2/	B3/	C4/	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Treatment Minutes larvae were on tape																		
120	-	-	-	-	-	-	-	-	-	705/	506/	0	-	-	-	-	-	-
60	99	100	46	45	75	65	0	0	10	557/	0	0	-	-	-	-	-	-
30	85	94	31	15	55	10	-	-	-	-	-	0	-	-	-	-	-	-
15	79	94	14	50	45	15	-	-	-	-	-	-	-	-	-	-	-	-
10	78	76	29	40	15	40	0	0	0	0	0	-	0	0	0	0	0	0
5	61	74	19	20	35	30	5	5	0	0	0	0	0	0	0	0	0	0
2	73	73	35	20	0	10	0	0	0	0	0	0	0	0	0	0	0	0
1	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0	0
0	0	0	0	-	-	-	-	-	-	0	0	0	-	-	-	-	-	-

1/ 20 larvae/container.
2/ A tape saturated with insecticide in latex solution.
3/ B tape saturated with insecticide in latex solution and overcoated with insecticide in acetone solution.
4/ C tape saturated with latex and overcoated with insecticide in acetone solution.
5/ At 2-hour check, 67 percent of larvae knocked down with symptoms of poisoning; at 48 hours, all larvae recovered.
6/ At 2-hour check, 50 percent of larvae knocked down with symptoms of poisoning; at 48 hours, all larvae recovered.
7/ At 2-hour check, 58 percent of larvae knocked down with symptoms of poisoning; at 48 hours, all larvae recovered.

TABLE 2. Percent mortality of 4th and 5th instar gypsy moth larvae placed on Fenvalerate tape, 10% A.I. Tape exposed to UV light at various intervals. (Controls - treated fabric tape not exposed to UV light).

Treatment	0.25[1]		0.75		1.5		2.5		3.75		5.25		7.0		9.0[2]		11.5		14.0		17.0	
	Hours after exposure that mortality readings were made																					
	96		72		48		48		96		72		144		144		120		120		96	
	A[3]	B[4]	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Minutes larvae were on tape																						
60 UV exposed	100	100	100	100	100	100	100	100														
Control	100	100	100	100	100	100	100	100														
30	100	100	100	100	100	100	100	100	100	95	100	100	100	75	93	93	100	100	80	100	100	100
	95	100	100	100	100	100	100	100	75	100	95	100	75	85	100	93	87	80	87	80	94	100
15	100	100	100	100	100	100	100	95	100	95	100	95	100	100	100	100	100	81	74	80	94	74
	70	90	85	100	100	100	100	100	100	100	85	95	100	90	100	80	67	80	67	54	87	74
10	95	100	100	100	100	100	100	100	100	100	75	100	65	100	100	100	100	94	80	80	80	94
	70	85	95	100	95	90	100	100	85	80	90	100	80	95	100	80	54	54	67	60	74	67
5	65	85	100	100	100	100	100	100	100	100	100	95	65	100	94	94	80	100	87	80	100	100
	75	35	95	100	90	90	100	100	100	85	75	95	55	75	80	80	54	54	34	60	67	87
2	90	100	100	100	100	100	100	100	80	80	100	95	100	90	100	94	54	100	94	94	67	94
	50	100	90	100	85	85	100	100	60	60	85	85	60	65	74	67	87	80	74	74	54	87
1									98	85	70	90	90	80	100	67	67	87	87	87	87	74
									25	30	90	95	35	80	80	94	54	47	74	87	60	47
1/4																					60	58
																					47	34
0	0	0	0	0	0	0	0	0	0	0	0	0	45	15	14	20	7	14	14	20	14	27

1/ UV treated tapes exposed from 0.25 to 7 hrs, 20 larvae/container.
2/ UV treated tapes exposed from 9.0 to 17.0 hrs, 15 larvae/container.
3/ Treatment A, fabric tape saturated with insecticide in latex solution.
4/ Treatment B, fabric tape saturated with insecticide in latex solution and overcoated with insecticide in acetone solution.

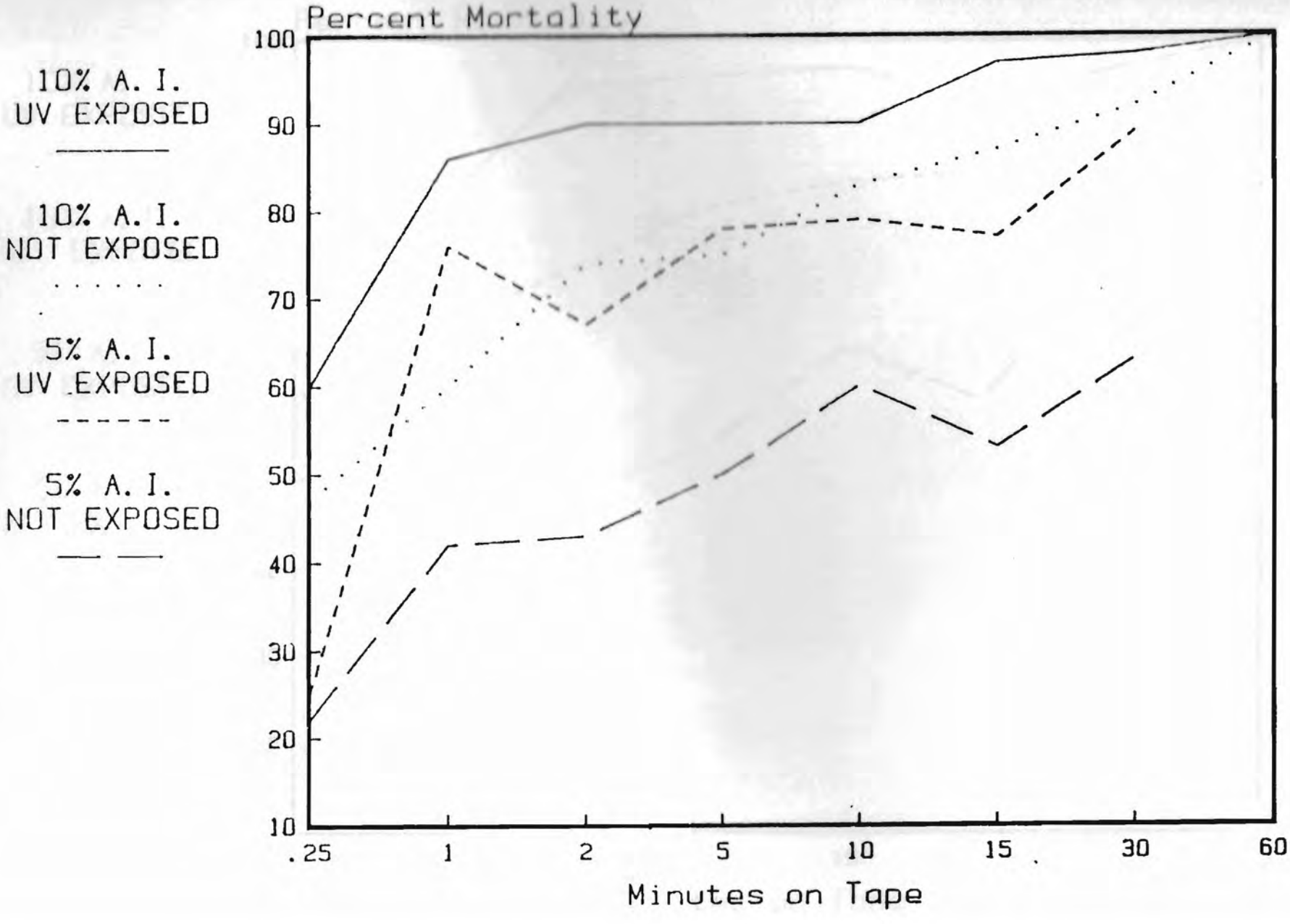
TABLE 3. Percent mortality of 4th and 5th instar gypsy moth larvae placed on Fenvalerate tape, 5 percent A.I. tape exposed to UV light at the following hourly intervals. (Controls - treated fabric tape not exposed to UV light).

Minutes larvae were on tape	5.25 1/		7.0		9.0 2/		11.5		14.0		17.0	
	Hours after treatment that mortality readings were made:											
	72		144		144		120		120		96	
	A 3/	B 4/	A	B	A	B	A	B	A	B	A	B
30 UV exposed	100	100	80	90	100	100	80	74	87	80	87	87
Control	100	95	55	70	80	80	34	74	60	47	46	100
15	100	95	85	100	100	87	54	74	47	80	74	74
	100	95	65	70	40	34	47	74	40	27	27	54
10	100	100	85	100	67	100	94	67	54	80	74	54
	95	95	55	85	47	60	60	54	47	54	54	40
5	100	100	80	100	94	80	54	87	47	47	94	74
	90	95	70	60	27	40	20	40	20	34	74	54
2	100	95	75	70	66	94	60	60	60	87	40	40
	80	80	55	50	34	54	20	34	34	40	34	27
1	95	100	85	55	67	87	40	40	80	47	87	74
	55	75	60	65	40	67	7	40	47	-	40	34
1/4											25	20
											22	16
0	0	0	45	15	14	20	7	14	14	20	14	27

1/ UV treated tapes exposed from 5.25 to 7 hrs, 20 larvae/container
2/ UV treated tapes exposed from 9 to 17 hrs, 15 larvae/container
3/ Treatment A, fabric tape saturated with insecticide in latex solution
4/ Treatment B, fabric tape saturated with insecticide in latex solution and overcoated with insecticide in acetone solution.

Fenvalerate Tapes - Treatment A

% Mortality of 4th Instar GM



Fenvalerate Tapes - Treatment B

% Mortality of 4th Instar GM

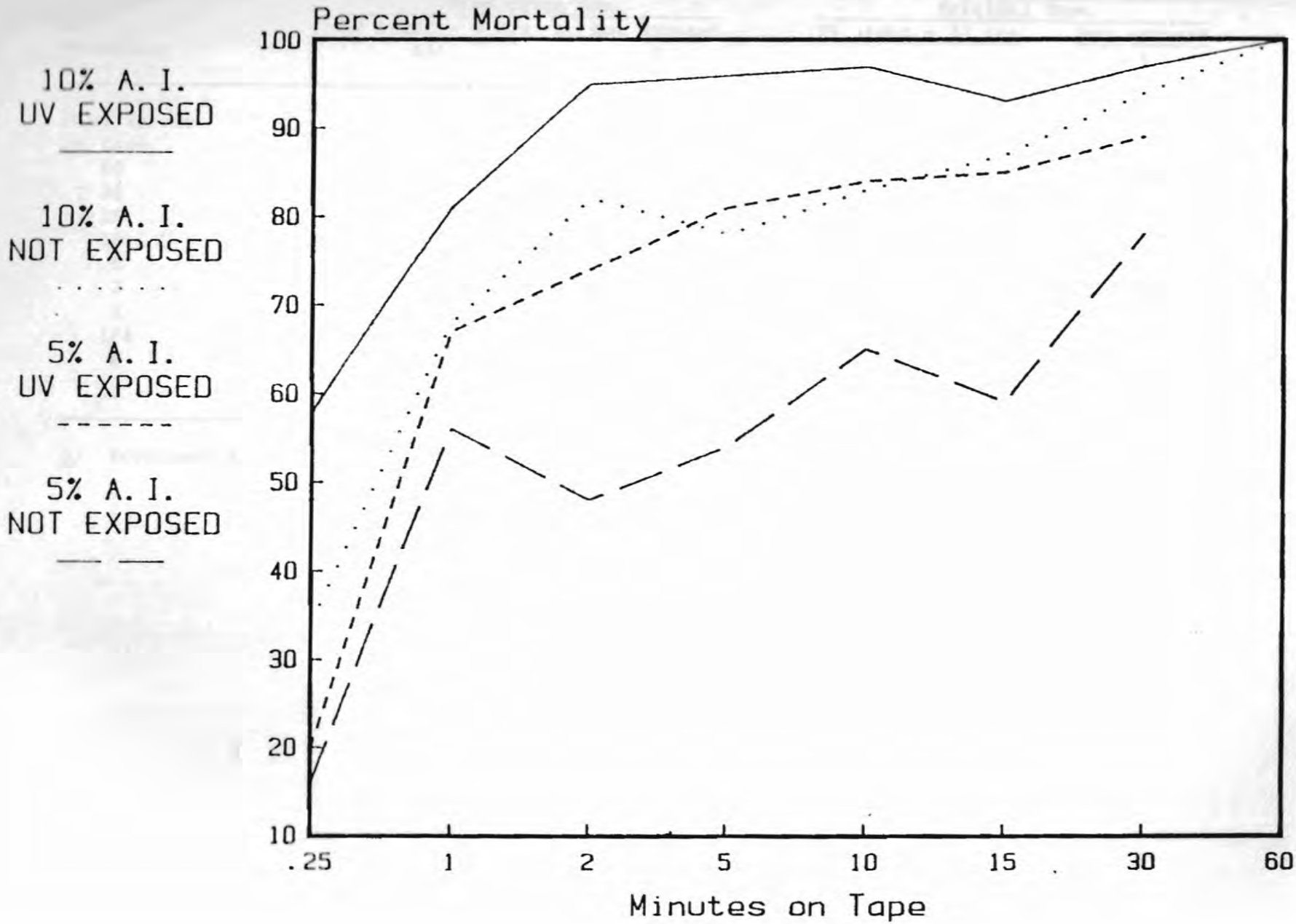


TABLE 6. Percent mortality of 4th and 5th instar gypsy moth larvae placed on 1st production run Fenvalerate tape, 10 percent A.I.

Treatment	Production Run		Original Tape	
	UV light - 4 hrs	Not exposed	UV light - 17 hrs	Not exposed
	A[1/]	A	A	A
Min. larvae on tape				
60	90	65	50	90
30	40	40	70	20
15	10	25	40	10
10	20	15	30	30
5	15	15	70	20
2	5	0	100	0
1	0	20	40	20
1/4	0	0	30	0
0	0	0	0	0

1/ Treatment A, tape saturated with Fenvalerate in latex solution.

A bioassay of Fenvalerate tapes was done in a natural infestation by banding oak trees to determine if this would prevent or reduce defoliation. Gypsy moth larvae migrate away from their feeding stations to resting places throughout all larval instars. In the 1st and 2nd instars, when they are feeding in the canopy of the trees, the migrating distance is short, usually from the underside of an expanding leaf to its stem. The migrating distance lengthens in the 3rd instar; they rest under bark flaps, niches on the underside of branches and on the shaded sides of upper tree trunks of host trees. By the 4th instar, most of the larvae have a well-established trail pattern leading down to their resting places on the trunks of trees and into the forest litter below. They pupate in these resting places. Trees banded with loose burlap provide excellent, protected artificial niches for the migrating larvae. The Fenvalerate-treated tapes, 10 percent A.I., were bioassayed in 2 plots with the following 4 treatments, 25 trees/treatment:

(1) Single band, 3" wide tape
(2) Double band, 5.5" wide tape
(3) Band under burlap, 3" wide tape placed under 12" wide burlap band
(4) Check - no treatment

The tapes were wrapped around tree trunks (minimum diameter 5") and stapled 4 ft. above the forest litter. They were placed on randomly designated red and white oak trees whose crowns did not touch one another. The plots were set up by May 18, 1983, and the trees were observed several times. On May 23rd, bands on taped trees were covered with dead 1st and 2nd instar larvae that were blown onto them. On June 13th some of the white oaks were being defoliated right after bud break. The red oaks had very little defoliation. On June 21st, the average defoliation estimate was 18 percent on red oaks and 66 percent on white oaks. The final defoliation estimate was made on June 29th. Table 7 shows the percent defoliation for both plots. The analysis of variance indicates that there are significant differences between the treatments. In the later defoliation period, the tapes were not effective in reducing defoliation. This was due to a high population density with many indications of larval wilt disease and a population collapse. The band-under-burlap treatment looked the most promising; many of the dead larvae remained under the burlap.

TABLE 7. Oak tree bandings with Fenvalerate-treated tape, 10 percent A.I., June 29, 1983.

Treatment	Tree Specie	Plot	Percentage defoliation*	N
Single	R. oak	1	77.0	(15)
Single	R. oak	2	61.2	(21)
Single	R. oak	1 + 2	67.8	(36)
Single	W. oak	1	100.0	(10)
Single	W. oak	2	100.0	(4)
Single	W. oak	1 + 2	100.0	(14)
Double	R. oak	1	82.5	(12)
Double	R. oak	2	62.6	(23)
Double	R. oak	1 + 2	69.4	(35)
Double	W. oak	1	99.2	(13)
Double	W. oak	2	100.0	(2)
Double	W. oak	1 + 2	99.3	(15)
Under burlap	R. oak	1	57.6	(17)
Under burlap	R. oak	2	29.0	(21)
Under burlap	R. oak	1 + 2	41.8	(38)
Under burlap	W. oak	1	98.8	(8)
Under burlap	W. oak	2	100.0	(4)
Under burlap	W. oak	1 + 2	99.2	(12)
Control	R. oak	1	50.3	(20)
Control	R. oak	2	48.7	(23)
Control	R. oak	1 + 2	49.4	(43)
Control	W. oak	1	100.0	(5)
Control	W. oak	2	100.0	(2)
Control	W. oak	1 + 2	100.0	(7)

* analysis of variance indicates that there are significant differences between treatments (F = 8.735)

In this study, there was a high population density partially caused by larval blow-in into the plots. In this type of infestation, the larvae usually feed out in the mid to late instars and mass migrate in search of food. These starving larvae become stressed and usually have a high incidence of disease and parasitism, and are susceptible to population collapse. It is not recommended to band trees when the population builds up to 2,000-3,000 egg masses per acre. The Fenvalerate-latex tapes in the laboratory experiments were exposed to up to 17 hours of UV light and were highly effective killers of 4th and 5th instar larvae at screening intervals of 1, 2, 5, 10, 15, 20, and 60 minutes. The banding of trees to prevent or reduce defoliation with these treated tapes should be done in low to medium infestations where there are less than 1,000 egg masses per acre.

Project Number: GM 3.1.4
Project Title: Hatching of Feral Gypsy Moth Egg Masses Temporarily Brought Indoors in December - An Infested Christmas Tree Scenario
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leader: E. C. Paszek

Christmas trees infested with egg masses shipped to uninfested States must be fumigated. The fumigation process is not always practical to use. The infestation hazard might be reduced by the fact that egg masses will hatch on these trees when brought indoors during the holidays, or dessicate if diapause hasn't been completed. To test this, we conducted a small experiment which simulated these conditions.

On December 17, 1981, 125 egg masses were collected from Sandwich, Massachusetts. One group (25) was kept in an outdoor insectary; another group (25) was held at ca. 25°C constantly thereafter; the remainder were returned outdoors after being incubated at 25°C for 1, 2, or 3 weeks. Hatch rates were recorded and are tabulated below in table 1.

TABLE 1. Rates of 5 groups of egg masses collected in Sandwich December 17, 1981.

	Controls		Weeks at 25°C Before return to outdoors		
	Continual outdoor	Continual indoor	1	2	3
Number larvae hatched/egg mass	413 1/	290 2/	316 1/	308 1/	88 3/
Percent of control (continual outdoor)	100	70.2	75.5	74.5	21.3

1/ hatch began May 10, 1982.
2/ hatch began December 29, 1981
3/ hatch began May 13, 1982, although 6.7 percent occurred before eggs were returned outdoors.

Warming up egg masses for 3 weeks in December and January causes an 80 percent reduction in hatch the following spring. This won't be of much consolation to uninfested states and infested Christmas trees must still stand as items of regulatory concern.

Project Number: GM 8.2.2
Project Title: Radiological Sterilization of Male Gypsy Moths
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: V. C. Mastro

In 1980, we initiated a study to define the dose of irradiation and age of male treatment which would result in the greatest number of F-1 sterile progeny. Test design and methodology are described in the Otis Methods Center Annual Report covering the period from October 1, 1980 to September 30, 1981. Briefly, male pupae in six age groups were exposed to seven different doses of radiation (total of 42 treatment groups and controls) and then mated to untreated females (50 pairs/treatment group). Progeny (F-1) of these matings were evaluated in the egg, larval, pupal and adult stages. In treatment groups where F-1 adults were available (survived to the adult stage), they were outcrossed with untreated adults and incrossed within their treatment group. Resulting F-2 eggs, larvae, pupae and adults were evaluated and adults were again outcrossed to normal insects and incrossed within their own treatment group. All treatments were evaluated through the F-3 egg stage and selected treatments were studied through the F-4 egg stage (Figure 1).

Currently all data on all life stages through the F-3 generation have been compiled and analyzed. Data collection for the F-4 generation will be complete by September of 1984. Only data from selected treatments will be presented in this report, however, the entire results of this study will be available from the Otis Methods Development Center to anyone who is interested. The full body of data is contained in fifty separate tables comparable to Table 1.

Table 1 presents the F-1 egg data from all of the treatment groups. Generally, as would be expected, when males are irradiated and mated to normal females, larger doses of radiation result in lower percentages of eggs which hatch in the F-1 generation. Also the younger the age of the male pupae when irradiated, the lower the percentage hatch. Debilitating effects of irradiation are noted in the youngest age class (0-1 days after pupation) when pupae are treated with a dose as low as 4 krads, (i.e. only 22 of 50 pairs mated; see Table 1). These debilitating effects are not seen in the next older age class (2-3 day old) until the treatment dose is increased to 10 krads. The relationship between age of treatment and hatch (e.g. the older the pupae, the higher the percentage hatch or vice versa) appears to be consistent except for the oldest treatment age class (10-11 day old pupae) in which the percentage egg hatch is lower than the next younger age class (8-9 day old pupae).

Table 2 presents survival and development data for F-1 larvae from the different treatment groups. All larvae were reared under standard Otis Methods Development Center rearing conditions on Bell high wheat germ artificial diet. Generally, survival of neonates from all treatment groups is high. Within age classes the proportion of F-1 larvae which survive to the adult stage decreases as the radiation dose increases. Between age classes within the same irradiation dose the proportion of survival is fairly constant. Length of larval development in the F-1 generation also generally increases as dose rate increases. For example, male progeny of P-1 males treated with 2 krads required 30.5 days (mean of all age classes) to complete development compared to a mean development time of 35.05 days for male progeny of 15 krad treatments. Males whose parents received no radiation treatment (control) required an average of 30.03 days for larval development.

Egg mass data from outcrossing and incrossing F-1 adults for some selected treatments are presented in Table 3. (All possible mating types were made, data are available on request). Because individual matings could not be observed, F-2 egg mass data were included in the summary only when females produced embryonated eggs. Data were not included if no eggs were produced or if all eggs were unembryonated because it was unknown if the female had mated. Generally, as irradiation dose was increased, the proportion of non-productive F-1 pairs also increased. Whether this indicates that the F-1's did not mate, or mated without oviposition or fertilization, remains to be determined.

Marked effects of radiation on F-1 fertility (e.g. F-2 egg mass viability) can be detected in treatment groups receiving doses as low as 4 krads. The data for the 6 krad group presented on Table 3 demonstrate that when F-1 males were outcrossed to control females, the resulting egg masses contained smaller proportions of embryonated eggs; and further, smaller proportions of these eggs hatched than for control matings. The reciprocal mating type (F-1 females x control males) usually resulted in an egg mass in which embryonation was near normal but in which hatch was reduced.

Figure 1. Mating types completed in dose x age irradiation study. All mating types, where possible, were carried through the F-3 egg stage; selected treatments, not shown, were carried through the F-4 egg stage.

P1 (Irradiated ♂ x Control ♀)

Seven irradiation doses were administered to six pupal age classes = 42 treatments.

F1 (F1♂x C♀) (F1♂x F1♀) (C♂x F1♀)

F2 (F2♂x C♀) (F2♂x F2♀) (C♂x F2♀)

(F2♂x C♀) (F2♂x F2♀) (C♂x F2♀) (F2♂x C♀) (F2♂x F2♀) (C♂x F2♀)

Table 1a. Viability and size of F-1 egg masses which resulted from mating males (treated with various doses of radiation in various age classes) with untreated females.

DOSE	AGE	PERCENT EMBRYONATION	PERCENT EMB. THAT HATCHED	PERCENT TOTAL THAT HATCHED	TOTAL EGGS	N
2 KRADS						
	0-1 DAY PUPAE	95.87	82.08	79.07	920.64	45
	2-3 DAY PUPAE	93.35	82.31	77.12	944.22	45
	4-5 DAY PUPAE	95.60	82.34	79.74	994.76	50
	6-7 DAY PUPAE	96.60	83.14	80.69	976.68	50
	8-9 DAY PUPAE	97.78	83.59	81.74	1013.8	48
	10-11 DAY PUPAE	94.23	81.87	77.28	925.28	39
MEAN		95.5691	82.5537	79.2725	962.56	
SUM						277
4 KRADS						
	0-1 DAY PUPAE	75.34	57.20	50.82	790.36	22
	2-3 DAY PUPAE	95.14	74.84	71.47	919.94	47
	4-5 DAY PUPAE	95.42	73.92	70.60	926.78	50
	6-7 DAY PUPAE	94.78	79.59	76.18	980.69	49
	8-9 DAY PUPAE	94.46	78.19	73.97	997.06	49
	10-11 DAY PUPAE	94.73	75.25	70.87	916.63	40
MEAN		91.6441	73.1674	68.9867	921.91	
SUM						257
6 KRADS						
	0-1 DAY PUPAE	41.84	28.09	20.17	448.67	6
	2-3 DAY PUPAE	92.69	56.67	53.06	884.64	45
	4-5 DAY PUPAE	94.55	65.52	62.41	905.04	46
	6-7 DAY PUPAE	93.91	72.77	67.92	985.43	49
	8-9 DAY PUPAE	96.63	71.35	69.00	990.76	46
	10-11 DAY PUPAE	96.92	69.65	67.55	989.82	38
MEAN		86.0923	60.6777	56.6842	867.39	
SUM						230
8 KRADS						
	0-1 DAY PUPAE	.92	0.0	0.0	794.00	1
	2-3 DAY PUPAE	90.50	41.09	37.21	879.05	39
	4-5 DAY PUPAE	90.07	55.68	50.20	900.58	45
	6-7 DAY PUPAE	94.03	62.31	59.30	979.44	48
	8-9 DAY PUPAE	92.55	65.49	59.92	1012.1	49
	10-11 DAY PUPAE	91.84	55.08	50.70	979.35	40
MEAN		76.6511	46.6089	42.8872	924.08	
SUM						222

Table 1b. Viability and size of F-1 egg masses which resulted from mating males (treated with various doses of radiation in various age classes) with untreated females.

DOSE	AGE	PERCENT EMBRYONATION	PERCENT EMB. THAT HATCHED	PERCENT TOTAL THAT HATCHED	TOTAL EGGS	N
10 KRADS						
	0-1 DAY PUPAE	12.82	0.0	0.0	223.50	2
	2-3 DAY PUPAE	83.27	23.41	21.19	862.83	18
	4-5 DAY PUPAE	88.19	40.10	35.92	897.15	48
	6-7 DAY PUPAE	91.60	51.93	47.66	993.60	48
	8-9 DAY PUPAE	92.60	50.64	47.63	974.71	49
	10-11 DAY PUPAE	88.78	40.32	35.97	970.08	39
MEAN		76.2100	34.3980	31.3943	820.31	
SUM						204
12 KRADS						
	2-3 DAY PUPAE	89.47	9.83	8.45	775.50	4
	4-5 DAY PUPAE	83.16	29.73	25.86	897.00	39
	6-7 DAY PUPAE	85.71	41.25	36.63	980.86	49
	8-9 DAY PUPAE	90.18	38.64	35.92	972.18	50
	10-11 DAY PUPAE	91.93	31.21	28.91	900.62	50
MEAN		88.0896	30.1309	27.1564	905.23	
SUM						192
15 KRADS						
	2-3 DAY PUPAE	91.67	7.44	6.82	773.00	1
	4-5 DAY PUPAE	76.97	15.05	10.61	880.48	25
	6-7 DAY PUPAE	82.75	22.47	18.07	899.48	44
	8-9 DAY PUPAE	86.02	26.35	22.77	952.43	50
	10-11 DAY PUPAE	84.58	12.22	10.67	1026.7	47
MEAN		84.3983	16.7066	13.7878	906.41	
SUM						167
0 KRADS						
	0-1 DAY PUPAE	98.17	88.66	87.10	975.51	49
	2-3 DAY PUPAE	97.61	89.08	86.98	978.82	50
	4-5 DAY PUPAE	94.79	86.79	82.32	996.22	49
	6-7 DAY PUPAE	97.21	88.77	86.52	955.20	49
	8-9 DAY PUPAE	97.85	83.20	81.48	1025.6	47
	10-11 DAY PUPAE	94.08	84.85	81.65	883.78	50
MEAN		96.6196	86.8926	84.3411	969.18	
SUM						294

Table 2. Survival and development of F-1 larvae, progeny of irradiated males and control females.

DOSE	AGE	NEONATES INFESTED	MALE PUPAE	FEMALE PUPAE	RATIO M:F(1)	%TOTAL SURVIVAL	AVE.MALE DEVEL.	AVE.FEMALE DEVEL.
2 KRADS								
	1	521	271	233	1.16	96.74	30.30	32.69
	3	521	257	235	1.09	94.43	30.59	32.37
	5	520	253	246	1.03	95.96	30.80	32.54
	7	522	242	250	.97	94.25	30.35	32.40
	9	520	275	230	1.20	97.12	30.24	32.26
	11	520	245	238	1.03	92.88	30.86	32.58
4 KRADS								
	1	518	283	209	1.35	94.98	31.28	33.98
	3	520	273	211	1.29	93.08	31.81	33.35
	5	520	266	232	1.15	95.77	31.33	33.35
	7	520	287	210	1.37	95.58	30.56	33.01
	9	520	269	229	1.17	95.77	30.84	33.12
	11	520	291	198	1.47	94.04	31.87	33.26
6 KRADS								
	1	98	57	32	1.78	90.82	32.10	35.31
	3	520	270	196	1.38	89.62	32.54	34.32
	5	520	284	200	1.42	93.08	31.99	34.02
	7	522	282	218	1.29	95.79	31.31	33.36
	9	520	279	198	1.41	91.73	31.43	34.03
	11	521	279	196	1.42	91.17	31.66	33.93
8 KRADS								
	3	520	302	171	1.77	90.96	33.37	35.56
	5	520	276	190	1.45	89.62	32.40	34.84
	7	520	279	185	1.51	89.23	32.38	34.77
	9	520	262	197	1.33	88.27	32.79	35.98
	11	520	261	192	1.36	87.12	32.72	34.72
10 KRADS								
	3	357	211	82	2.57	82.07	33.18	36.86
	5	521	296	165	1.79	88.48	33.59	36.05
	7	520	268	190	1.41	88.08	32.61	35.31
	9	520	290	186	1.56	91.54	33.26	35.51
	11	520	314	153	2.05	89.81	33.10	35.46
12 KRADS								
	3	47	27	6	4.50	70.21	33.74	34.78
	5	520	291	138	2.11	82.50	34.24	36.31
	7	522	311	143	2.17	86.97	33.43	36.13
	9	521	317	149	2.13	89.44	33.31	36.36
	11	520	311	134	2.32	85.58	34.01	36.49
15 KRADS								
	3	8	3	2	1.50	62.50	35.50	35.00
	5	312	159	59	2.69	69.87	35.19	38.44
	7	520	286	110	2.60	76.15	34.59	37.70
	9	520	298	109	2.73	78.27	34.98	37.43
	11	432	261	73	3.58	77.31	35.01	37.57
0 KRADS								
	ALL	3124	1587	1393	1.14	95.39	30.03	31.88

Table 3. Viability and size of F-2 egg masses which resulted from incrossing (within treatment groups) and outcrossing F-1 adults which were progeny of an irradiated male and an untreated female.

Treatment of P-1 Parent							
Dose	Age at irradiation	Mating type ♂ x ♀	Mean No. of total eggs produced	Mean percent of eggs embryonated	Mean percent of embryonated eggs which hatched	Mean percent of total eggs which hatched	No. successful mating pairs
6	8-9	F1 x C (1)	809.5	43.9	15.9	6.5	24
		C x F1 (2)	747.5	74.7	20.1	13.0	21
		F1 x F1 (3)	572.2	44.1	2.3	1.3	20
6	10-11	F1 x C	673.0	46.1	21.1	13.0	21
		C x F1	823.3	77.2	14.8	13.6	26
		F1 x F1	659.4	31.8	2.9	1.2	24
8	8-9	F1 x C	612.4	22.1	4.2	1.2	21
		C x F1	735.0	79.6	5.7	5.5	25
		F1 x F1	495.2	13.6	0.0	0.0	12
8	10-11	F1 x C	625.5	23.5	8.2	1.9	19
		C x F1	920.4	79.3	5.7	4.5	22
		F1 x F1	505.4	13.7	0.6	0.1	18
10	8-9	F1 x C	607.0	39.8	11.0	7.4	17
		C x F1	661.4	74.1	0.5	0.5	25
		F1 x F1	548.6	9.7	0.4	0.1	20
10	10-11	F1 x C	460.1	16.8	0.3	0.1	19
		C x F1	622.8	66.8	3.1	0.9	22
		F1 x F1	584.3	10.9	0.3	0.1	16
Control		C x C	947.3	93.7	80.9	76.5	145

When comparing a particular mating type (e.g. F-1 male x C female) between radiation treatments, the sterility of the F-1 progeny generally increased with the radiation dose administered to the P-1 male parent. In other words the F-1 progeny of a 10 krad-treated male were more sterile than the progeny of a 4 krad-treated male. Also when F-1 females (progeny of irradiated males x control females) were outcrossed the mating was more fertile than when a F-1 male was outcrossed. When considering an age x dose treatment for a particular objective (e.g. eradication vs population suppression), the fertility of the F-1 female and the survival of any her F-2 progeny should be closely scrutinized. Selection of a lower treatment dose (6 krads) provides a greater number of F-1 adults, however, the resulting adults are more fertile than those of higher radiation treatments. Approximately 68% of the eggs in an F-1 mass will hatch when males are treated with 6 krads as pupae between 8 and 11 days of age. These F-1 larvae survive nearly as well as control insects and development times are shorter than progeny of males treated with 10 krads, perhaps indicating they are of a better quality. Hatch rates of F-2 egg mass produced by either incrossing or outcrossing the 6 krad F-1 adult progeny are greater than F-1 progeny of a 10 krad treatment (Table 3). Although 6 krads may not be considered as a treatment dose when the objective is eradication, it may be a better choice for population suppression. Survival of F-2 progeny from 6 krad treatment groups is nearly the same as normal insects; length of development of both males and females is more like the control group than progeny from treatment groups which received higher doses.

Treating P-1 males with 10 krads in either the 8-9 or 10-11 age groups provides F-1 progeny which are very sterile (see Table 3). However larval survival of the F-1 progeny is lower than control larval survival, and F-1 larval development times average approximately three days longer than normal. The small number of F-2 larvae resulting from outcrossing F-1 adults (either male or female) have longer development times and smaller proportions survive to the adult stage when compared to normal insects (Table 4).

Table 4. Survival and development of F-2 larvae.

Treatment of P-1 male parent Dose	Age	Mating type of F1 parent ♂ x ♀	No. of neonates infested	Percent survival	No. of male pupae	Mean Male larval development time	No. of female pupae	Mean female larval development time	Sex ratio M:F(1) 2/
6	8-9	F1 x C (1)	216	59.7	63	29.5	66	32.6	0.95
		C x F1 (2)	244	60.7	73	29.8	75	32.7	0.97
		F1 x F1 (3)	9	88.9	3	35.0	5	38.8	0.60
6	10-11	1	264	75.8	115	30.2	85	32.8	1.35
		2	260	70.4	113	30.6	70	32.0	1.61
		3	52	76.9	20	29.7	20	32.4	1.00
8	8-9	1	18	55.6	2	32.0	8	37.2	0.25
		2	74	68.9	26	29.5	25	33.9	1.04
		3	None 1/	-	-	-	-	-	-
8	10-11	1	26	53.9	6	34.4	8	35.9	0.75
		2	77	71.4	31	30.5	24	34.1	1.29
		3	None 1/	-	-	-	-	-	-
10	8-9	1	136	60.3	54	30.0	28	34.6	1.93
		2	6	33.3	1	34.0	1	38.0	1.00
		3	None 1/	-	-	-	-	-	-
10	10-11	1	None 1/	-	-	-	-	-	-
		2	10	40.0	3	30.5	1	32.0	3.00
		3	None 1/	-	-	-	-	-	-
Control group			3118	76.6	1382	28.6	1007	30.5	1.37

1/ Treatments where no F-2 neonates were available for rearing.

2/ All sex ratios calculated based on females equaling one.

Effects of irradiation apparently are carried over into at least the F-2 adult stage. Although egg masses produced by outcrossing F-2 adults were embryonated nearly as well as control mating egg masses, the proportion of eggs which hatched was lower (Table 5). Viability generally was the lowest in egg mass produced by incrossing F-2 adults (mating types 3, 6 and 9). The proportion of eggs which embryonated and which hatched was generally the smallest in these groups. Within all inbred groups the lowest viability was usually in egg masses produced inbreeding progeny of irradiated males for two generations (mating type 9). These effects can be seen in treatment groups when as little as 2 krads was used to treat the P-1 male parent (data not shown).

Survival and development of F-3 progeny, regardless of the parentage, appears to be more similar to the control group than to the F-2 generation (Table 6). The proportions of neonates surviving to the pupal stage for nearly all treatment groups are very similar to control insects. Male and female development times, however, appear to be longer than development times of control insects. This disparity, however, is not as great as in the F-2 generation. Although the radiation effects are not as pronounced in the F-3 larval stage, they still are apparent. Results of incrossing and outcrossing F-3 adults (now being evaluated) will determine if any sterility effects can be detected in the adult stage.

Table 5. Viability and size of F-1 egg masses which resulted from inbreeding (within treatment group) and outbreeding F-2 adults.

Treatment Group of F-1 male parent		Mating type F-1 parents ♂ x ♀	Mating type F-2 parents ♂ x ♀	Mating code	Mean no. of total eggs produced	Mean percent of eggs embryonated	Mean percent of embryonated eggs which hatched	Mean percent of total eggs which hatched	No. of mating pairs
Dose	Age								
6	8-9		F2 x C	1	897.9	72.1	47.1	36.3	15
		F1 x C	C x F2	2	873.7	79.4	56.4	44.6	15
			F2 x F2	3	626.6	70.3	26.5	20.6	11
			F2 x C	4	897.8	72.6	43.4	30.6	19
		C x F1	C x F2	5	798.0	82.2	41.8	34.7	14
			F2 x F2	6	866.5	64.3	15.4	11.6	11
6	10-11		F2 x C	1	919.9	78.7	53.9	47.7	14
		F1 x C	C x F2	2	817.3	86.5	52.0	48.1	15
			F2 x F2	3	731.5	62.6	34.2	19.0	13
			F2 x C	4	811.1	70.2	38.9	33.6	18
		C x F1	C x F2	5	879.8	81.4	37.2	33.1	13
			F2 x F2	6	758.7	71.4	36.2	28.1	12
			F2 x C	7	634.2	53.4	58.1	24.3	8
		F1 x F1	C x F2	8	missing	100.0	42.4	42.4	2
			F2 x F2	9	537.5	40.0	21.6	14.6	4
8	8-9	F1 x C	C x F2	2	872.3	54.9	4.5	4.1	4
			F2 x C	4	812.4	83.7	56.5	44.1	5
		C x F1	C x F2	5	525.0	60.8	48.2	25.9	6
			F2 x F2	6	691.2	82.9	43.9	35.5	4
8	10-11	F1 x C	C x F2	2	925.5	70.3	3.8	3.7	2
			F2 x C	4	1094.1	72.2	33.2	26.1	13
		C x F1	C x F2	5	953.2	72.9	42.1	37.4	9
			F2 x F2	6	332.8	34.4	12.2	2.3	4
10	8-9		F2 x C	1	891.7	72.6	52.1	40.1	10
		F1 x C	C x F2	2	957.9	79.7	55.3	46.9	7
			F2 x F2	3	986.8	67.2	22.1	12.6	4
10	10-11	C x F1	F2 x C	4	1194.0	99.2	81.0	80.3	1
Control			C x C	-	1073.5	93.4	71.3	67.8	141

Table 6. Survival and development of F-3 larvae - progeny of irradiated males x control females.

Treatment of P-1 male parent									
Dose	Age	Mating type 1/ of F1 parent	No. of neonates infested	Percent survival	No. of male pupae	Mean Male larval development time	No. of female pupae	Mean female larval development time	Sex ratio 2/ M:F(1)
6	8-9	1	390	71.3	108	28.7	94	30.0	1.57
		2	397	83.1	174	27.3	49	29.8	.90
		3	194	69.1	48	29.8	40	34.3	1.79
		4	448	76.3	166	27.8	71	31.2	1.06
		5	388	74.0	141	28.9	50	31.9	1.04
		6	134	71.6	57	27.9	12	32.2	.68
6	10-11	1	410	57.6	152	27.8	84	31.4	1.81
		2	397	81.4	201	28.7	122	32.3	1.65
		3	246	68.3	93	28.9	75	32.8	1.24
		4	342	84.8	162	27.9	128	32.0	1.27
		5	232	90.5	130	27.9	80	30.6	1.63
		6	196	84.7	73	28.9	93	32.4	0.78
		7	208	75.0	88	29.2	68	34.7	1.29
		8	61	91.8	29	29.5	27	31.1	1.07
		9	69	82.6	31	31.5	26	32.7	1.19
8	8-9	4	166	46.4	44	28.0	33	32.4	1.33
		5	200	81.5	81	29.7	82	32.5	0.99
		6	72	79.2	22	29.1	35	33.2	0.63
8	10-11	2	6	66.7	0	-	4	34.5	0.00
		4	224	73.7	115	29.2	50	32.6	2.30
		5	210	86.2	108	28.1	73	31.7	1.48
		6	7	42.9	2	27.0	1	29.0	2.00
10	8-9	1	203	80.8	111	28.5	53	31.0	2.09
		2	208	77.9	103	27.9	59	30.9	1.75
		3	61	90.2	33	30.4	22	34.6	1.50
10	10-11	4	96	62.5	31	31.7	29	31.7	1.07
Control		C x C	3032	84.4	1501	27.2	1058	29.9	1.42

1/ See Table 5 for description of mating types.
2/ All sex ratios calculated based on females equalling one.

Project Number: GM 0.2.7
Project Title: Sterile Male Trial, Berrien County, Michigan
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: V. C. Mastro

Sterile males (15 krads) were released in a small isolated population for 2 consecutive years in Berrien County, Michigan to determine if population suppression was feasible using this technique. This project is nearly complete and most information is summarized in the last annual report. Results of trapping the site (32/sq.mi.) in 1983 were negative (i.e. no males were trapped) and it appears the population was eradicated using the sterile male release technique. The area will again be trapped at the same rate in 1984 to demonstrate that population elimination has been achieved.

Project Number: GM 0.2.8
Project Title: Factors Affecting Sexual Periodicity in Male Gypsy Moths
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leaders: D. R. Lance, T. M. ODell, V. C. Mastro and A. P. C. Toles

Actograph work for this report period had been planned along 3 general lines of inquiry: (1) How does temperature during the course of a test affect the activity rhythms of male gypsy moths, (2) What was the periodicity of activity for the sub-sterilized males that were released as part of the Maryland IPM project, and (3) Is it possible to reduce background "noise" in the actograph to the point where males can be tested individually?

Unfortunately, actograph work has been seriously hampered by recurring problems with the floppy disk recorders. These problems appear to be occurring because, during the entire course of actograph tests, the disk drives must remain in a "write" mode; i.e., the recorder head is continuously in contact with a spinning disk. This may be causing excessive wear or other problems. We now are developing software (and/or hardware, if necessary) to allow the actograph to turn the recorder "on" to a write mode for only the ca. 10 seconds per hour in which actual data uptake occurs. As a result of these problems, data sets for (1) and (2) are not complete.

To reduce background vibration within the actograph, environmental control equipment was removed from the top of the actograph room and was suspended independently from the ceiling. Again, due to problems with the floppy disks, we have not yet successfully run tests to determine whether the background noise has been reduced to a point where the activity of individual moths can be quantified.

Project Number: GM 1.2.1
Project Title: Behavior and Ecology of Immature Gypsy Moths: Population Quality and Competitiveness of F1 Sterile Individuals
Report Period: October 1, 1982 - September 10, 1983
Report Type: Interim
Project Leaders: D. R. Lance, J. S. Elkinton, V. C. Mastro and C. P. Schwalbe

Gypsy moths are behaviorally and physiologically plastic organisms. Larval behavior, development and survival vary from population to population. In large part, this variation arises from differences in such environmental factors as food quality, population density and temperature. For the gypsy moth, genetics appear to play only a minimal role in causing interpopulation differences. Population quality has been used as a catch-all term to refer to the behavioral, physiological and ecological nature of a population.

Understanding population quality is important for workers in insect rearing, pest management, insect-plant interactions, biological control and other fields. For example, by understanding interactions between the rearing environment and insect behavior, workers could "program" optimal behavior into sterile male insects. Also, when gypsy moth populations increase, the forest environment becomes altered. Changes in the forest, in turn, result in changes in gypsy moth behavior, survival and fecundity. Understanding these interactions may help us to explain and predict gypsy moth population trends. In this series of studies, we are attempting to quantify and explain the reasons for density-correlated shifts in the quality of gypsy moth larvae.

Study #1 - Dispersal of first instar gypsy moth larvae that are exposed to previously defoliated vs. undefoliated host plants.

Dispersal of first instar larvae may be an important factor in determining the survival and success of gypsy moth populations. Foliage quality has been shown to affect first instar dispersal (Lance and Barbosa 1981), and foliage quality, in turn, can decline in response to defoliation (Schultz and Baldwin 1982). This study was set up to see if first instar larvae disperse more or less frequently from trees which were or were not defoliated in the previous year.

Methods and Materials

Larval dispersal from black oak foliage was tested in 4 oak-dominated sites with differing histories of defoliation (see Table 3). The techniques were similar to those described by Lance et al. (1982). For this study, eggs were collected from a moderately high density, building population in the Moody Pond area of Mashpee, Massachusetts. Twenty marked first instar larvae (1-2 days old) were released daily on each of 3 branches at each of the 4 sites. This year, different branches were selected for each of 8 day's release. After 24 h, larvae that were on the branches were collected, returned to the laboratory and examined under a UV light for the presence of fluorescent marking powder.

Results and Discussion

The proportions of larvae that remained on the branches after 24 hrs are shown in Table 1. As in 1982, the proportion of larvae which remained on the branches did not appear to be related to the degree of defoliation in the year before the study. Again this year, dispersal at the Crane Quail/Ashumet Road site was lower compared to the other 3 sites; local variation in foliage quality may be important in determining the incidence of dispersal. Also, there was a highly significant negative correlation between the low temperature on the night of the test and numbers of larvae that remained on the branches. However, previous defoliation history did not appear to influence the incidence of dispersal.

Table 1. Proportions of gypsy moth larvae remaining on black oak branches after 24 h (8 release dates).

Site	Defoliation history (% defoliated) 1981	1982	1983	Mean no. of larvae leaving release containers (per site per day)	Mean percent of larvae remaining after 24 hrs[a]
Ashumet Road	<10%	<10%	40%	43.8	41.7 a
Mashpee River	<10%	<10%	60%	43.3	33.8 b
Cotuit Watershed	25%	100%	<10%	45.5	35.2 b
Lee Road	<10%	70%	80%	43.8	32.0 b

a Means that are not followed by the same letter are significantly different at $p<0.05$, as determined by the Student-Neuman-Keuls test.

Study #2 - Behavior of late instar gypsy moths in outbreak and endemic populations.

The literature suggests that there are distinct qualitative differences in behavior and physiology between gypsy moth larvae in endemic populations and larvae in outbreak populations. Apparently, these differences in population quality result from environmental changes which occur when forests are attacked heavily by gypsy moths (e.g., more sunlight penetrating the canopy, defoliation-induced changes in leaf chemistry, greatly increased numbers of gypsy moth larvae). While population quality has been the subject of numerous investigations, there is little quantified information on density-correlated variation in the behavior of late instar larvae. This information is essential for the development and evaluation of tests to assess population quality, and for understanding how qualitative variation affects the dynamics and control of gypsy moth populations. In this study, feeding, food selection, resting and locomotor behaviors are compared in late instar larvae at 2 sites with different population densities.

Methods and Materials

The behavior of late instar larvae was observed in the Massachusetts National Cemetary, Otis Air National Guard Base, Massachusetts, and in an endemic site in the Ashumet area of East Falmouth, Massachusetts. At the high density site, small (7-10 m high) black oaks (*Quercus vetutina* Lam.) and white oaks (*Q. alba* L.) accounted for ca. 75% of stems over 4 cm dbh; most of the remaining trees were pitch pine (*Pinus rigida*). The endemic site was dominated by the same 3 tree species, but pitch pine was much more prevalent (ca. 50% of stems over 4 cm dbh). At the high density site, the trees surrounding the study area were 80% defoliated by the time pupation was occurring. The trees at the endemic site suffered little noticable defoliation.

At both sites, 8-10 m high black oaks were surrounded by a 4 x 4 x 8 m high observation tower of galvanized steel pipe. At night larvae were observed using ambient light and a (borrowed) light amplifier. At the endemic site, larvae rested during the photophase, and so observations at Ashumet were made primarily during the scotophase and crepuscular periods.

Results and Discussion

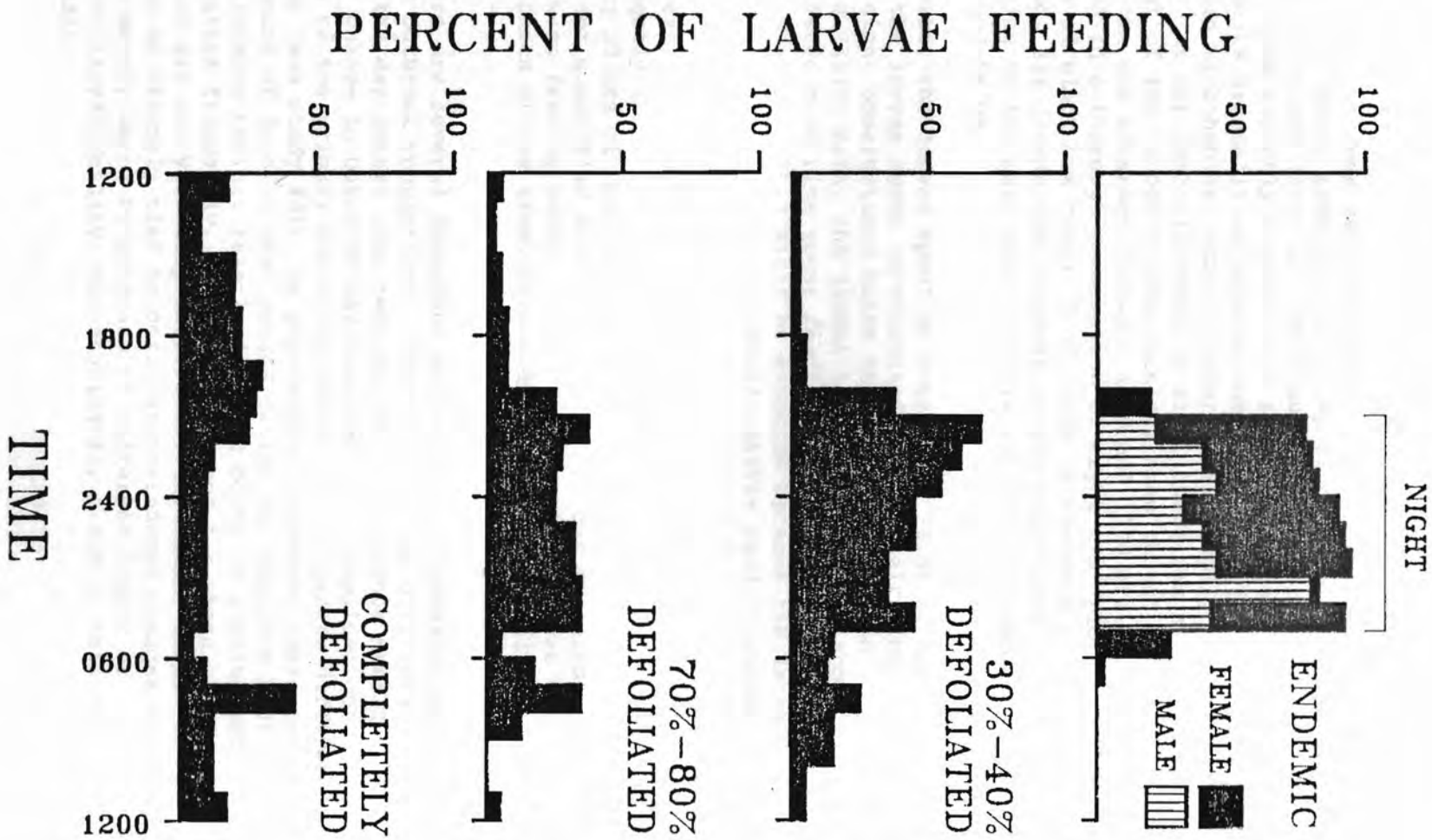
Last year, we described the basic food-seeking and feeding behaviors of gypsy moth larvae in outbreak and endemic populations (Lance et al. 1982). These basic observations were found to apply to this year's insects as well.

At our endemic site this year, we followed individual larvae from the time they became active (2020-2035 hrs; this was at or just after sunset) until they settled down and began feeding. The larvae contacted a mean of 4.6 ± 2.3 clusters before settling down to feed (n=10). The larvae sampled the petioles of 2.8 ± 1.2 leaves per cluster; thus, the larvae contacted an average of 13.2 leaves before settling in to feed. During this process, larvae occasionally fed for a minute or so (removing a pinhead-sized chunk) before rejecting a leaf. Two of the 10 larvae settled to feed on the first cluster they contacted. In 1982, we didn't see any larvae settle to feed on the first cluster. Still, this year's larvae appeared to be selective in their choice of feeding sites.

Once the larvae "settled in" to feed, they went through a series of relatively long bouts of fairly continuous feeding (a mean of 69 minutes per bout for females, 35 minutes for males). The duration of feeding bouts was extremely variable -- some females fed almost continuously for up to 6 hours). Between feeding bouts, the larvae often rested without leaving the leaf on which they had been feeding. In other cases, larvae switched feeding sites, but almost all larvae fed on no more than 3 leaves during the course of a night.

Sixth instars (females) fed throughout most of the night (30.9% of their total 24 hr day was spent in feeding), while males spent less than half the night eating (14.4% of the 24 hr day). The feeding rhythms of the larvae are shown in Figure 1.

Figure 1. Relationship between population density and feeding rhythms of late-instar gypsy moth larvae in the field. Data for 30-40% defoliated site was taken from Leonard (1970).



In the outbreak population, larval size was more variable, and a subjective determination of instar (and sex) was not possible. Feeding was primarily nocturnal, although the larvae remained in the canopy and did feed occasionally during the day (Fig.1; 70-80% defoliated). There was a peak of feeding between 0700 and 0800 hrs, (2-3 hrs after sunrise) which appears to be typical of high density populations. Overall, feeding was less nocturnal this year than last year when the trees were completely stripped. However, at a site that was 30-40% defoliated, Leonard (1970) reported higher percentages of larvae feeding during the night (Fig. 1). This suggests that the density-related shift from strictly nocturnal to relatively aperiodic feeding is not abrupt, but occurs gradually as populations increase. The corresponding switch in microhabitat occurs either more abruptly or at lower population densities. At our 1983 high-density site, there was no net movement of larvae out of the canopy in the morning, even though feeding was primarily nocturnal. On one afternoon between 1500 and 1700 hrs, a total of 5 larvae were discovered in a thorough examination of eight 1 m^2 plots in the litter near the bases of black oak trees (2 of these larvae were crawling). During the same period, 224 larvae were present in the study tree. Of these 224, only 4 were resting on the main stem (a habit typical of endemic larvae), and no larvae were found below 3 m.

At the high density site, the larvae spent an average of 16.5% of their time feeding. Last year, the larvae spent an estimated 15% of their time feeding, although the lack of night observations makes this figure uncertain. According to Leonard's (1970) data, the larvae in his study spent about 22% of their time feeding. Percent of time spent feeding, then, may decrease with increasing population density. This might be expected because the larvae are smaller in higher density populations, and smaller larvae tend to spend less of their time feeding.

During the day, outbreak larvae fed in short bouts ($\bar{x}$ = 7.8 minutes), and switched leaves frequently (actively feeding larvae fed on a mean of 2.3 leaves per hour during 30 hrs of observation on individual larvae). Duration of feeding bouts was not quantified during peak periods, but appeared to be somewhat longer. Between feeding bouts, larvae would rest on leaves or twigs, would crawl to other parts of the tree, or would go through typical food-finding behavior.

In general, then, there are several important differences in behavior between larvae in endemic and outbreak populations. First, larvae in outbreaks remain in the canopy during the day rather than resting in hidden niches near the bases of trees. This change in behavior may have important implications regarding the effects of the primary mortality factors that operate different in density populations (see study #6). As populations increase, there is also an increase in the amount of feeding that occurs during the daylight hours. Further, larvae in outbreaks exhibit relatively short bouts of feeding and change their feeding sites frequently. While the reasons for these differences in behavior are not yet fully understood, laboratory tests indicate that they may be strongly tied to defoliation-induced changes in leaf chemistry rather than merely being by-products of different levels of inter-larval contact or larval density (see studies #3, 4 and 5, this section; Lance et al. 1981, 1982).

Study #3 - Development and behavior of gypsy moth larvae reared on black oak foliage.

Gypsy moths from different types of populations show qualitative differences in development and behavior. These differences may be related to differences in the quality of host foliage. In other systems, host plant quality has been shown to be affected for up to 3 years following heavy defoliation.

This study was undertaken to compare development, food utilization, and behavior among gypsy moths that were fed on foliage from previously defoliated trees, trees that were undergoing heavy defoliation, or trees with no recent history of defoliation.

Methods and Materials

During April, 1983, gypsy moth egg masses were collected from a moderately high density population near Moody Pond in Mashpee, Massachusetts. The eggs were held at 4°C until they were used. On May 9, eggs were dehaired, surface-sterilized, and held at 25° pending hatch. On May 13, neonates were transferred onto black oak foliage. The foliage collection sites, and their defoliation histories, are summarized in Table 2. The leaves were left attached to short twigs and were surface-sterilized in a 0.2% sodium hypochlorite solution. The twigs were placed into aqua-piks that had been pushed through the bottom of 480 ml unwaxed paper cups. The foliage was replaced every other day for the remainder of larval development. Rearing was carried out at 23°C with 5 larvae in each container.

A group of these larvae was set aside for feeding efficiency studies. Between apolysis and ecdysis of the IV-V molt, these larvae were removed from their rearing containers and were placed individually into 16 oz. cups. They then were provided daily with a single leaf, the area of which had been previously measured using a LiCor electronic planimeter (old leaves and frass were removed from the cups daily). On each day, 10 additional leaves from each site (see Table 4) were measured, dried (65°C for several weeks) and weighed, and the resulting data were used to develop regression equations of dry weight vs. leaf area. These regressions were used to estimate the amount of food which was given to each larvae. When a larva began to molt or spin up for pupation, it was placed into the drying oven, along with its frass and uneaten portions of the leaves. After several weeks, these samples were weighed, and feeding indices were computed using previously described methods (Lance et al. 1982; also see Table 4).

A second group of eggs was set up for hatch, and the neonates were infested on artificial diet on June 4. After 10 days (most of the larvae were 3rd instars at the time), the larvae were transferred to cut foliage from either an endemic site (Crane Wildlife Management Area/Hayway Road) or an outbreak site (Moody Pond area, Mashpee). On July 5, 40 larvae (V-VI instars) were taken from each defoliation treatment, were placed into individual 16 oz. cups, and were provided with foliage from either the endemic or the outbreak site (20 larvae per treatment on each type of foliage). Then, the larvae were observed 3 times hourly for 24 h starting at 1700 h. At each observation, the activity of each larva (resting, feeding or crawling) was recorded. During the scotophase, observations were made using light-amplifying equipment. A low wattage light bulb, painted red, was left burning on the opposite side of the room to provide the slight illumination necessary for using the night-vision equipment.

Results and Discussion

In feeding tests, larvae showed relatively poor development when they were provided with foliage from heavily attacked trees. Female pupal weights were significantly lower for larvae that were fed on foliage from heavily attacked trees (Table 2). Foliage from the twice-defoliated stand (Lee Rd) was the least suitable, as it resulted in the lowest female pupal weight and longest development times. When defoliation occurred only in the year prior to the study, foliage suitability was not significantly affected. These data are in agreement with previous studies (Lance et al. 1982, Valentine et al. 1983).

Table 2. Development of gypsy moth larvae on excised black oak foliage from stands with different defoliation histories.

	Percent defoliation		Male development[a]			Female development[a]		
Site	1982	1983	n of larvae	Mean days as larvae	Mean pupal weight (mg)	n of larvae	Mean days as larvae	Mean pupal weight (mg)
Crane	<20	<20	47	36.5 ab	435 a	49	41.9 a	989 a
Mashpee	<20	70-80	51	36.0 ab	421 a	47	42.5 a	772 b
Cotuit	100	<20	20	35.4 a	410 a	12	42.7 a	883 a
Lee Road	70	80	10	37.6 b	422 a	20	44.8 b	673 c

a Within a column, means that are not followed by the same letter are significantly different ($p < 0.05$) as determined by the Student-Neuman-Keuls test.

Larvae in the earlier series of tests (set up on May 13) completed development 1-3 weeks ahead of larvae in field populations; this difference probably was due to the relatively warm temperatures of the laboratory rearing environment. In contrast, larvae that were set up on June 4 completed development ca. 2 weeks later than wild larvae. In the latter tests, the effects of defoliation were more pronounced, and both males and females showed highly significant reductions in pupal weight and increases in development time (Table 3). In general, foliage quality declines as the season progresses; however, this decline appears to be much more rapid and severe in stands that are being heavily defoliated.

Table 3. Development of gypsy moth larvae that were reared on artificial diet for 12 days before being transferred to excised black oak foliage.

Defoliation at foliage collection site	Male development[a]			Female development[a]		
	n of larvae	Mean days as larvae	Mean pupal weight (mg)	n of larvae	Mean days as larvae	Mean pupal weight (mg)
Light (20%)	47	36.3 a	289.6 a	40	45.3 a	561.8 a
Heavy (70-80%)	19	45.2 b	182.6 b	25	54.7 b	323.9 b

a Within a column, means that are not followed by the same letter are significantly different ($p < 0.001$), as determined by ANOVA.

Quantitative analysis of feeding efficiency indicates that foliage from heavily attacked trees is not converted to biomass as efficiently (ECI) as foliage from trees in endemic sites (Table 4). Although differences in digestibility (AD) were not significant, most of the reduction in ECI appeared to result from the low AD of foliage from the outbreak site. This is in agreement with current theory, which suggests that defoliation-induced increases in leaf tannin content cause a reduction in the digestibility of foliage (Schultz and Baldwin 1982). Because their low ECI is not accompanied by a relatively high consumption rate (RCR), larvae from the outbreak-foliage treatment grew significantly more slowly (RGR) than larvae from the other treatments.

Table 4. Quantitative analysis of feeding for fifth instar gypsy moth larvae provided with black oak foliage from sites with various defoliation histories[a].

Defoliation at foliage collection site		n of	Weight					
1982	1983	larvae	gained	AD	ECI	ECD	RGR	RCR
<20%	<20%	19	37.2 a	21.6 a	4.9 a	48.9 a	0.076 a	1.53 a
<20%	70-80%	12	22.6 b	17.2 a	3.1 b	40.1 a	0.049 b	1.58 a
100%	<20%	13	40.1 a	21.4 a	4.3 a	30.6 a	0.069 a	1.62 a

a All indices were computed using mg dry weight, according to the following formulas:

AD = approximate digestibility = ([food ingested-frass]/food ingested)*100
ECI = efficiency of conversion of ingested food = (weight gain/good ingested)*100
ECD = efficiency of conversion of digested food = (weight gain/[food ingested-frass])*100
RGR = relative growth rate = (ln [final dry wt.]-ln [starting dry wt.])/(days as larvae)
RCR = relative consumption rate = (RGR/ECI)*100

For AD, ECI and ECD, arcsine squareroot transformations were performed prior to ANOVA (actual means are shown). Within a column, means that are not followed by the same letter are significantly different ($p < 0.05$) as determined by the Student-Neuman-Keuls test.

In the feeding rhythm tests, the mean percentage of feeding that occurred during the scotophase was only 61.1% for larvae from the endemic-foliage treatment. This was lower than expected; however, suitability indices (see Table 3) indicate that these larvae developed on poor quality food (perhaps this resulted because the foliage was changed every other day instead of daily). Poor quality food can result in a decrease in the amount of feeding that occurs nocturnally (see Study #5). Accordingly, the percentage of feeding that occurred during the scotophase was 49.7% for larvae from the outbreak-foliage treatment. The Mann-Whitney U test indicated that feeding was significantly ($p < 0.01$) more nocturnal for larvae from the endemic-foliage treatment. This lends further support for the theory that the density-correlated shift in feeding rhythms is actually a response to defoliation-induced changes in leaf quality.

Study #4 - Laboratory studies on feeding rhythms of larvae from outbreak and endemic populations.

In high density populations, late instar gypsy moth larvae feed on and off throughout the day and night, with a sharp peak of feeding typically occurring ca. 2 hrs after sunrise. Larvae in endemic populations feed almost strictly nocturnally. Field observations (see Study #2) suggest that these differences in feeding rhythms are manifestations of qualitative physiological differences between larvae from high density populations and those from endemic populations. If this is true, then larval feeding rhythms could provide a useful tool for assessing gypsy moth population quality. The purpose of this study was to determine whether the respective feeding rhythms of high-density and endemic larvae are maintained when these insects are brought into a controlled laboratory environment.

Methods and Materials

Feeding and locomotor activity rhythms of field-collected late instars were tested using a previously described technique (Lance et al. 1982). Fifth instar larvae were collected from outbreaks in Cotuit, Massachusetts (1982) and the Massachusetts National Cemetary (1983), and from endemic populations in Ashumet, Massachusetts (1982) and Crane Wildlife Managemenmt Area (1983). These larvae were placed individually in the test chamber and were provided with a black oak leaf from one of the sites. They were allowed to feed for 24 hrs, starting at 1400-1500 hrs. These tests were conducted at 25°C, 0500-2100 Pp (EDT), with a chart speed of 15 cm/h. If larvae exhibited very little feeding during the test, they were held for 24 hrs in a 180 ml plastic cup and were provided with foliage from an endemic site. This was done to determine whether the lack of feeding was due to non-preference for the test foliage, or whether the larvae had not eaten for some other reason (e.g., they were diseased, parasitized, or about to pupate). Only healthy, "hungry" larvae were included in the analyses.

Results and Discussion

A total of 10 larvae were tested for each combination of population type and test foliage. The feeding rhythms of these larvae are shown in Fig. 2. In general, the feeding rhythms characteristic of the source population were maintained when the larvae were brought into the laboratory. Larvae from the endemic populations fed primarily nocturnally, although total feeding was reduced when the larvae were offered foliage from the outbreak site (2.1% of their time vs. 7.0% with "endemic" leaves; the difference probably was due to differences in the acceptability of the leaves from the 2 types of sites).

Larvae from the outbreak site also spent more time feeding on foliage from the endemic site, but they also showed considerable feeding on outbreak foliage (9.5% of the 24 hr day for "endemic" foliage vs. 7.0% for "outbreak" foliage). Regardless of the test foliage, the outbreak larvae fed on and off throughout the day, which agrees with our observations in field populations (Study #2).

Larvae from 3 of the 4 treatments showed a low level of locomotor activity throughout the day (Fig. 3). However, when endemic larvae were offered foliage from an outbreak site, they were very active during the scotophase. This indicates that this foliage is not readily accepted by endemic larvae; gypsy moth larvae tend to be more active in the presence of less preferred food (Lance and Barbosa 1982).

Figure 2. Feeding rhythms of fifth instar gypsy moth larvae that were field-collected from endemic and outbreak populations, and then offered foliage from endemic or outbreak site while being tested.

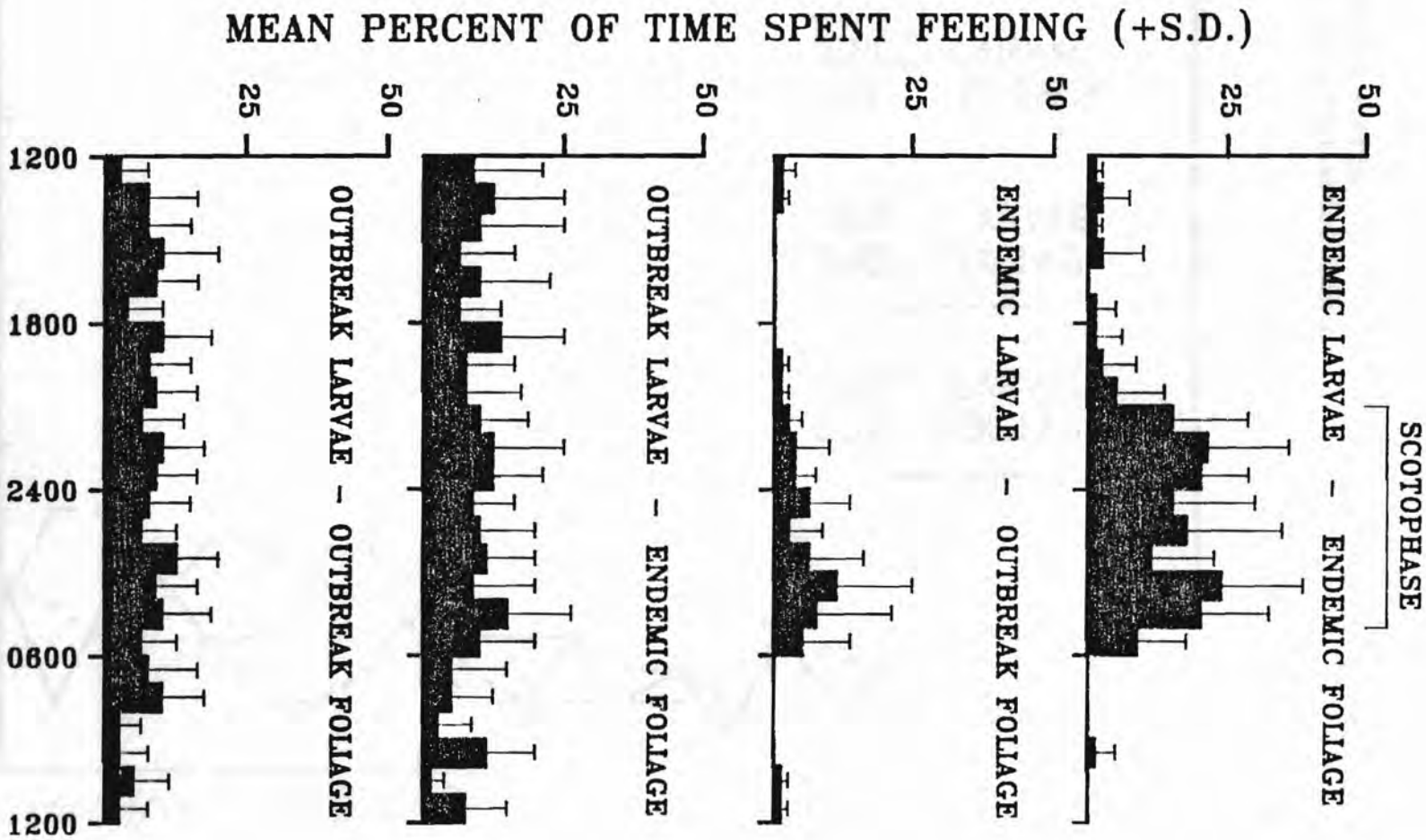
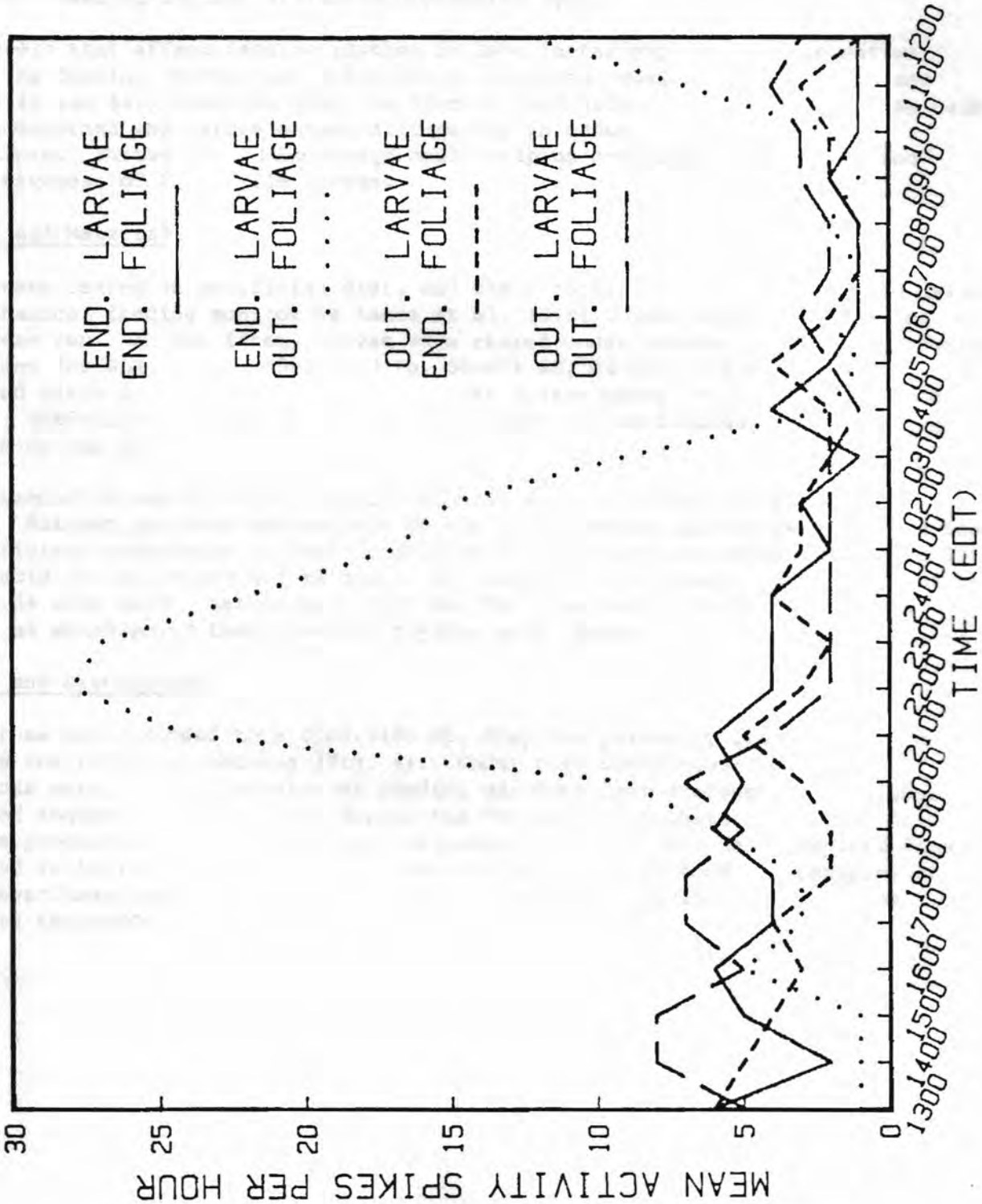


Figure 3. Locomotor rhythms of fifth instar gypsy moth larvae which were field-collected from outbreak and endemic populations; larvae were then exposed to foliage which was taken from either an outbreak or an endemic site.



Food quality (as affected by defoliation pressure), then, can have immediate effects on locomotor activity and feeding behavior. Other laboratory studies (see Study #5) indicate that, when larvae are exposed over long periods to high or low quality diets, their feeding rhythms reflect those of endemic or outbreak larvae, respectively. Thus, defoliation-induced changes in foliage quality appear to be very important in determining the behavioral as well as developmental aspects of gypsy moth population quality.

Study #5 - Feeding rhythms of laboratory-reared gypsy moths.

The factors that affect feeding rhythms in late instar gypsy moths are not well known. As feeding rhythms are different in different quality populations (see Studies #2 and #4), understanding the factors that affect feeding rhythms may help us to understand why larvae behave differently in endemic and outbreak populations. Further, this knowledge could help us evaluate the behavior and competitiveness of F1-sterile larvae.

Methods and Materials

Larvae were reared on artificial diet, and their feeding rhythms were tested using the mechanical feeding monitor of Lance et al. (1981). Two separate series of tests were run. In the first, larvae were reared under standard laboratory rearing conditions (or SLRC; 25°C, 0500-2100 Pp, 50-60% RH, B4 high wheat germ diet). Fifth and sixth instars then were tested over 3 days under one of 3 photoperiod regimes: 0500-2100 Pp (control), continuous light, or continuous dark (starting at 2100 hrs on the day the test was set up).

In the second series of tests, larvae were reared at SLRC through the second instar. Between apolysis and ecdysis to the third instar, larvae were removed from their original containers and were placed on fresh diet that contained 0, 2 or 10% tannic acid (8 larvae per 180 ml cup). In ensuing tests, tannin concentrations of 0 and 2.5% were used. Larvae were left on this diet until the fifth or sixth instar, at which point their feeding rhythms were tested.

Results and Discussion

When larvae were exposed to a 0500:2100 Pp, they fed primarily from 2100 hrs until 0600 hrs the following morning (Fig. 4). Under both continuous light and continuous dark, the periodicity of feeding was much less distinct. The higher levels of feeding that did occur during the "normal" scotophase period may have been the product of a weak circadian influence, or simply may have resulted from cycles of satiation alternating with complete digestion of food and emptying of the gut. Regardless, gypsy moth feeding rhythms appear to result primarily from immediate responses to environmental stimuli.

Food quality (as affected by defoliation pressure), then, can have immediate effects on locomotor activity and feeding behavior. Other laboratory studies (see Study #5) indicate that, when larvae are exposed over long periods to high or low quality diets, their feeding rhythms reflect those of endemic or outbreak larvae, respectively. Thus, defoliation-induced changes in foliage quality appear to be very important in determining the behavioral as well as developmental aspects of gypsy moth population quality.

Study #5 - Feeding rhythms of laboratory-reared gypsy moths.

The factors that affect feeding rhythms in late instar gypsy moths are not well known. As feeding rhythms are different in different quality populations (see Studies #2 and #4), understanding the factors that affect feeding rhythms may help us to understand why larvae behave differently in endemic and outbreak populations. Further, this knowledge could help us evaluate the behavior and competitiveness of F1-sterile larvae.

Methods and Materials

Larvae were reared on artificial diet, and their feeding rhythms were tested using the mechanical feeding monitor of Lance et al. (1981). Two separate series of tests were run. In the first, larvae were reared under standard laboratory rearing conditions (or SLRC; 25°C, 0500-2100 Pp, 50-60% RH, B4 high wheat germ diet). Fifth and sixth instars then were tested over 3 days under one of 3 photoperiod regimes: 0500-2100 Pp (control), continuous light, or continuous dark (starting at 2100 hrs on the day the test was set up).

In the second series of tests, larvae were reared at SLRC through the second instar. Between apolysis and ecdysis to the third instar, larvae were removed from their original containers and were placed on fresh diet that contained 0, 2 or 10% tannic acid (8 larvae per 180 ml cup). In ensuing tests, tannin concentrations of 0 and 2.5% were used. Larvae were left on this diet until the fifth or sixth instar, at which point their feeding rhythms were tested.

Results and Discussion

When larvae were exposed to a 0500:2100 Pp, they fed primarily from 2100 hrs until 0600 hrs the following morning (Fig. 4). Under both continuous light and continuous dark, the periodicity of feeding was much less distinct. The higher levels of feeding that did occur during the "normal" scotophase period may have been the product of a weak circadian influence, or simply may have resulted from cycles of satiation alternating with complete digestion of food and emptying of the gut. Regardless, gypsy moth feeding rhythms appear to result primarily from immediate responses to environmental stimuli.

Figure 4. Effect of photoperiod on the feeding rhythms of laboratory-reared late instar gypsy moth larvae over a period of 3 days. Double underscoring of the abscissa denotes the scotophase.

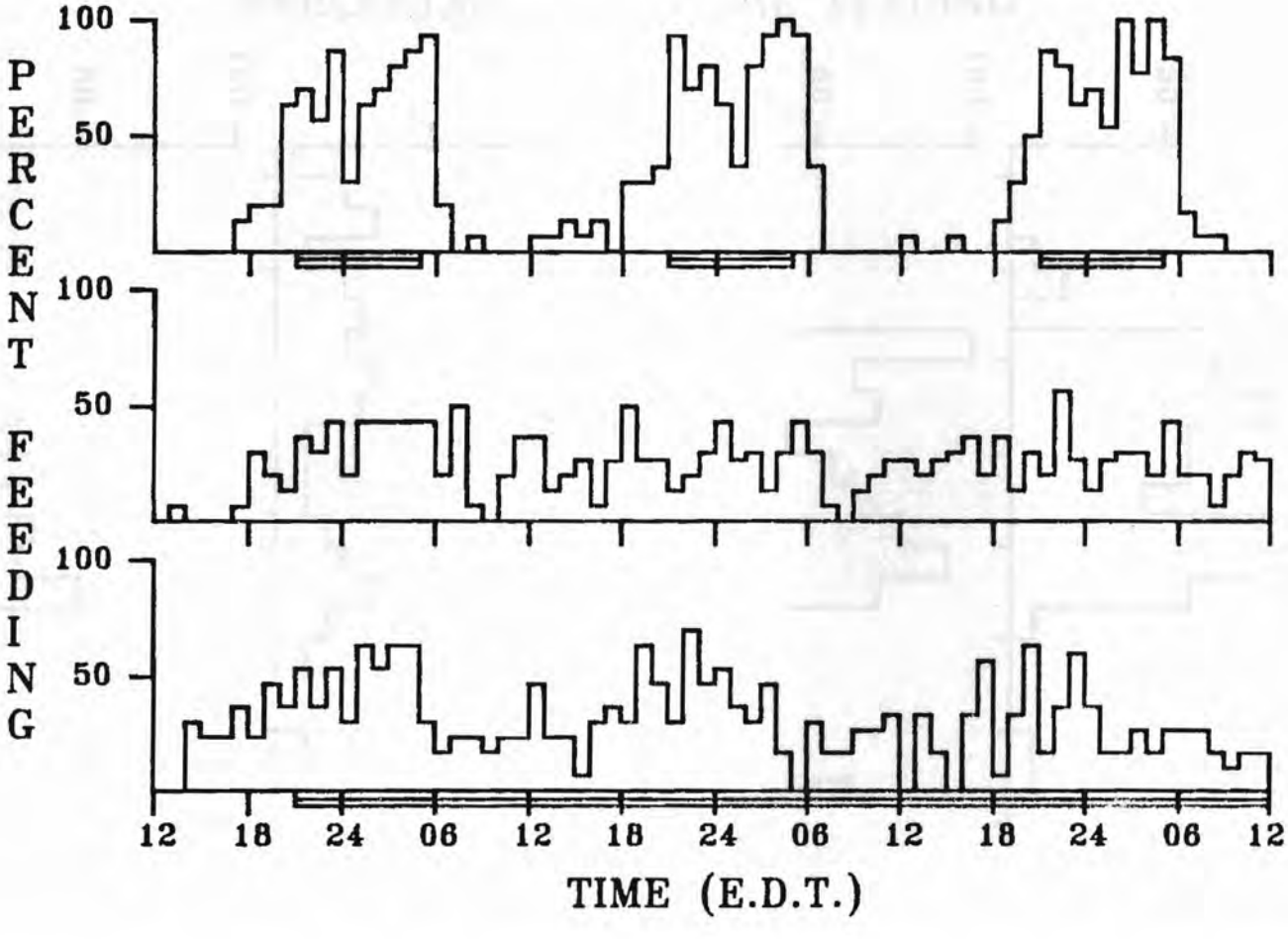
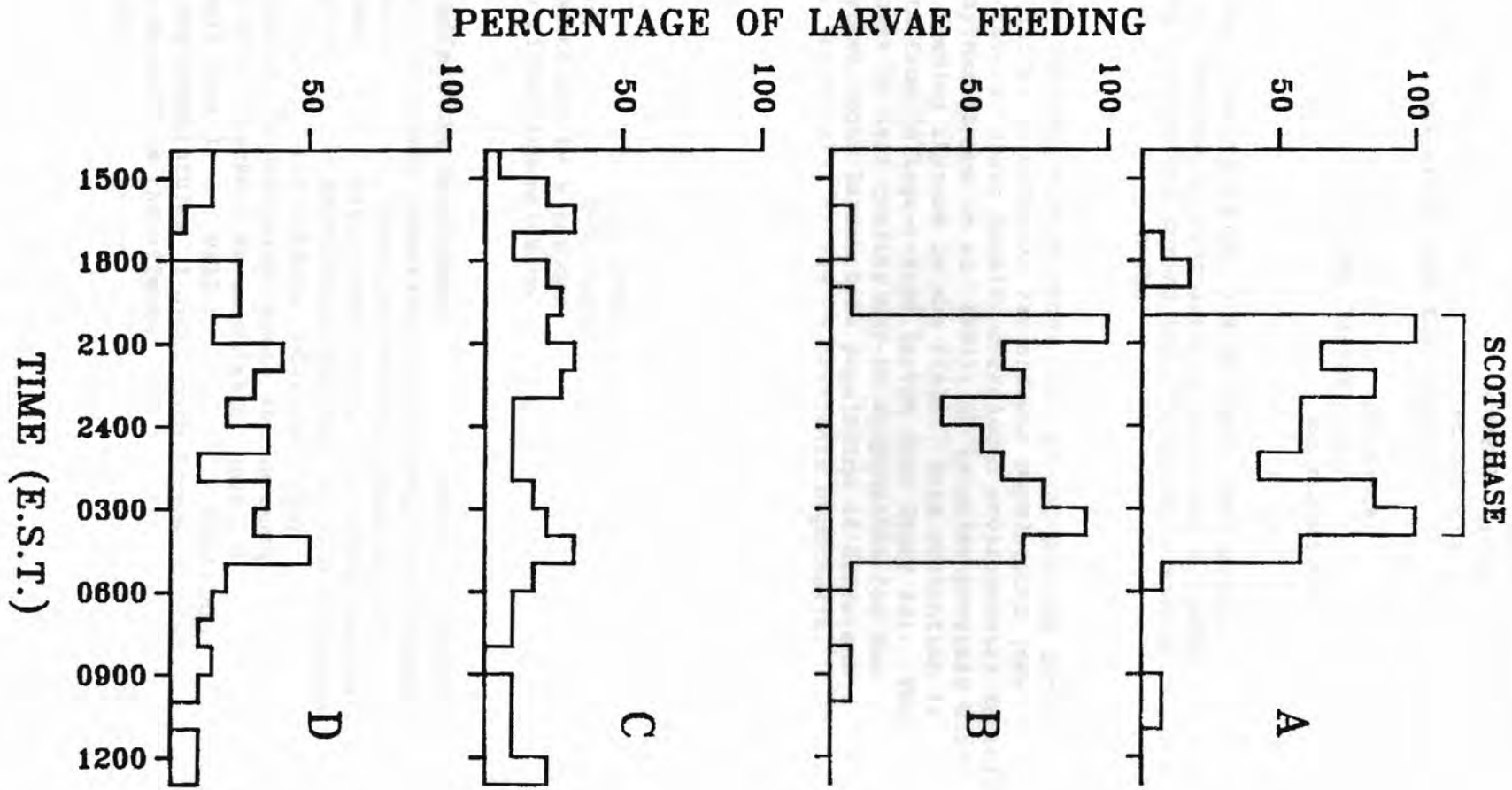


Figure 5. Feeding rhythms of laboratory-reared late instar gypsy moth larvae. (A) and (B): Larvae that were reared on standard B4 diet and were offered B4 diet or diet containing 2.5% tannic acid, respectively, during the test. (C) and (D): Larvae that were reared on diet containing 2.5% tannic acid and were offered diet containing 0% or 2.5% tannic acid, respectively.



In the tannic acid tests, the diet offered during the actual tests had very little influence on feeding rhythms (compare Fig. 5, a vs. b, and c vs. d). However, when larvae were reared on diets that contained tannic acid, they showed much less feeding periodicity compared to control larvae (Fig. 5, a and b vs. c and d). The proportion of feeding-hours that occurred during the scotophase was computed for each larva, and the resulting percentages were subjected to the Mann-Whitney U test. In comparisons within rearing treatments, the tests did not detect significant differences resulting from the diet used during the test ($p = 0.89$ for larvae reared on control diet, $p = 0.32$ for the tannin-reared larvae; the statistic Z, corrected for ties, was used). However, larvae from the control rearing treatment showed a highly significantly ($p < 0.0001$) greater tendency to restrict their feeding to the scotophase. Data from the 0% vs. 2.0% vs. 10% TA tests (not presented here) further suggested that a reduction in diet quality can cause a reduction in the proportion of feeding that occurs during the scotophase. However, the magnitude of the effect did not increase when the tannin level was elevated from 2.0% to 10%.

The difference in feeding rhythms between tannin-reared and control larvae was similar to that between larvae in outbreak vs. endemic populations (see Studies #2 and #4). As several other density-correlated environmental factors do not affect feeding rhythms (Lance et al. 1981), it is quite possible diet quality also determines feeding rhythms in the field. This contention is supported further by data from foliage-reared larvae (see Study #3). Thus, defoliation-induced changes in leaf quality may be responsible for the variation in feeding rhythms among larvae from population of different densities. Further experiments will be run to test this hypothesis.

Study #6 - Population Density, Microhabitat and Development Time in Gypsy Moth Larvae.

The gypsy moth periodically defoliates large areas of deciduous woodlands in the northeastern United States. During these outbreaks, larvae develop up to 3 weeks faster than they do in low-level (endemic) populations (Campbell 1978). Some authors (e.g., Leonard 1968, Campbell 1978) have suggested that this accelerated development may be a response to larval crowding, but laboratory data has been either inconclusive or contradictory (Leonard 1968, Lance et al. 1981).

Temperature is known to affect the development rate of insects. Microhabitat can have a profound effect on insect temperatures; in fact, some lepidopterous larvae can thermoregulate by altering their microhabitat and/or their orientation to the sun (Wellington 1949, Casey 1976). In endemic populations, gypsy moth larvae feed at night but rest during the day in protected sites near the bases of trees (e.g., in the litter, under bark flaps or in crevices in the bark). However, larvae in outbreaks remain in the canopy throughout the day and night (Study #3). Further, as defoliation opens the canopy, it becomes increasingly likely that larvae will be exposed to sunlight. In this study, we have examined the temperatures of gypsy moth larvae in high and low-density populations, as well as the effects of several temperature regimes on larval development rates.

Methods and Materials

Field studies were carried out in an outbreak population within the Massachusetts National Cemetery, Otis Air Base, Massachusetts, and in an endemic population in Crane Wildlife Management Area, Falmouth, Massachusetts. Both sites consist of mixed woodlands that are dominated by 6-11 m h black oak (*Quercus velvtina* Lam.), white oak (*Q. alba* L.) and pitch pine (*Pinus rigida* Mill.). At the outbreak site, almost all larvae spent the day in the canopy and the oaks were ca. 80% defoliated by the time pupation occurred. Defoliation at the endemic site was very light and larvae spent the day resting in sites near the bases of the trees.

The body temperatures of larvae (T_b) were measured to the nearest 0.1°C, using a Baily BAT-8 thermometer with a microthermister probe. The probe was applied to a fold in the dorsal integument and the temperature was allowed to stabilize for several seconds before a reading was taken. After reading the temperature of each larva, ambient temperature (T_a) was measured by rapidly revolving the probe on a ca. 0.5 m radius until the temperature stabilized. For each larva, the following data were recorded: height off of the ground, degree of insolation, structure on which the larva was located, activity in which the larva was engaged, larval instar (determined subjectively), and time of day. On 7 occasions, all temperature readings at both sites were made within a period of 90 minutes; the resulting data were used for inter-population comparisons. Scaffolding of 10 cm diameter pipes was built around trees to allow access to the canopy.

In the laboratory, larvae were reared on artificial diet (Bell et al. 1981) at 8 per 180 ml container under several temperature regimes. During June, 1982, on Otis Air Base, mean temperatures were 15°C between 2100 and 0700 h, and 20° from 0700 to 2100 h. For rearing, a 12:12 h thermoperiod of 15°:20° was used as the control temperature regime. Even in endemic populations, larvae remain in the canopy for the first 2-3 stadia (Campbell 1978). Thus, all larvae were held under the same temperature regime (15°:20°) for 20 days, at which point most of the larvae were 3rd instars. Then, groups of larvae were transferred to 12:12 h thermoperiods of 15°:22° and 15°:24°. These temperature regimes were designed to reflect the differences in the temperatures of larvae in outbreak vs. endemic populations (see below). After 40 days, the rearing containers were checked for pupation on a daily basis.

Results and Discussion

Over a period of 12 days, the temperatures of 366 larvae were measured. On sunny days, larvae in the outbreak population were 2°-6° above ambient (T_b-T_a), while larvae from the endemic population were near ambient (Table 1). On cloudy days (e.g., J.D. 186), all larvae were near ambient and the difference between populations was less pronounced or absent. Similarly, at night, all of our measurements of larval temperatures fell within the range of $T_a \pm 0.7°$ (n=30), regardless of site.

Table 1. Mean temperature data for instar gypsy moths in high and low-density populations.

			Low density site[a]			High density site[a]		
Julian date[b]	Time	Percent cloud cover	n of larvae	T_b	T_b-T_a	n of larvae	T_b	T_b-T_a
181*	1200	0	7	22.7	-2.6	9	29.6	3.2
182*	1700	75	13	21.8	0.4	19	24.3	1.8
186	1000	100	14	23.2	0.6	19	23.1	0.8
186*	1300	100	19	24.3	0.2	19	25.4	0.8
188*	1300	25	19	22.1	0.0	19	26.3	3.1
191*	1300	33	19	23.0	0.4	19	24.7	2.7
192*	1000	25	19	22.4	-0.4	19	25.0	2.6

[a] T_b represents the temperature of larvae, while T_b-T_a represents the difference in temperature between the larvae and ambient.

[b] On dates followed by an asterisk (*), both T_b and T_b-T_a differed significantly between sites ($p < 0.01$).

In the endemic population, almost all larvae were in the shade. However, larval temperatures did vary with microhabitat. For larvae under bark flaps, T_b-T_a averaged -0.80° (n=21), while larvae in bark fissures (+0.04, n=74) and in the litter (+0.63, n=30) were slightly warmer than ambient; these 3 means were significantly different from one another (Student Newman-Keuls test, $p < 0.05$).

On sunny days in the outbreak population, larvae which were fully exposed to sunlight were an average of 3.1° above ambient (n=45), while shaded larvae had a mean T_b-T_a of 1.3° (n=73). Other larvae were either half in and half out of the shade, or were alternately in and out of the sun as overhead leaves moved in the wind; for these partially insolated larvae, T_b-T_a averaged +2.6° (n=42). The means from the 3 insolation categories were statistically distinct (S.N.K. test, $p < 0.05$).

On 4 days at the outbreak site, we measured the temperatures of larvae that were resting in locations similar to those utilized by larvae in endemic populations (i.e., low on the boles of trees). For these larvae, T_b-T_a was 1.2° (n=23), which was significantly lower ($p < 0.001$) than T_b-T_a for the larvae in the canopy on those same 4 days (2.1°, n=54). On 3 of these 4 days, temperatures also were measured for larvae on tree boles at the endemic site. On 2 of the 3 days, T_b-T_a was significantly ($p < 0.01$) lower for larvae in the endemic populations as compared to larvae that were low on the boles of trees at the outbreak site (0.6° and -0.5° at the endemic site vs. 1.5° and 2.2° at the outbreak site, respectively). The third day (J.D. 186, a.m.) was overcast and the difference between sites was negligible. Thus the higher temperatures of larvae in outbreaks appear to be a function of both the difference in the locations of the larvae and the increase in the amount of sunlight penetrating the canopy. Within the canopy (over 3 m off of the ground), larval temperature did not vary significantly with height off of the ground, plant structure on which the larva was situated (leaf, twig or branch), or activity in which the larvae was engaged (resting, crawling or feeding).

In a number of lepidopterous species, larvae thermoregulate through microhabitat selection or by changing their posture with respect to incoming thermal radiation (Casey 1976). In our observations in the outbreak population, gypsy moth larvae did not appear to respond to cool weather by "basking" in the sunlight. We did not attempt to characterize minor variations in the postures of the larvae, although they can have significant effects on the temperatures of gypsy moth larvae that are exposed to thermal radiation (Casey and Hegel 1981). However, larval temperature did not increase with decreasing ambient temperature (e.g., see Table 1), which indicates that the larvae were probably not using posture to maintain an "optimal" temperature. Still, positional thermoregulation may be important when ambient temperatures are more extreme than those encountered in this study.

When larvae were exposed to a 15°:22° or a 15°:24° temperature regime in the laboratory, they developed 1 or 2 weeks faster, respectively, compared to larvae from the 15°:20° control group (Table 2). These differences in development time are comparable to those observed between high and low-density populations in the field (Campbell 1978, D. R. Lance personal observation). Thus, density-related differences in development time appear to result largely from the shift in microhabitat selection that occurs as population increases.

Table 2. Effect of temperature on development time in laboratory-reared gypsy moth larvae.

Rearing temperatures[a]		Male development		Female development	
0700-1900h	1900h-0700h	n of larvae	Mean days as larvae[b]	n of larvae	Mean days as larvae[b]
20°	15°	78	64.4 a	53	69.6 a
22°	15°	76	56.1 b	66	60.4 b
24°	15°	88	51.2 c	51	55.0 c

[a] For the first 20 days of development, larvae from all 3 treatments were under a 15°:20° thermoperiod.

[b] Within a column, means that are not followed by the same letter are significantly different ($p < 0.05$) as determined by the Student-Newman-Keuls test.

Diet quality and larval density also have been suspected as factors that potentially could affect development rate. Leonard (1968) reared larvae singly and under crowded conditions, and he reported that the crowded larvae developed a few days faster. However, his comparisons were made only between groups of larvae which had gone through the same numbers of stadia before pupating. A higher percentage of the crowded larvae underwent extra-numerary molts, and development time increased with the number of stadia. When Leonard's data are averaged for all larvae (which would be more relevant to the field situation), differences in development time become negligible. We have also found that development time does not increase with density in laboratory cultures (Table 3).

Table 3. Mean development times for gypsy moth larvae that were reared under crowded and isolated conditions.

	Mean days as larvae					
	Test 1a[a]		Test 1b[a]		Test[b]	
Rearing treatment	male	female	male	female	male	female
Isolated	39.1	44.5	44.3	51.4	35.2	39.6
Crowded	40.5	50.7	43.0	50.2	37.2	40.1

[a] Data from Leonard, 1968.

[b] Data from Lance, Elkinton, Mastro and Schwalbe, unpub. manuscript; "crowded" larvae were reared at 10 per 180 ml container.

Differences in diet quality also are not likely to result in the relatively rapid development which occurs during outbreaks. Defoliation-induced changes in oak leaf chemistry cause foliage quality to decline as defoliation proceeds (Wallner and Walton 1979, Schultz and Baldwin 1981, Study #3, this report). Exhaustion of local food supplies and utilization of secondary hosts also can act to reduce diet quality (Barbosa and Greenblatt 1979, Lance et al. 1981). Declines in food quality typically tend to, if anything, extend development time (Barbosa and Greenblatt 1979, Wallner and Walton 1979, see also Study #3). However, because the quality of the available diet deteriorates rapidly as defoliation progresses, larvae that develop slowly will have only poor quality food (or no food) on which to finish their development. During outbreaks, individuals that remain in warmer microhabitats should develop faster and therefore may not be as severely affected by defoliation-related reductions in diet quality.

When larvae spend the day resting in relatively protected sites, they enjoy a reduced risk of being located by parasitoids and avian predators (Campbell and Sloan 1976). These natural enemies are important in maintaining populations at endemic levels. However, during outbreaks, a nuclear polyhedrosis virus is typically the major mortality agent of the late instars (Campbell 1967, Campbell and Sloan 1978). If larvae in outbreaks did not remain in the canopy by day, they likely would form dense aggregations near the bases of trees. Campbell (1978) suggested that such aggregations could enhance the spread of virus. Because viral mortality (and consequently, inoculum) is typically greatest in the late stages of development (Doane 1970), we further suggest that slowly developing larvae would have an increased risk of ingesting a large dose of viral innoculum. By remaining in the canopy during the day, larvae also may have a better chance of pupating before pending viral epidemics reach their full level of impact.

Thus, the gypsy moth larvae's density-related shift in microhabitat selection appears to have implications for avoiding natural enemies (Campbell and Sloan 1976) and perhaps for assimilating poor quality food. During outbreaks, this shift also results in rapid development, which may allow larvae to escape the full impact of viral epidemics and defoliation-induced reductions in foliage quality.

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Project Number: GM 1.2.4
Project Title: Mass Trapping
Report Period October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: V. C. Mastro and C. P. Schwalbe

Introduction

Studies conducted during the 1982 field season demonstrated that, at a given insect density, the proportion of the male population trapped (recaptured) generally increased as trap density increased from 0.75 trap/ha to 25.0 trap/ha. Roughly the same proportion of males were trapped at the two most dense trap configurations (7.5 - 25/traps/ha), however, mating success of placed females was always the lowest in plots which were arrayed with trap densities of 25 traps/ha. Thus, as trap density increases, the proportion of males trapped levels off, but mating success continues to decrease. One explanation for this is that males may be trapped faster (e.g. before they can mate) at the higher trap densities. To determine if rapidity of capture was influencing mating success, a series of tests was conducted during the 1983 field season.

Methods and Materials

Four plots were established in wooded areas near Otis ANG Base with a minimum of one mile between plots. Traps were arrayed in three of these plots at densities of 2.5, 7.5 and 25 traps/ha. The fourth plot served as a control and was not trapped. Traps used in this study were standard USDA delta traps baited with 500 ug (+) disparlure in the standard plastic laminate formulation. Within each plot a grid (10 m between points) was established to facilitate insect release. Insect release grids were positioned so that the minimum distance between an insect release point and an outside trap position was one-half of the inter-trap spacing.

Insect population density was held constant throughout this test at 2.5 pairs per ha. Males and females were both released as one day old adults (i.e. the day following eclosion). Positions for male and female release were selected randomly within the grid. For any particular release day, the same randomization scheme was used for all four treatment blocks. Release positions were rerandomized for each replicate (total of 5 replicates).

Females for release were placed in standard A-frames which were suspended ca. 1.5 m above the ground. Males, which had been marked with fluorescent powder, were shaken from cups at the release site. Releases were normally completed by 8:00 AM EST. Following release of adults, traps were checked on an hourly basis for male captures for the remainder of the day (9:00 AM - 3:00 PM EST). Males were removed, checked for marking and recorded.

Females were recovered after the last round of trap checks and returned to the laboratory for oviposition. Any resulting eggs were held (30 days) for embryonation and determination of mating success.

Results and Discussion

As in previous studies, when traps were placed in more dense configurations, higher proportions of males were recaptured and female mating success decreased. The most dense configuration of traps (25/ha) resulted in recapture of an average of 86% of all males and mating was suppressed to an average of only 4% of the females placed. Unlike 1982 trials, an array of 25 traps per hectare recaptured more males than the plots arrayed with only 7.5 traps per hectare. Other results are similar for both years' studies.

Table 1. Mating success of females and recapture rates of males in simulated populations trapped at three densities.

Trap density per hectare	Mean percent of males recovered	Mean percent of females mated	Mean elapsed time for male recapture in hours
0 (control)	-	67.1	-
2.5	67.5	23.2	3.5
7.5	75.5	8.9	2.5
25	86.0	4.0	2.2

These studies demonstrate that as trap density increased, released males were recaptured more quickly. A short pre-capture period (PCP) allows males less searching time and therefore a lower probability of finding a female before finding a trap. With increasing trap density, the decrease in PCP parallels the decrease in female mating success. Although it appears to be possible to reduce mating success to zero, the nature of the relationship between trap density and successful mating indicates a very dense placement of traps would be required. At this insect density, twenty five traps per hectare provided approximately a 94% reduction in mating compared to the control group. If applied to the same population for several years, this rate of trapping should, assuming the annual rate of increase is no greater than 15 X, provide for a systematic reduction of the population to extinction.

Further mass trapping studies in 1984 will define the best trap density to apply to various insect densities. Because the effectiveness of trapping is affected by insect density, a well defined relationship would enhance the use of this technique for treatment of sparse isolated populations.

Project Number: GM 1.2.7
Project Title: Gypsy Moth Mating Disruption - Oconomowoc, Wisconsin Demonstration Project
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: C. P. Schwalbe

The history of this project since detection of the infestation in 1977 was summarized in the last Progress Report. While the initial intent of the program was to demonstrate eradication by mating disruption with aerially broadcast disparlure, 1982 and 1983 actions have employed mass trapping tactics.

1982 trapping yielded only 19 moths in a very localized area near Okauchee Lake. This area was again mass trapped (3 traps/acre) in 1983, and only 4 males were captured. However, delimitation trapping (32 traps/mi^2) over the surrounding area resulted in the surprising find of 17 males on the western edge of what was the original infestation. Clearly, a population is maintaining itself in that area, in spite of Sevin and disparlure treatment there in 1981; 1982 delimitation trapping in that vicinity was negative.

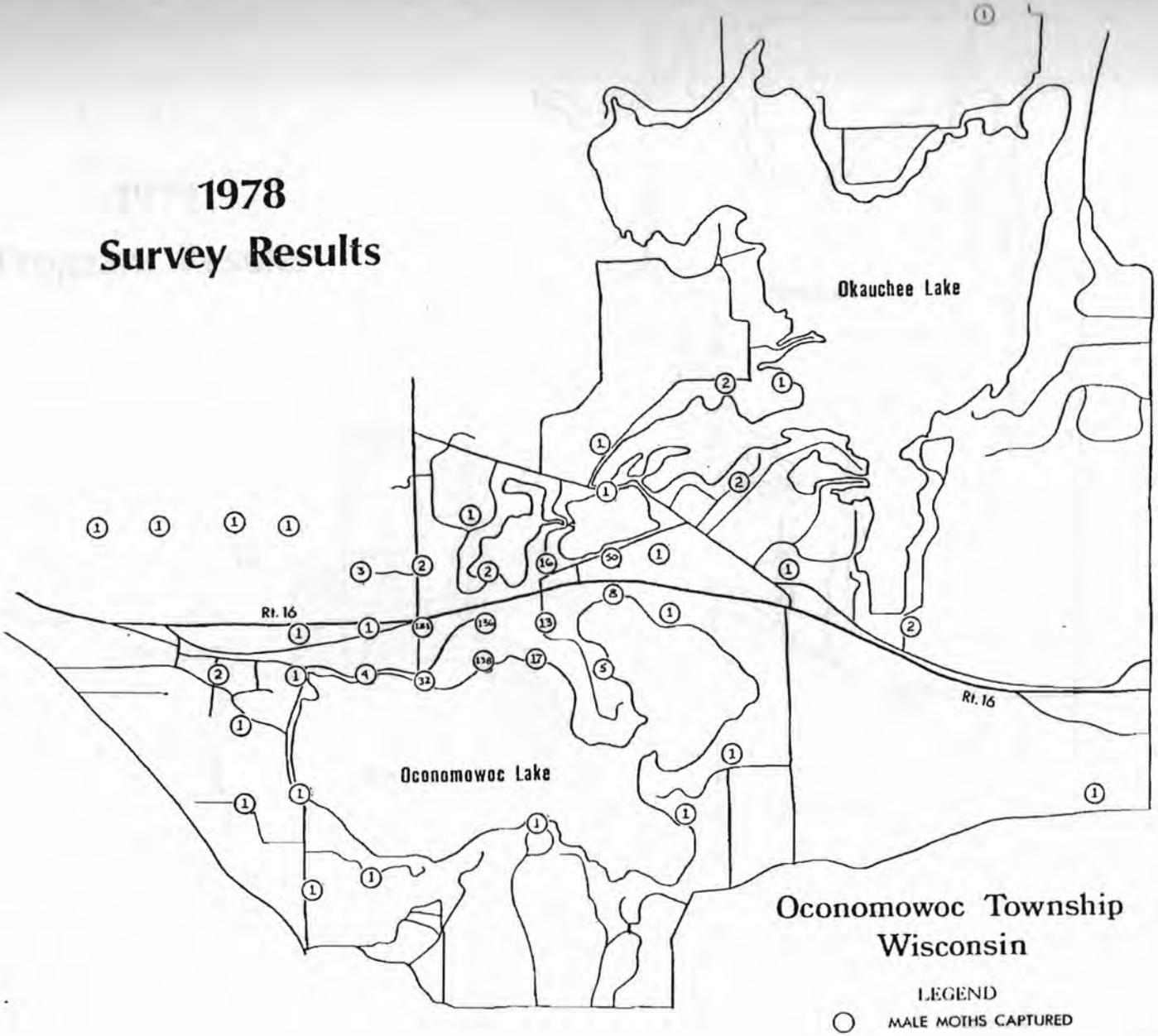
Based on these findings we conclude:

1) Mass trapping is associated with a general reduction of population density since 1981 near Okauchee Lake.

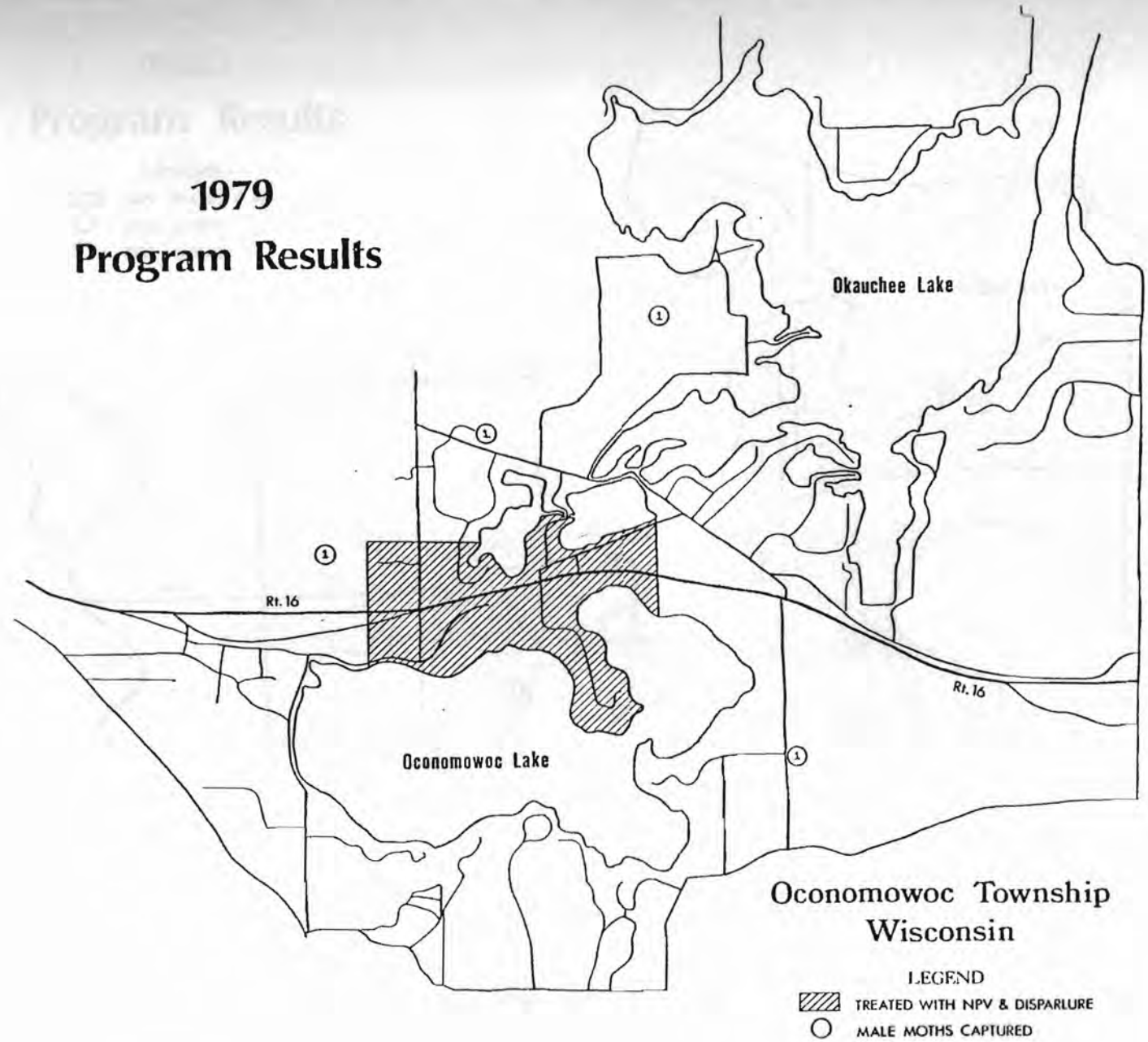
2) The combined effects of broadcast disparlure (Hercon flakes) and limited ground application of Sevin resulted in large parts of the original 1978 infestation being reduced to non-detectable levels. In 1978, 700 moths were captured and the infestation was estimated to occupy 425 acres.

3) A population (which is probably reproducing) has been located on the western edge of the project area.

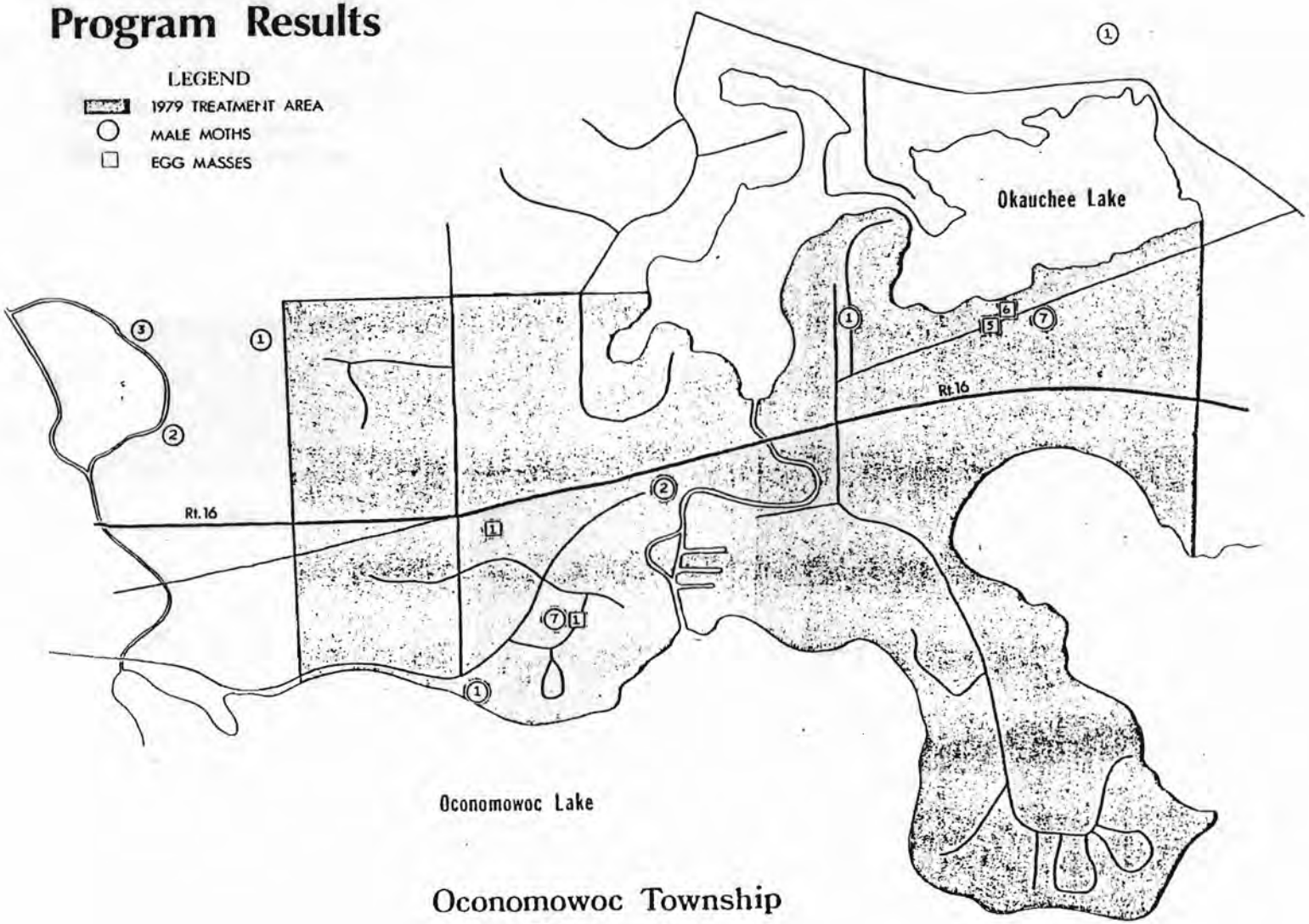
1978
Survey Results



1979
Program Results

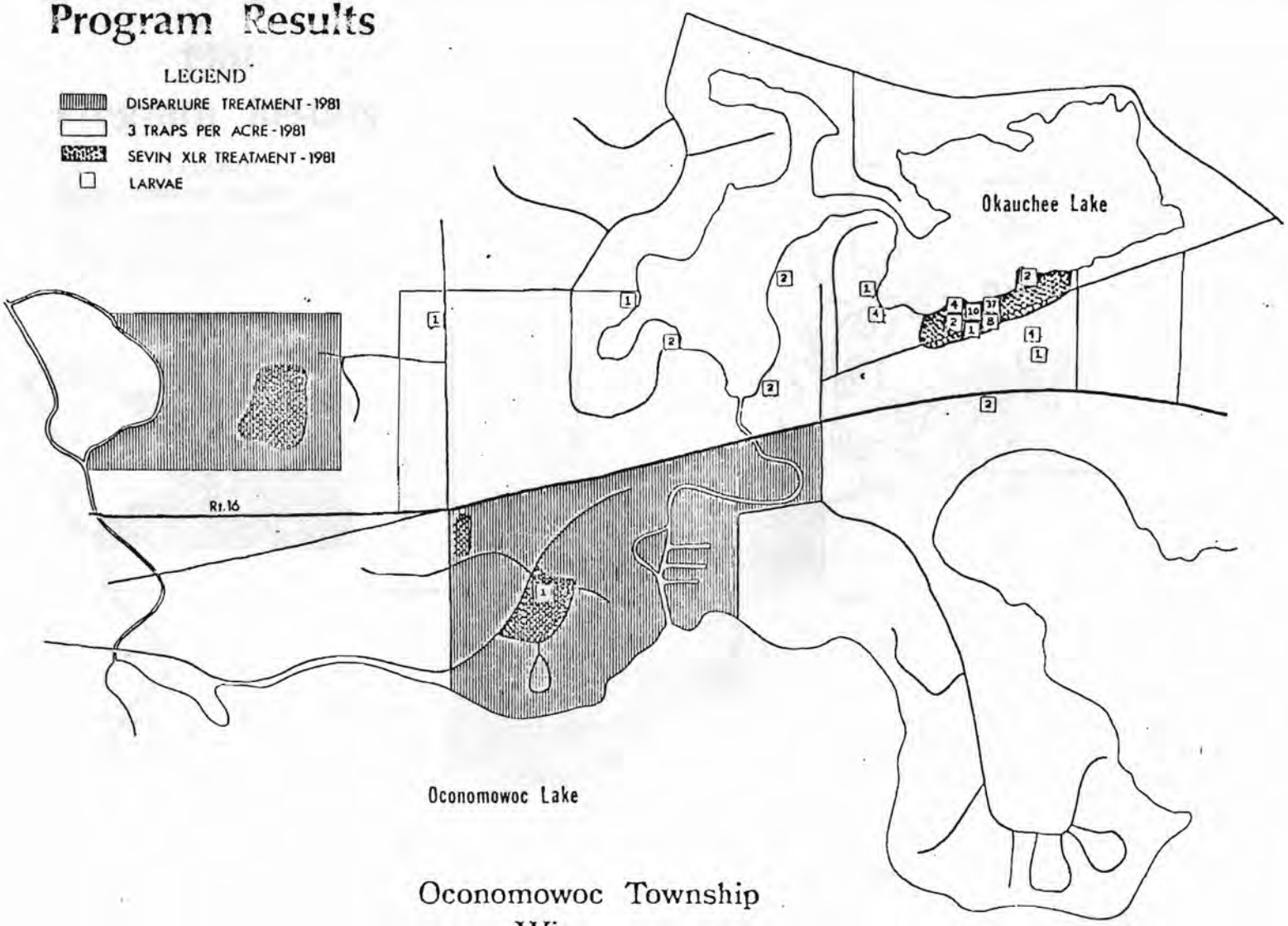


1980
Program Results



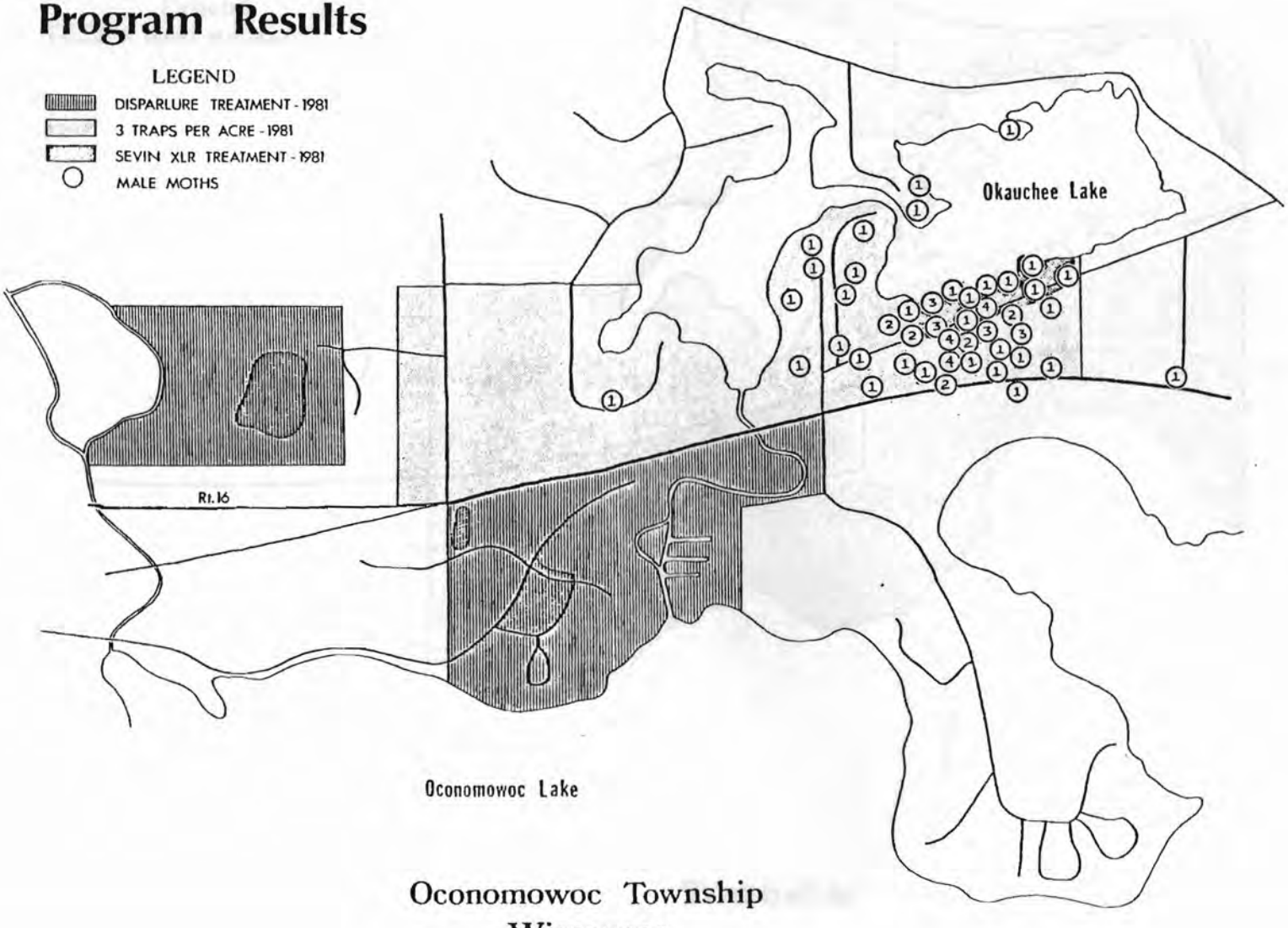
Oconomowoc Township
Wisconson

1981
Program Results



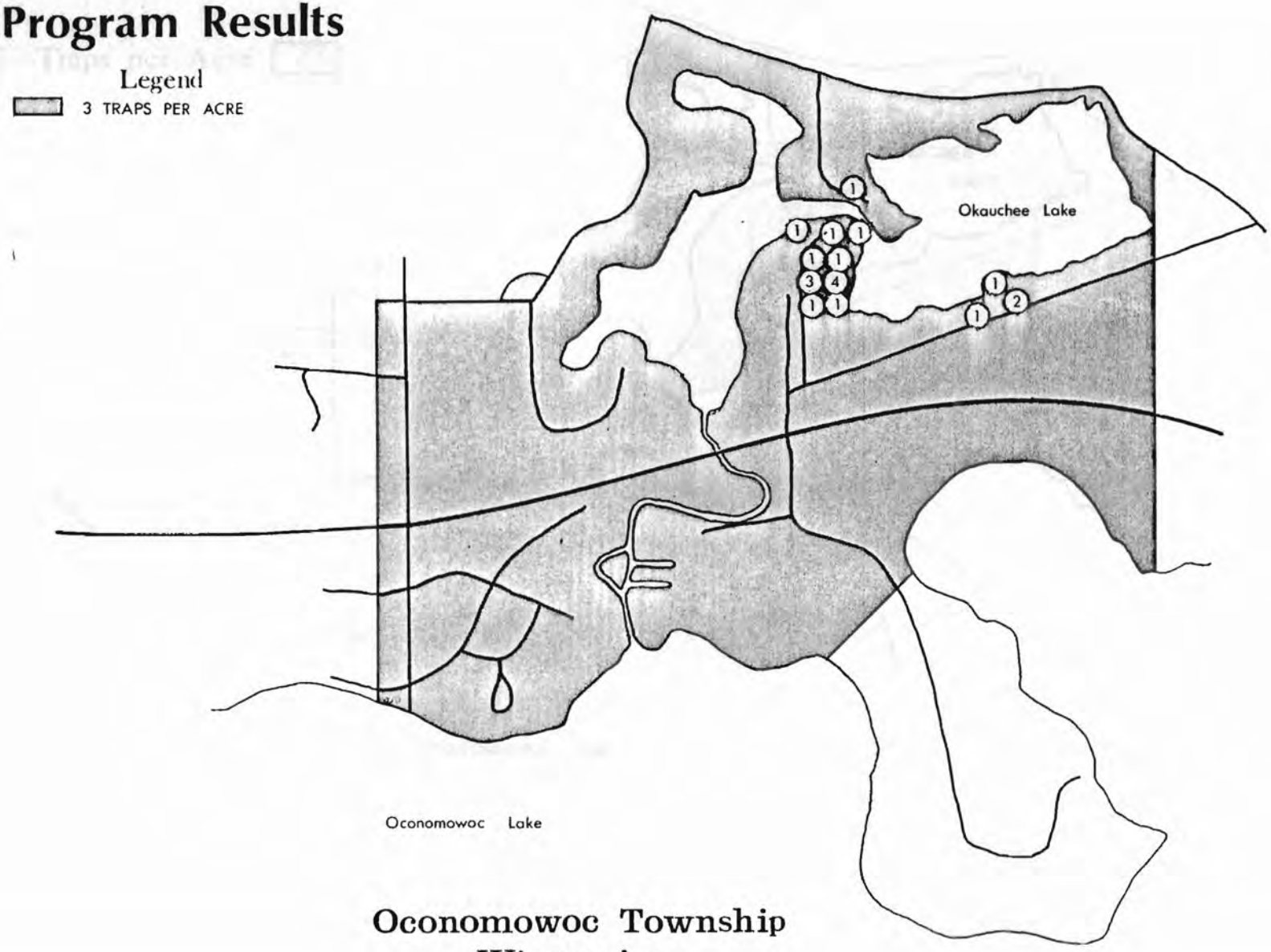
Oconomowoc Township
Wisconson

1981
Program Results



Oconomowoc Township
Wisconson

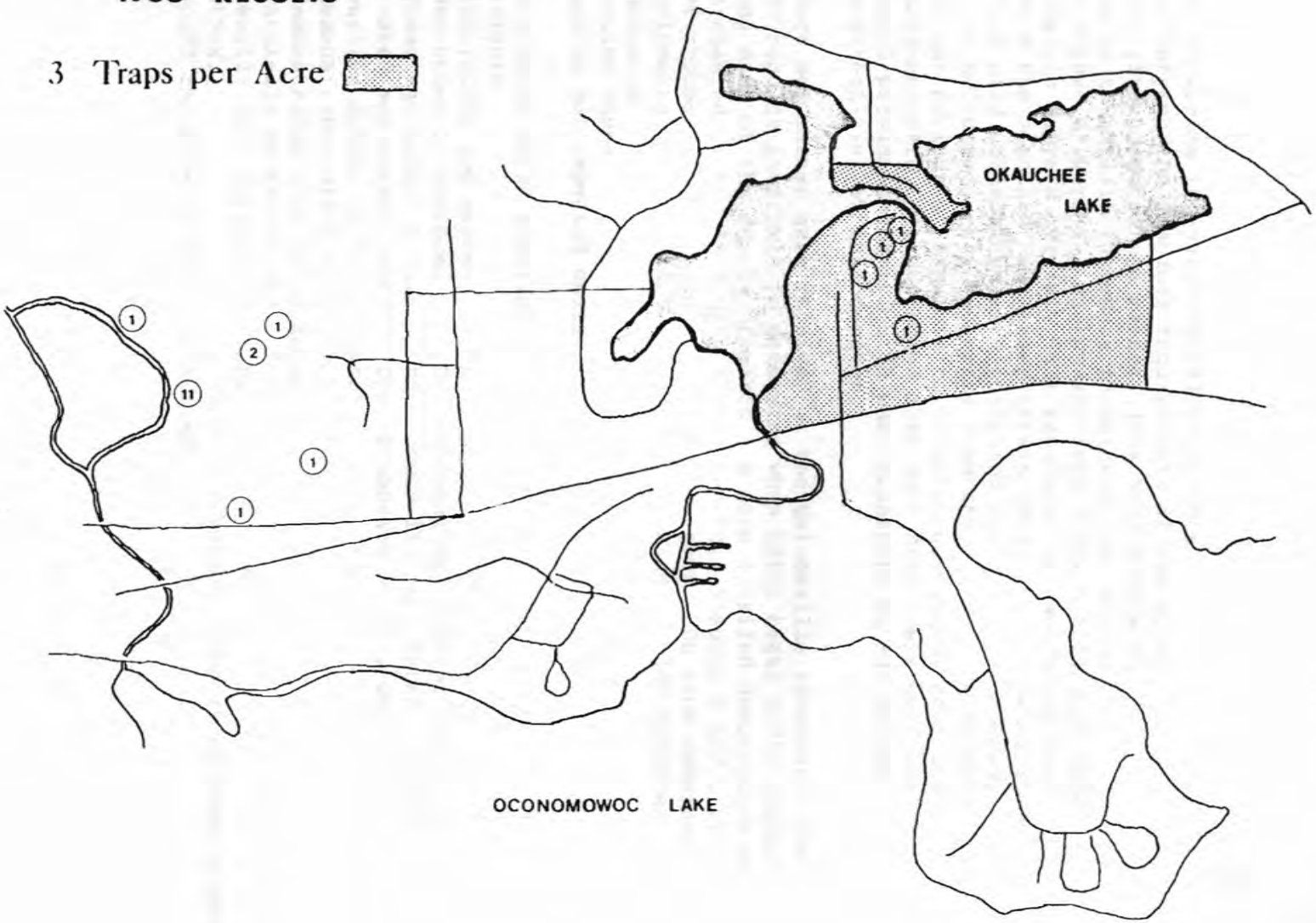
1982
Program Results



Oconomowoc Township
Wisconsin

1983 RESULTS

3 Traps per Acre



Project Number: GM 2.2.1
Project Title: Ecological Range of the Gypsy Moth
Report Type: Final
Report Period: October 1, 1982 - September 30, 1983
Project Leaders: V. C. Mastro, W. Dixon and A. P. C. Toles

Introduction

To determine if environmental conditions in Florida are suitable for satisfying the diapause requirements of the gypsy moth, ten newly laid laboratory-reared egg masses (less than 8 days old) were shipped every two weeks to a field insectary in Gainesville, Florida. Initial shipments (June 9 - August 6) were F-1 egg masses from a newly colonized laboratory strain (Devils Hopyard strain). The remainder of laboratory reared egg masses were from the standard New Jersey strain (F-23). Parallel shipments of egg masses from wild collections were initiated on August 20. Because feral egg masses were collected every two weeks from the wild, later shipments experienced longer periods of exposure to climatic conditions near Otis ANG Base, Massachusetts. It was hoped that data from these wild egg mass shipments would provide information on when transport of egg masses provided the greatest risk of introduction.

Once egg masses were received at the Gainesville insectary, they were checked at regular intervals to determine when hatch began and a subjective estimate was made of the degree of hatch. A more detailed description of methods is presented in the last Otis Annual Report. Figures 1 and 2 present the result of shipments of the two types of egg masses. On each examination date all shipments were examined and scored for hatch: 1-100 neonates (1), 100-500 neonates (2), and for greater than 500 neonates (3), (Figures 1 and 2). A rating number is presented in Figures 1 and 2 when hatch was first observed and is not repeated unless there was some significant change in hatch.

The first hatch observed from either type of egg mass was on February 7 (Figure 1). On this date less than one hundred larvae were observed on laboratory egg masses (NJ-strain) which were received in Gainesville on September 17 (shipped from Otis Methods Development Center on September 15). These egg masses initiated hatch only 150 days after oviposition. Laboratory reared egg masses from the Devils Hopyard strain, which were shipped on earlier dates, initiated hatch much later (March 28). Although this demonstrates differences in response of these two laboratory cultures, it also demonstrates that the diapause requirements of both strains were at least partially satisfied under Florida climatic conditions. Although hatch was usually only partial (i.e. rated 1 or 2) in shipments of laboratory reared stock, it is highly probable that percentage hatch in succeeding generations would increase as the strain adapted.

No feral egg masses initiated hatch until February 21 (shipments received October 15 and 28) Figure 2. More general hatch was noted on February 28 (shipments received on December 3 through February 7 all had hatch on this date). Hatch of egg masses shipped after February 7 began to hatch within a few weeks of when they were received indicating diapause requirements were satisfied and only warm temperatures were required for hatch to begin. All feral egg masses shipped initiated hatch at some point after receipt. In terms of establishment and regulatory risk, those egg masses hatching during a period when native vegetation (oaks) begins to foliate in the spring are the most likely to successfully establish. Oaks in the Gainesville area begin to foliate in mid March and by the first of April all decidious hardwoods have leafed out. Feral egg masses arriving in Gainesville between August 20 and December 3 and after January 20 would appear to be the greatest risk. However, other non typical host material may be available to substain larvae hatching before the oaks begin to foliate or after the spring flush of leaves.

Any definition of these dates as the highest risk dates for transport of eggs would only be based on one years data from one shipment location. Shipment of egg masses from another location or in other years will probably change the results because the environmental conditions the eggs experience before shipment would change.

In summary, the Florida (i.e. Gainesville) environment provides conditions suitable for egg development, diapause and hatch. Hatch at a time when suitable host foliage is available almost certainly will result in establishment and once established the strain will adapt to local conditions and the percentage of eggs in succeeding generations which hatch in synchrony with native host material will be greater.

Figure 1. **Hatch of Gypsy moth eggs held in a Gainesville, FL insectary: Laboratory Strains**

Date Received Gainesville	Examination Date																					
	FEBRUARY				*MARCH*							*APRIL*									*MAY*
	7	14	21	28	3	8	14	18	21	25	28	4	8	11	15	18	22	25	29		2
Devils Hopyard Strain																					
1982 9 June														1							
23 June											1										
12 July												1									
29 July												1	2								
6 August											1		2								
NJSS Strain																					
1982 20 August					1							2		2							
2 September					1							2		2							
17 September	1										2										
29 September				1					2												
15 October			1									2									
28 October		2										3									
12 November											1		2								
3 December												1		2							
10 December																					
23 December											1	2	3								
1983 6 January											1				2						
20 January																					
7 February																					
17 February																					
3 March																					
16 March																					
1 April																					
15 April																					
29 April																					

1: 1–100 larvae
2: 100–500 larvae
3: >500 larvae

Data collected and compiled by W. N. Dixon

Figure 2. **Hatch of Gypsy moth eggs held in a Gainesville, FL insectary: Feral Eggs**

Date Received Gainesville			Examination Date																			
			FEBRUARY				*MARCH*							*APRIL*								*MAY*
			7	14	21	28	3	8	14	18	21	25	28	4	8	11	15	18	22	25	29	2
1982	20	August												1								
	2	September								1												
	17	September												1								
	29	September									1				2							
	15	October			1									1								
	28	October			1									1	2							
	12	November			1	2																
	3	December				2										2						
	10	December				2																
	23	December				3																
1983	6	January				3																
	20	January				3																
	7	February				2		3														
	17	February						1	2													
	3	March								1	2	3										
	16	March											1	2		3						
	1	April													2	3						
	15	April																1	2	3		
	29	April																				2

1: 1–100 larvae
2: 100–500 larvae
3: >500 larvae

Data collected and compiled by W. N. Dixon

Project Number: GM 2.2.4
Project Title: Induced Inherited Sterility Trial - Horry County, South Carolina
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: V. C. Mastro and T. M. ODell

Background

Males treated as pupae (between 8-11 days old) with 10 krads were released in 1982 in a small, isolated gypsy moth infestation in Horry County, South Carolina to pilot test the induced-inherited sterility principle. Results of laboratory studies indicate that when males treated with 10 krads in the 8-11 day age class are mated to normal females, a proportion of the progeny survive to become sterile adults.

The release site was monitored intensively in 1983 for both larval and adult stages to determine if F1-sterile moths could be detected. The larval survey was conducted in much the same manner as in 1982, i.e. burlap bands were placed around host trees and checked on a weekly schedule. Over most of the treatment area, the rate of banding for larval survey was the same as for the 1982 survey (3 bands per point on a 47 m^2 grid). However, because it was anticipated that few larvae would be found, all host trees around the core area (sites of 1982 larval finds) were banded. Checks of bands began on May 11, 1983 and were finished on June 1, 1983. Also, because it was expected that few adult males would be trapped in 1983, nine additional adult traps were added to the core area, which resulted in an inter-trap spacing of 144 m. All traps used in the treatment block in 1983 were of the live-male type so males could be returned to the laboratory for mating tests. Traps were checked hourly during the expected male flight period.

Results and Discussion

Three larvae were located under bands in 1983; 16 were found in 1982 with a less intensive burlap survey. All of the larvae were found on separate trees in the center of the core area (grid point 10-21). Two of the three larvae completed larval development and pupated (1 male and 1 female). The female failed to eclose; the male, however, did eclose and was mated to a normal laboratory-reared female for determination of male type (F1 sterile or feral).

A total of 5 adult males were captured in 1983 compared with 13 in 1982. Again, these numbers are not directly comparable because the intensity of trapping in 1983 was greater. Captured males were taken to the laboratory where they were paired with normal laboratory-reared females. Including the one male collected as a pupae, a total of six matings were done (four of these mating pairs produced egg masses). Resulting egg masses were returned to the Otis laboratory and held for embryonation and diapause, and then were allowed to hatch for determination of mating type (i.e. F1 male or feral male).

Three of these egg masses were judged to be fertilized, indicating those male parents were feral. One of the remaining three pairs was conclusively the result of a sterile mating. The two remaining pairs probably resulted from F1 sterile male matings. However, because no egg masses were produced, the male type cannot be positively determined.

Indications are that a very small residual population may be present in the Horry County, South Carolina site in 1984. To determine if there is a remaining population, a square mile area will be trapped at a rate of 32 traps/mi^2 in 1984. If trapping indicates any population remains, it may be necessary to apply an additional treatment to a small area in 1985. This decision will be made at the conclusion of the 1984 trapping program.

Project Number: GM 2.2.5
Project Title: Mass Trapping Pilot Study - Monona, Wisconsin
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: C. P. Schwalbe, V. C. Mastro

Delimitation survey in 1981 defined a small, isolated gypsy moth infestation in Monona, Wisconsin. Because the infestation was located adjacent to Lake Monona, and in a highly residential area, it was decided to attempt eradication by mass trapping. Additional background is provided in the October 1, 1981 - September 30, 1983 Progress Report. Results of the 1981 delimitation survey are summarized on the attached map. Based on the survey, the project site was divided into 3 areas where trapping and larval sampling were conducted in 1982-1983.

Area I Mass trapped - 7.5 traps/ha; burlap banded - 7/.5 burlaps/ha
Area II Mass trapped - 7.5 traps/ha
Area III Delimitation survey - 32 traps/mi^2

Results of the 1982-1983 surveys are depicted on the attached maps and it is noted that while moths were captured over ca. 88 ha (Area I) in 1981, more intensive trapping in 1982 indicated that only ca. 13 ha were actually infested. Additionally, trap catch was dramatically reduced in 1982, presumably due to extremely low 1981-1982 winter temperatures. Data are summarized in Table I.

TABLE 1. Results of male moth trapping and larval and pupal sampling in Monona, Wisconsin.

	1981	1982	1983
No. male moths captured	236	113	40
Estimated male population	2360 1/	151 2/	53 2/
No. larvae and pupae		31	34
Estimated ha infested		13	8
Estimated no. pairs (season-long)		11.6 3/	6.7 3/
Flight period (days)		7/20-8/9 (21)	7/21-8/10 (21)
Estimated no. pairs/ha/day		0.6	0.3
Estimated no. females mated 4/		13.3	4.7

1/ Assuming 10 percent capture rate with 32 traps/mi^2.

2/ Assuming 75 percent capture rate with 7.5 traps/ha.

3/ Assuming 1:1 male:female sex ratio.

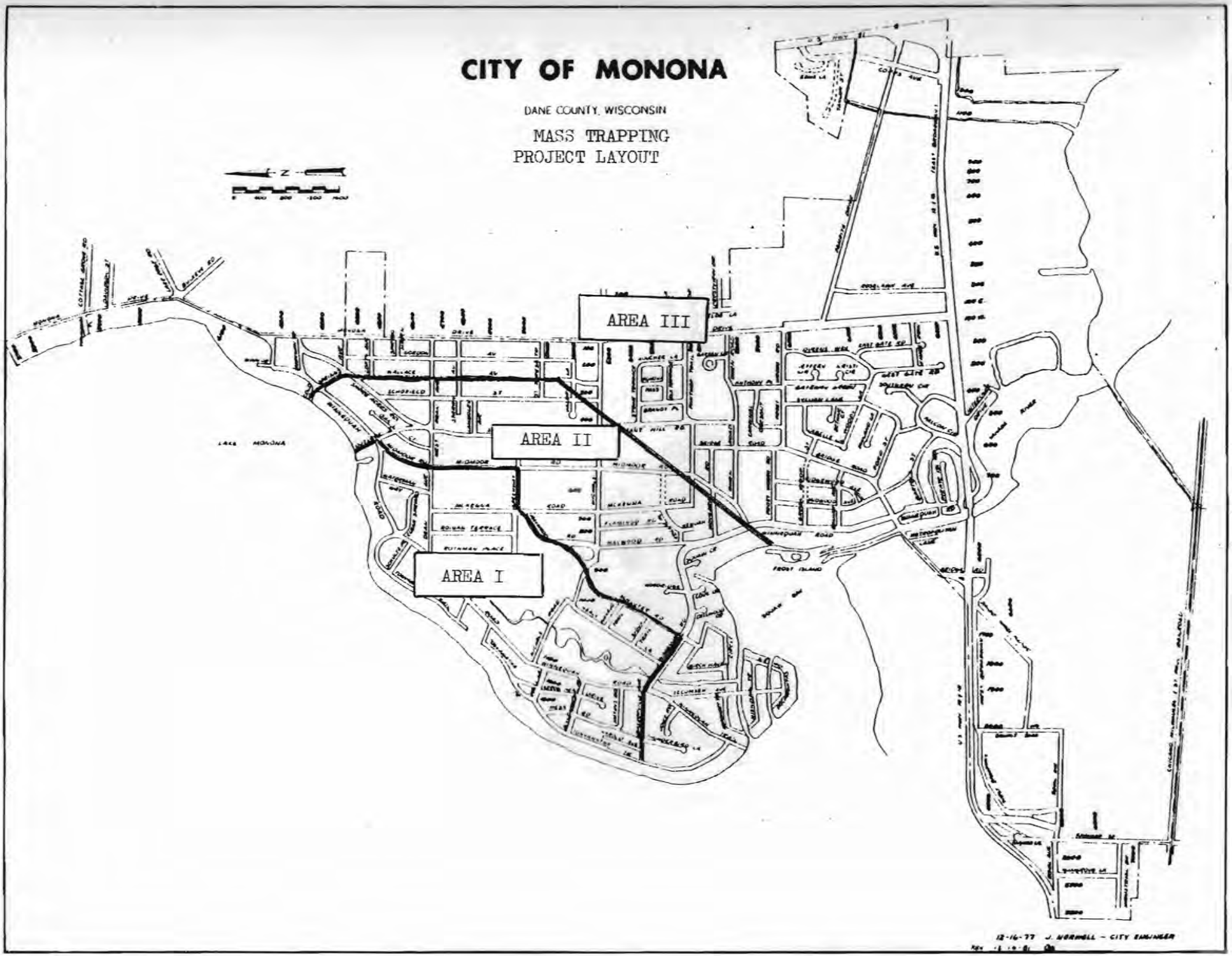
4/ No. females mated/season = (no. pairs/ha/day)(.088)(flight period)(ha infested).

Note: .088 = mating success of 2.5 females/ha in area containing 7.5 traps/ha.

CITY OF MONONA

DANE COUNTY, WISCONSIN

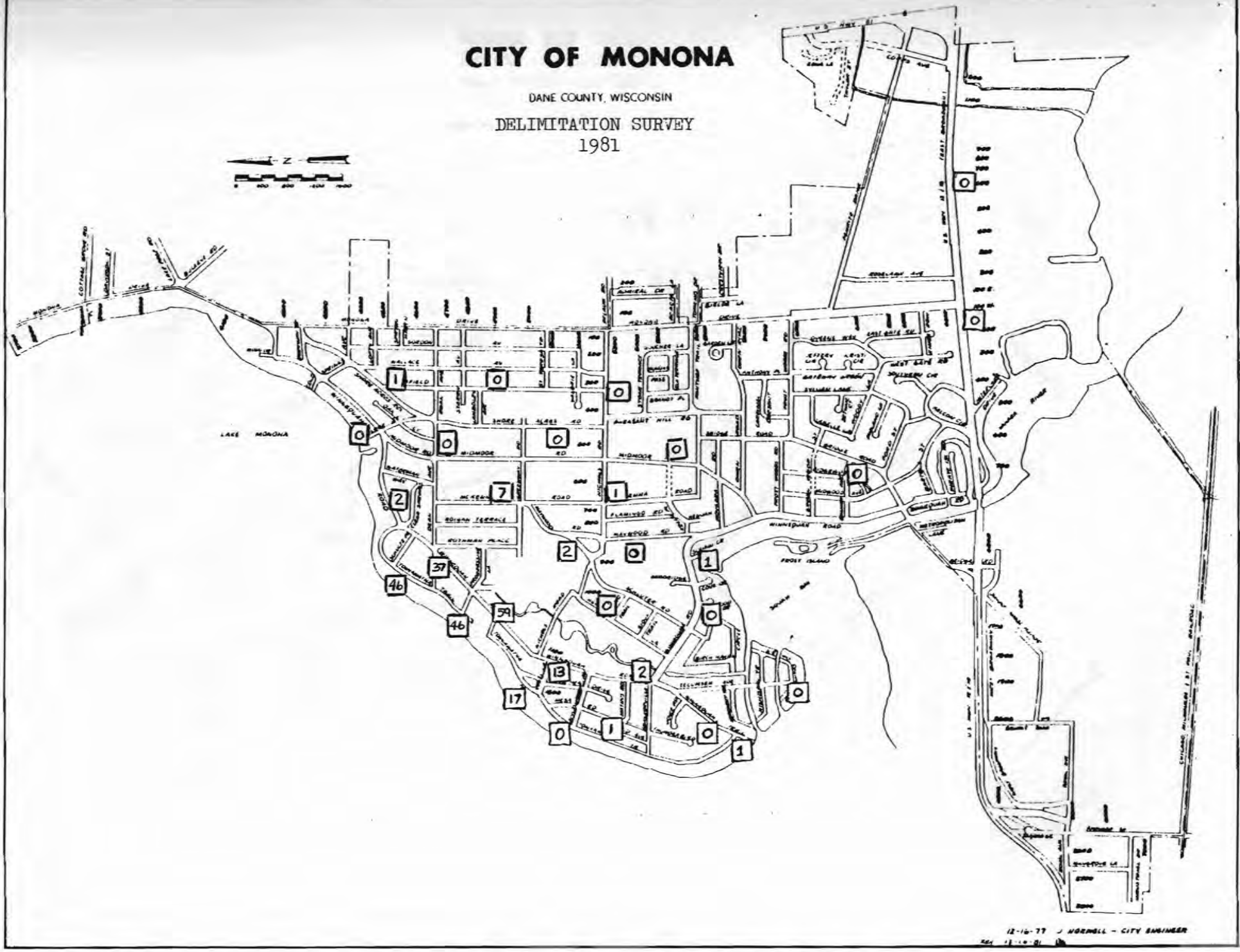
MASS TRAPPING
PROJECT LAYOUT



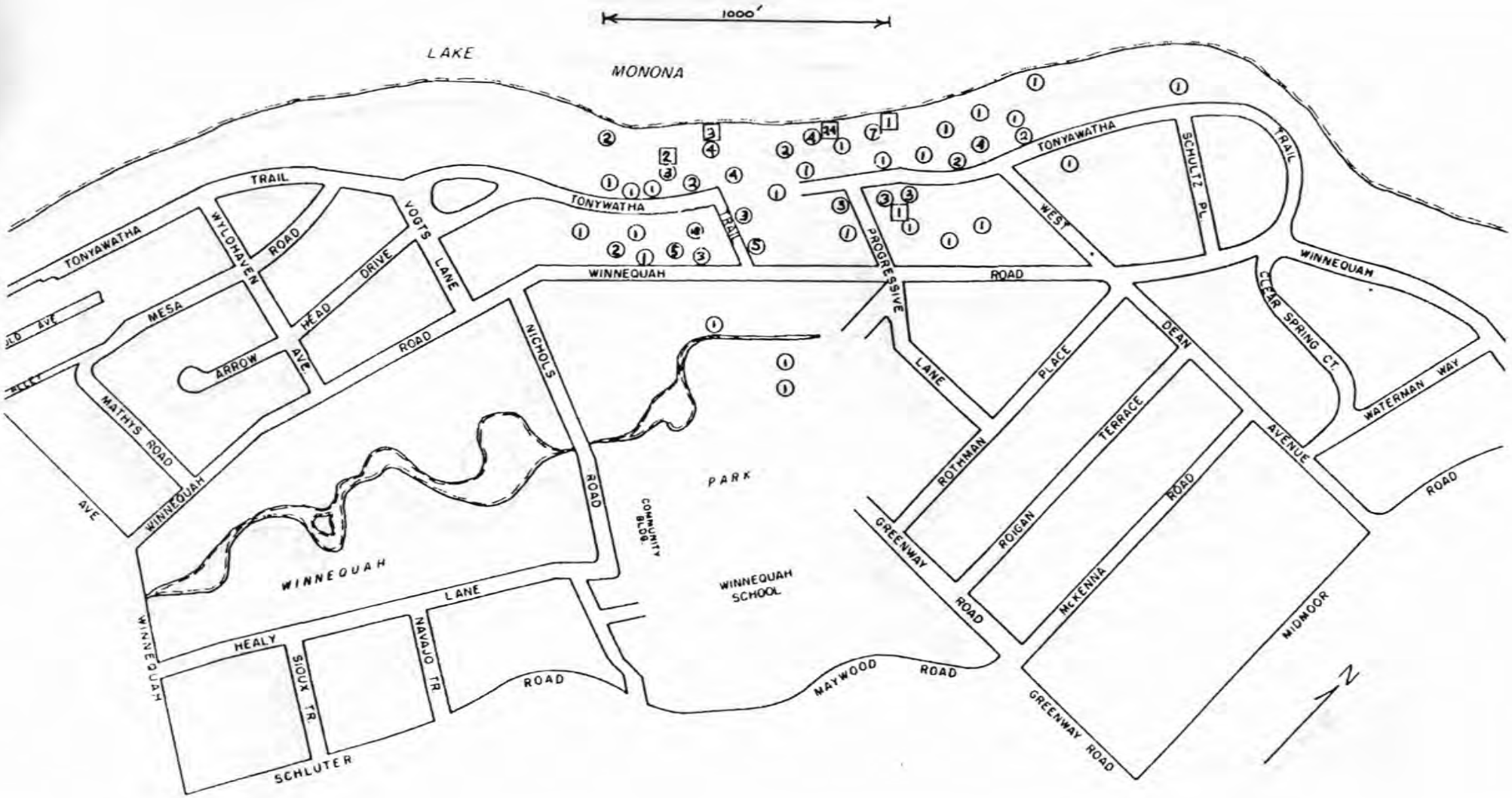
CITY OF MONONA

DANE COUNTY, WISCONSIN

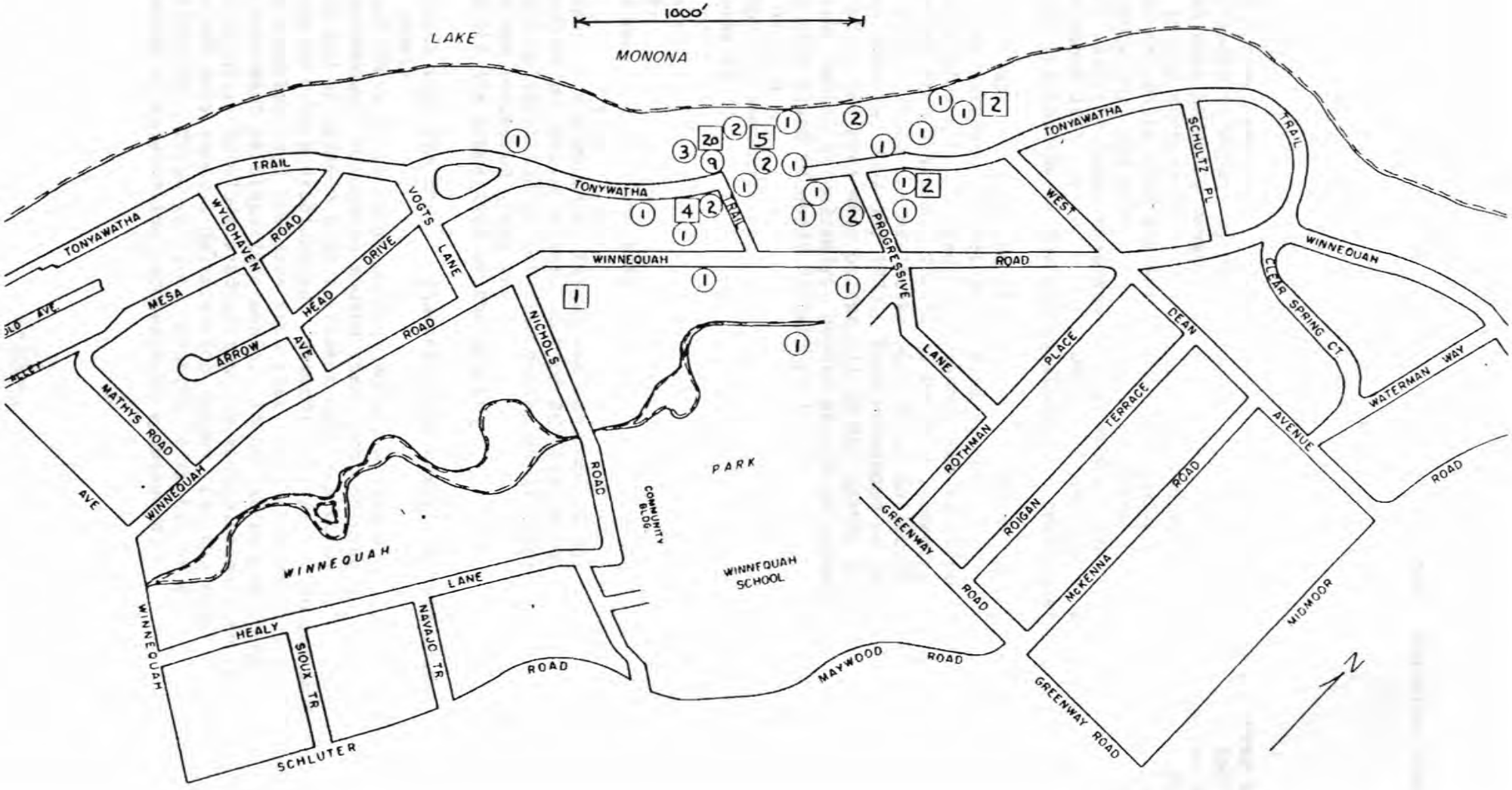
DELIMITATION SURVEY
1981



MONONA, WIS 1982
LARVAE CAPTURED □
MALE MOTHS CAPTURED ○



MONONA, WIS 1983
LARVAE CAPTURED [ ]
MALE MOTHS CAPTURED ○



Project Number: GM 3.2.1
Project Title: Mating Disruption Demonstration Project - Carteret County, North Carolina
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: C. P. Schwalbe

Detection survey results in 1981 indicated that an infestation of gypsy moth had established in Beaufort, North Carolina (Carteret County) near a log yard associated with Atlantic Veneer. In 1982 a more intensive delimitation survey resulted in the capture of 38 moths in a fairly distinct pattern; from this pattern we estimated that the infestation was confined to 150 acres and was, indeed, isolated. Egg mass survey discovered three egg masses (which were collected) near the traps that caught most moths. These results are presented on the attached 1982 map. This site was subsequently selected for a trial to demonstrate eradication by the mating disruption technique.

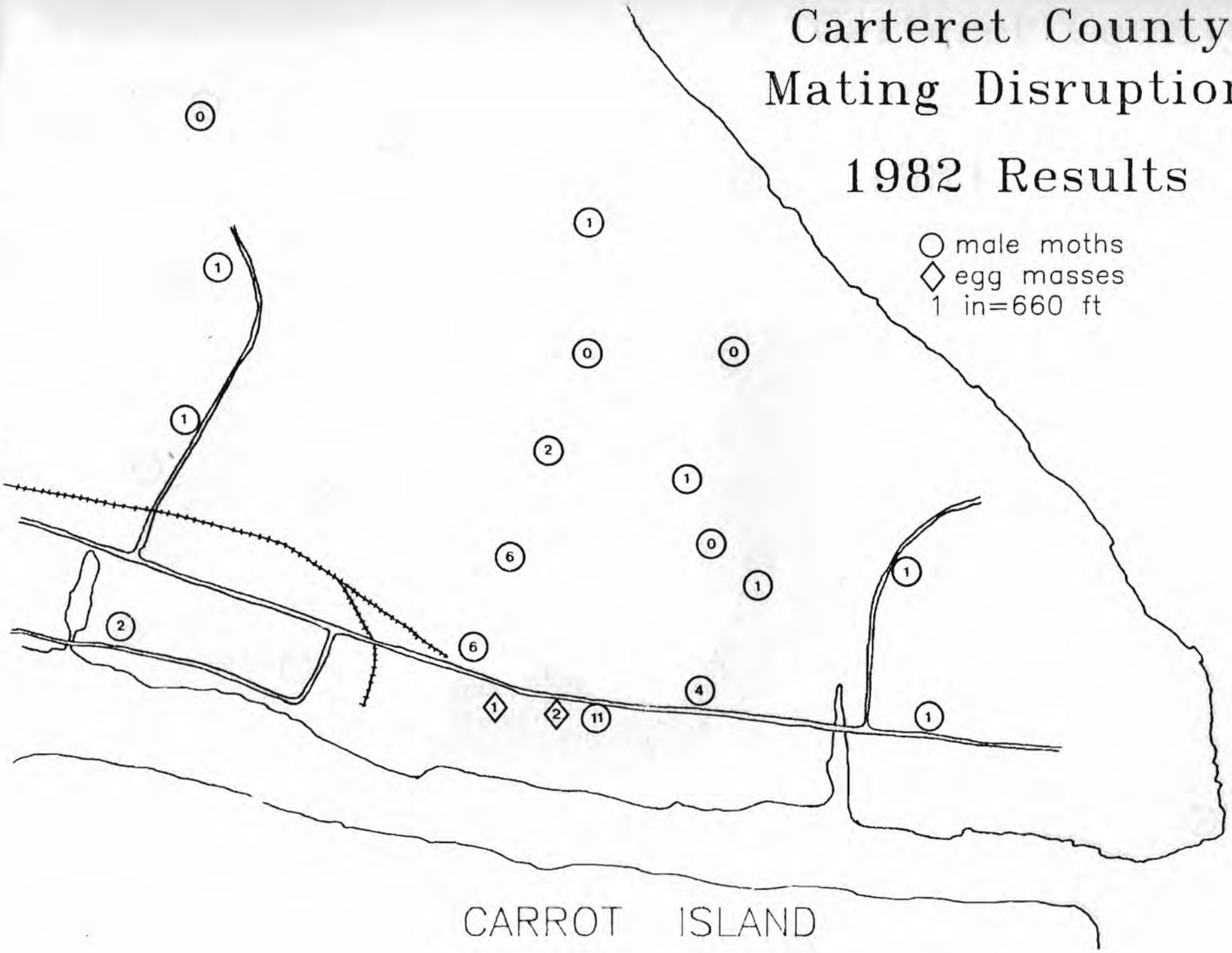
In 1983 about 20 acres (in the vicinity of egg mass finds and highest moth catches) were surveyed for larvae by placing burlap bands around boles of host trees. Approximately 200 trees were banded and 42 larvae and pupae were found and collected from 7 trees. The first hatch was observed on a caged egg mass April 7, 1983. Third instar larvae were first collected on May 9, 1983 and pupae were observed June 13, 1983. This convinced us that a population had established in the area and, unless all of the larvae present were captured under burlap (which is unlikely), adults should be present. With this, we proceeded with plans to apply disparlure in the Hercon flake formulation at the rate of 20 g a.i./acre. The material was aerially applied on May 25, 1983 with Hercon equipment mounted on a USDA plane piloted by Dick Dyer. Some fringe areas of the spray block were not suitable for aerial treatment (thickly residential or sparsely forested) and these areas received manually placed Lure-tape. In all cases, however we attempted to deliver 20 g a.i./acre. A large central area containing the mill buildings and devoid of trees was excluded from the treatment area.

During the period when adult flight was expected, virgin, sterile female moths were placed at monitoring sites. Out of 80 females collected, none were found mated. The delimitation survey grid contained 32 traps/mi^2 and only one male moth was recovered (near the site where egg masses were found in 1982). These results are summarized on the attached 1983 map.

These results indicate that an indeterminate number of male moths (and probably female moths) were present in the project area after treatment was applied and there is no evidence that mating occurred. However, a previous test in Oconomowoc, Wisconsin showed that such encouraging results can be misleading and we believe that treatment may have to be applied over two generations to ensure eradication. Accordingly, we are proposing to intensively burlap band the area this year and, if larvae are detected, apply the same treatment of disparlure again. If no larvae are found, however, we will provisionally conclude eradication was accomplished with the 1983 treatment and only conduct delimitation survey (for the next 2-3 years) for final evaluation. Three years of negative delimitation survey is the basis for declaring an infestation successfully eradicated.

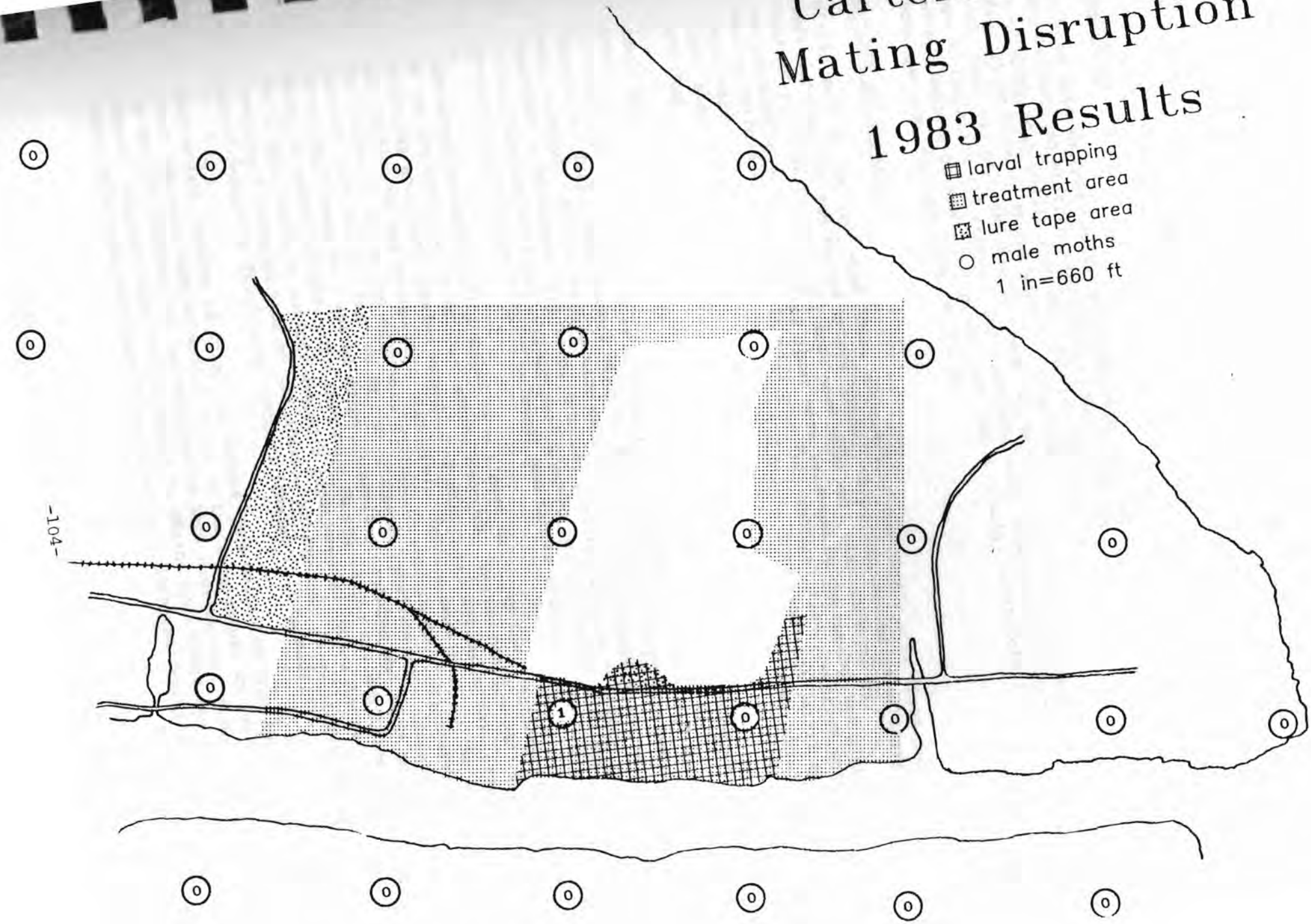
Carteret County Mating Disruption

1982 Results



Carteret County Mating Disruption

1983 Results



Project Number: GM 3.2.2
Project Title: Sterile Male (F-1) Pilot Study, Kent County, Maryland
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: V. C. Mastro, K. Tatum, T. M. ODell and R. Webb

Introduction

Four wood lots in Kent County, Maryland were selected for pilot testing of the induced-inherited sterility principle. This area was chosen because of it's sparse gypsy moth populations and relative isolation from surrounding infestations offered by the wood lot situation. One wood lot (ca. 96 acres) of the four was selected for a release of males treated with a substerilizing dose of radiation (10 krads). The remaining wood lots served as control plots. Results of the first pilot test (Horry County, South Carolina) of this technique are reported under GM 2.2.4.

Methods and Materials

Within each of the four wood lots, ten one-fortieth (1/40) acre fixed plots were surveyed for egg masses before hatch. To provide estimates of larval density and distribution, all four wood lots were also surveyed by burlap-banding trees. Banding sites were selected by overlaying the plots with a uniform 47 m^2 grid. At each grid intersection, five preferred host trees were burlapped. Bands were checked weekly throughout the larval and pupal stages and the numbers and sex (pupae only) found under each band were recorded.

Adult male flight was monitored in three of the plots using milk carton traps placed on a uniform grid (142 m^2). Only three of the wood lots were trapped; the fourth served as a control for the possible effects of adult male trapping. Traps were checked daily in all of the plots and males were removed and recorded. In the sterile male release plot all captured males were examined to determine type (e.g. internally marked sterile or feral) and during peak native flight, traps were checked hourly (1000 - 1500 hrs) to compare flight periodicity of released and native males.

Laboratory-reared virgin females were placed daily in the treatment plot and in one control plot to monitor mating success. Twenty one-day-old (day following eclosion) females were placed daily; the following day, females and any portion of egg masses were recovered. Females were held in the laboratory for oviposition and the eggs were allowed to embryonate. Egg masses were then held at 5-6° for 150 days to satisfy diapause requirements. Progeny from these egg masses were reared using standard procedures and adult progeny were mated to normal adults. The resulting F2 egg masses are now being held under chill conditions to satisfy diapause requirements. When the evaluation is complete a determination of the initial mating type (e.g. irradiated male x normal female or feral male x normal female) will be made.

Post-season samples of feral egg masses were collected from two wood lots. These egg mass samples were treated in the same manner as egg masses deposited by monitor females and the appropriate back crosses were made. The F2 egg masses resulting from back-crossing F1 adults from these egg masses are now being held under cold conditions to satisfy diapause requirements.

Males treated as pupae (9 to 11 days after pupation) with 10 krads were released in one of the wood lots (ca. 96 acres). Male release was initiated on June 22 and completed on July 31. Approximately 9,000 - 10,000 internally marked males were shipped daily from the Otis laboratory and released the following day in the treatment plot. Several shipments were delayed or lost in transit. Release was carried out by placing pupae in standard sterile male release containers arrayed in a uniform 47 m x 47 m grid over the entire plot. Within the grid, 4 contiguous points (two release cages/point) were used for an eight day release rotation. At the beginning of a new rotation (i.e. the ninth day) the container previously used on the first day was emptied of pupal skins and reprovisioned with fresh pupae. Estimates of the percent eclosion were made when cages were reprovisioned.

To determine if any significant population changes had occurred, postseason egg mass surveys were conducted in the 1/40 acre plots that had been established for the preseason egg mass survey.

Table 1 presents a summary of the activities conducted in the four plots.

Table 1. Activities performed in test plots in Kent County, Maryland.

Treatment	Plot 3	Plot 1	Plot 4	Plot 2
Pre and post season egg mass surveys	X	X	X	X
Larval burlap survey	X	X	X	X
Male trap survey		X	X	X
Monitor female placement			X	X
Feral egg mass samples collected			X	X
Sterile male release				X

Results and Discussion

During preseason egg mass surveys on the fixed 1/40 acre plots, only 4 egg masses were found in plot 3. In post season surveys, eight egg masses were found in both plot 2 (sterile male treatment plot) and plot 1.

Burlap-banding data indicated that larvae and pupae were present in all of the four wood lots, and also were useful in predicting the location of feral egg masses for post-season samples (Table 2).

Table 2. Results of 1983 burlap band larval survey, Kent County, Maryland.

Plot No.	Number burlap bands placed	1st Obs. (6/8-6/10) No. larvae	2nd Obs. (6/14-6/16) No. larve	2nd Obs. (6/14-6/16) No. pupae	3rd Obs. (6/23-6/29) No. larvae	3rd Obs. (6/23-6/29) No.1/ pupae	4th Obs. (8/17-8/31) Number egg masses
3	210	15	16	0	7	10	10
1	360	13	21	0	12	1	9
4	1069	23	24	0	10	10	1
2(sterile release)	936	259	352	1	287	48	73

1/ includes prepupae and pupae.

Male trap data are presented in Table 3. Traps were checked from 6/24 to 8/10 on a daily basis.

Table 3. Males captured in high capacity traps in Kent County, Maryland Plot 1983.

Plot No.	Number of traps	Number feral males captured	Number sterile males captured
1	18	665	-
4	35	299	-
2 (sterile release)	35	2,104	22,906

Results of the larval and adult surveys indicate that the plot chosen as the treatment site (plot 2) had the greatest native insect density. Although the season-long overflooding ratio, of sterile:feral males (computed from trap captures) varied considerably during peak native flight the ratio averaged 10.4 sterile:1 feral. However, on some particular days this figure was much lower. Some of these low overflooding ratios can be attributed to delayed or missing sterile male shipments; on other days, feral male flight simply was very high.

Although overflooding ratios were not optimum during peak male flight, the achieved ratios should provide some degree of population suppression in 1984. More importantly, the resulting progeny from feral egg masses in the treatment plot also will provide data on the survival, developmental synchrony and competitiveness of F-1 sterile insects.

Project Number: GM 3.2.3
Project Title: Bioassay of Gypsy Moth Pheromone Formulations
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leaders: E. C. Paszek, V. C. Mastro, A. P. C. Toles, C. P. Schwalbe, D. Lance

Hercon[R] Gypsy Moth Lure Tape containing (+) disparlure is used in gypsy moth survey traps. It was compared with Scentry[TM], and to the Albany (+) disparlure. The following 5 gypsy moth pheromone formulations were placed outdoors in traps on July 15, 1982, and aged for 30 days:

Hercon 1982 (Lot D0032)
Albany Plus GM-80
Albany Plus GM-45
Albany Plus GM-1
Sinclair Rush

On August 11, 1983, new, unaged samples of the above lures were field-tested with the aged samples in a natural infestation. USDA Standard, Schwartz-Plimmer (100 ug) and Albany Standard (100 ug) were used as controls. These formulations were bioassayed August 11-19, 1983, in milk carton traps randomly placed 50 m apart in a 12 X 7 grid. Each treatment was replicated 7 times and the cotton wicks were replaced on August 17.

TABLE 1. Mean number of moths captured/trap.

Attractant	Unaged	Aged
USDA standard (100 ug)	14.9 a	-
Albany standard (100 ug)	9.9 ab	-
Hercon 1982	3.4 bc	12.0 a
Albany Plus GM 80	4.6 bc	5.1 bc
Albany Plus GM 45	2.7 bc	4.1 bc
Albany Plus GM 1	1.7 c	4.4 bc
Sinclair Rush	3.0 c	1.3 c

Because the Albany standard consistently caught fewer moths than the USDA standard, a second test was conducted August 25, 1983. Traps baited with either of the two pheromone sources were alternately placed at 20 m intervals on a 70 m radius circular line and laboratory-reared moths were released at the center. Four separate releases were made.

Project Number: GM 3.2.3
Project Title: Bioassay of Gypsy Moth Pheromone Formulations
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leaders: E. C. Paszek, V. C. Mastro, A. P. C. Toles, C. P. Schwalbe, D. Lance

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TABLE 2. Mean number of moths captured/trap.

Attractant	Replicate 1	2	3	4	X
USDA standard	2.45	5.91	2.27	5.27	3.98 a
Albany standard	1.18	4.27	1.	1.45	1.98 b

In this test, Albany-baited traps caught significantly fewer males than USDA-baited traps.

The concentration of pheromone used in loading the cotton wicks was checked by gas chromatography. Peak height for a 10 ul injection of the Albany solutions was 12 mm. Similar injections with two standards containing 100 ug/100 ul yielded peak heights of 115 mm, indicating that the Albany material provided for the tests only contained 10 ug/100 ul. This most likely accounts for the reduced catch in traps baited with that material, although there is no way of determining if increasing pheromone concentration would have improved catch. Accordingly, the effectiveness of the Albany disparlure supply is not well defined.

Since all Albany formulations captured signifcantly fewer moths than the Hercon aged material, we are not able to recommend these dispensers for general program use in 1984.

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Albany standard	1.18	4.27	1.	1.45	1.98 b

In this test, Albany-baited traps caught significantly fewer males than USDA-baited traps.

The concentration of pheromone used in loading the cotton wicks was checked by gas chromatography. Peak height for a 10 ul injection of the Albany solutions was 12 mm. Similar injections with two standards containing 100 ug/100 ul yielded peak heights of 115 mm, indicating that the Albany material provided for the tests only contained 10 ug/100 ul. This most likely accounts for the reduced catch in traps baited with that material, although there is no way of determining if increasing pheromone concentration would have improved catch. Accordingly, the effectiveness of the Albany disparlure supply is not well defined.

Since all Albany formulations captured signifcantly fewer moths than the Hercon aged material, we are not able to recommend these dispensers for general program use in 1984.

Project Number: GM 3.2.4
Project Title: 1984 Evaluation of Klearfold Plastic Delta Type Traps
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leader: E. C. Paszek

In 1982, in a limited study comparing a clear plastic delta type trap made by Klearfold Company[1/] with the standard paper delta survey trap, significant difference in the number of moths captured was observed. The clear plastic trap had the advantage of being sturdier, impervious to water and transparent. This eliminated the opening of the trap for inspection. This year, in a more intensive study, the Klearfold delta was bioassayed in clear, yellow, opaque colors and in a modified version where a 7" X 3.5" plastic liner with tack trap adhesive was inserted in the bottom of the trap. The removable insert feature makes the trap permanent; by discarding the insert and folding up the trap for storage it can be reused.

This study was conducted in a very light natural infestation. The traps were baited with aged lures and stapled at breast-height to trees. The minimum diameter of the boles of these trees was 7 inches. They were replicated 10 times in a randomized 10 x 6 grid with points 50 m apart and checked every 24 hours for 6 days. The traps did not fill up with moths. The average daily catch varied from 1 to 3 moths/trap (Table 1).

1/ The Klearfold traps tested in this study were manufactured by Klearfold, 364 Valley Road, Warrington, Pennsylvania 18976. The design numbers were 01021-7320; material gauge .014 dated 6/28/83 and 8138-7320; material gauge .014 dated 6/14/83. The size of the trap was 5-3/4 x 2-1/2 x 7-1/4.

Table 1. Average number of moths captured in delta traps (10 replicates checked 6 times).

	Total number captured	Average number per trap
Yellow plastic	160	2.7
Clear plastic	159	2.7
Standard orange paper	112	1.9
Opaque plastic	110	1.8
Yellow plastic modified	85	1.4
Clear plastic modified	85	1.4

Because of the low number of moths captured per trap the statistical analysis was done on the comparison of the 10 replicates, not the individual traps.

Table 2. Mean number of moths captured per replicate ± standard deviation.

Yellow plastic	26.7 ± 27.4 a
Clear plastic	26.5 ± 28.7 ab
Standard orange paper	18.7 ± 13.7 ab
Opaque plastic	16.7 ± 16.0 abc
Yellow plastic modified	14.2 ± 13.5 bc
Clear plastic modified	14.2 ± 15.5 c

Means followed by the same letter not significantly different according to Duncan's Multiple Range Test at 5% level of significance.

The yellow and clear plastic delta traps captured more moths (not significant) than the standard orange paper delta. The opaque plastic trap captured fewer moths than the standard trap, but this difference was not significant. The yellow plastic delta captured significantly more moths than the yellow plastic modified delta and the clear plastic delta captured significantly more moths than the clear plastic modified delta. The smaller adhesive surface area of $26"^2$ (compared with $58"^2$ in the standard design) may account for the smaller number of moths captured in the two modified delta traps.

The plastic delta traps are sturdy permanent traps that are impervious to water. The trap adhesive clings better to their plastic surface than to the plasticized surface of the paper trap. The color of the standard delta paper trap fades out and excessive moisture causes the traps to sag. The plastic deltas are fade-proof. The clear plastic delta has the additional advantage of being a "see through" trap. This eliminates the necessity of opening it for inspection, saving time in checking trap lines. The yellow and clear plastic deltas should be used on a pilot scale in the gypsy moth survey program along with the paper deltas. This would allow for a final evaluation of the trap by field personnel.

Project Number: GM 3.2.5
Project Title: Sterile Male Technique - Studies on the Feasibility of Releasing F1 Sterile Gypsy Moths as Eggs
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: D. R. Lance, V. C. Mastro, C. P. Schwalbe and T. M. ODell

In many Lepidoptera, complete sterility occurs in otherwise viable F1 individuals when male P1 pupae are treated with a proper sub-sterilizing dose of gamma radiation. Thus, a release of radiation-treated adults causes the same reduction in the number of fertile insects, regardless of whether the released insects have been partially or completely sterilized. However, if sub-sterilized males are released, there potentially could be many completely sterile F1 progeny to further interact with the next generation in the targeted population. Alternatively, sub-sterilized males could be outcrossed in the laboratory, and the resulting eggs could then be placed in the field. Possible advantages of this latter technique include simplified logistics of release, ability to "stockpile" eggs, reduction of rearing cost, reduction of waste in the rearing process (females would be used), and elimination of many of the non-genetic effects that the laboratory may have on insect quality. In either case, the efficacy of a release program would increase with the number of competitive F1-sterile adults that were present when feral moths were mating.

The gypsy moth is an early-season univoltine defoliator that overwinters in the egg as a pharate first instar. Because adults live only a few days, it would be essential that F1-sterile moths develop in synchrony with wild insects. Initially, F1-sterile first instars must eclose more or less in synchrony with wild eggs, and at a time when young, tender foliage is available. Secondly, F1-sterile larvae must survive to adulthood and develop in synchrony with wild insects. In this study, hatch and development were examined in gypsy moths from various irradiation, rearing and egg diapause treatments.

Materials and Methods

Hatch Synchrony Tests. On several dates during the summer and fall of 1982, laboratory-reared female gypsy moths were mated with laboratory-reared males and were allowed to deposit egg masses onto brown paper. These masses were held at 25°C for 28 days and were then transferred to 1 of 3 diapause temperature regimes (2-3°, 5-6°, or 8-9°) until the following spring. Also, egg masses from outcrossings of substerilized males (IR 10K as 8-11 day old pupae) were allowed to overwinter in an outdoor insectary and in the laboratory. In April 1983, wild egg masses were collected on small (ca. 5 cm^2) pieces of bark and were held in an outdoor insectary until they were used. Egg mass treatments are summarized in Table 1.

On May 3, 1983 (Julian date 123, approximately, the initiation of hatch in the field), whole egg masses from all treatments were stapled onto plywood boards and were held in an outdoor insectary pending their hatch. Each egg mass was surrounded by double-sided sticky tape to deter inter-mass movement of 1st instars. Newly eclosed larvae were counted and removed daily.

Also on May 3, several egg masses from each treatment were dehaired, and 50 eggs from each mass were glued with contact cement to a piece of brown paper. The 50-egg samples then were treated in the same manner as the whole egg masses.

Rearing Tests. Eggs from 3 sources (the NJ laboratory strain, NJS male IR 10K as 8-9 day-old pupae X NJS female matings, and the Mashpee Wild population) were surface-sterilized and were held at 25° until they hatched. Neonates were placed onto black oak foliage and were reared using one of two methods. In the first, larvae were held in 150 mm diameter X 36 mm deep petri dishes. A ca. 80 mm diameter hole was cut in the top and bottom of each dish and was covered with 0.6 mm mesh plastic screening. A 10 mm wide slot was cut into the side of each dish to allow the dish to be closed over the tips of branches. For each treatment, 10 larvae were placed into each of 12 dishes on 4 trees (a total of 480 larvae per treatment). The cages were held closed with large rubber bands, and the 10 mm slots were sealed with foam rubber and rope caulking to prevent the escape of larvae. The dishes were placed onto small (less than 5 m high) black oaks in an open, sunny area (Deer Horn Hill, Massachusetts National Cemetery) and were checked on a weekly basis. At each check, the numbers of larvae in each instar were recorded, and dishes were moved if the food supply was becoming exhausted.

In the second rearing method, larvae were reared at 10 per 480 ml cup using the method of Lance et al. (GM 1.2.1, Study #3, this report). Fourteen-day-old larvae were then transferred to 42 x 42 x 57 cm high screen cages (ca. 50 larvae per cage), and the cages were placed in an outdoor insectary. Foliage was left attached to ca. 0.4 m long black oak terminals. These were surface-sterilized (5 min. in 0.2% sodium hypochlorite) and placed into the cages in water-filled flasks. The foliage was changed every other day throughout the course of development. For both rearing methods, the cages were checked daily after pupation commenced, and all pupae were removed. Pupae were weighed and allowed to eclose.

Results and Discussion

Hatch Synchrony. The timing of hatch in gypsy moth eggs is affected by the length of the diapause (chill) period and the temperature during both diapause and post-diapause periods. In terms of releasing F1-sterile insects as eggs, we have demonstrated two methods by which hatch can be adequately synchronized with that of wild eggs. First, moths were reared in the laboratory during the period when feral moths were developing in the field. Then, the laboratory-reared egg masses were immediately placed outside, where they embryonated and went through diapause under conditions similar to those of the feral eggs (Lance et al. 1982, and Table 1). In practice, this method would be disadvantageous because the sites to be treated would have to be known a year in advance (differences in local temperatures would affect the time of hatch if eggs were held at one site and then transferred to another), and the moths would have to be reared at a time when there are many other demands on the rearing facility. In the second method, moths were reared in the late summer and fall, and the resulting egg masses were held at 6-8° for diapause before being placed outside in the late spring (Lance et al. 1982).

Unfortunately, only a very narrow window of production time yielded synchrony in hatch between wild and laboratory-reared eggs. Placing eggs in the field two, four or six weeks prior to the expected hatch date did not have a consistent effect on the time of hatch, but merely created further unpredictability (Lance et al. 1982).

In the current study, we attempted to delimit this "production window" and determine if it could be widened by using several temperatures for over-wintering. To eliminate variation due to release date, we placed eggs outside just prior to the expected onset of hatch in the field. Using this method, eggs from only one treatment (182 days at 8-9°) had an acceptable hatch period (Table 1). With chill periods of less than 161 days, holding eggs at 5-6° vs. 8-9° had little effect on the time of hatch; eggs from the 2-3° treatments (less than 181 days) tended to hatch up to eight days later (Table 1). Still, manipulation of chill temperature alone does not appear to offer a promising (read "simple") method of extending the production period. Fortunately, many of the treatments resulted in viable eggs and a 13-22 day lag in hatch (Table 1). We now believe that the simplest method of achieving hatch synchrony is to pre-condition these eggs with several days of warm temperatures and release them just prior to expected field hatch. This method could extend the production period to up to four months, which would give the current rearing facility a capacity of over one million egg masses (ca. 400 million F1-sterile larvae) per year. For example, infestation dates could run from July 1 to August 31 for moths whose egg masses were to be held at 2-3°, and from September 1 to October 31 for the 5-6° or 8-9° treatments. The actual time for release could be determined by following phenological indicator species (e.g., red oak) and "readiness to hatch" in field-collected eggs. Work needs to be done on pre-conditioning temperatures, although one or a few egg masses from each group could be started into pre-conditioning one, two or three days early; the rest of the masses could then be returned to cool temperatures (pending release) after the early-conditioned masses started to hatch.

Table 1. Timing of hatch in gypsy moth egg masses that were subjected to various radiation and chill treatments.

Source of eggs[a]	Chill treatment	Days in chill	n of masses sampled	Mean larvae per sample	Duration of hatch (days)	Mean Julian date of hatch (± S. D.)	Compatibility code[b]
wild	field	--	9	285	33	130.4 ± 3.4	--
NJS IR 10K	outside	(210)	10	148	23	134.0 ± 5.5	a
NJS IR 10K	5-6°	ca. 210	10	65	17	152.2 ± 2.1	b
NJSS	8-9°	182	5	840	27	132.3 ± 4.6	a
	8-9°	161	5	708	29	138.8 ± 4.2	b
	8-9°	137	5	814	39	145.7 ± 5.3	b
	8-9°	116	5	766	21	152.1 ± 3.0	b
	8-9°	96	5	944	32	157.9 ± 3.5	b/c
	8-9°	74	5	651	26	167.4 ± 4.4	c
	5-6°	272	5	3	9	148.1 ± 3.7	c
	5-6°	251	5	11	12	143.6 ± 2.5	c
	5-6°	223	5	58	16	147.0 ± 3.6	c
	5-6°	203	5	166	16	146.2 ± 3.4	c
	5-6°	182	5	895	19	143.0 ± 1.6	b
	5-6°	161	5	832	17	145.6 ± 3.1	b
	5-6°	137	5	884	17	147.8 ± 3.3	b
	5-6°	116	5	886	16	150.1 ± 2.1	b
	5-6°	96	5	851	23	151.7 ± 1.8	b
	5-6°	74	5	966	28	159.6 ± 6.7	c
	2-3°	251	5	428	23	151.0 ± 2.4	c
	2-3°	181	5	763	16	151.6 ± 1.3	b
	2-3°	154	5	711	20	152.0 ± 1.9	b
	2-3°	119	5	798	35	156.1 ± 4.1	b/c
	2-3°	77	5	638	29	162.5 ± 6.7	c

a Wild eggs were collected in Forestdale, Massachusetts; NJS-IR 10K refers to egg masses from crosses of irradiated (10 Krad) laboratory-reared males X laboratory-reared females: NJSS = New Jersey Standard Strain (F24).

b Compatibility code: a = eggs hatch on going out; b = pre-conditioning needed for synchrony; c = not usable.

One possible means of releasing sterile F1 individuals is to drop dehaired eggs from airplanes. In this study, the timing of hatch in dehaired eggs was similar to that of eggs in intact masses. However, the percentage of hatch was generally low in all of the 5-6° and 2-3° eggs. Dehaired eggs are readily subject to dessication and humidity should be checked in the above chambers. Oddly, percent hatch did not decrease with the length of time spent in the insectary prior to hatch. We recommend that, at present, only eggs chilled at 8-9° be dehaired prior to release.

Table 2. Timing of hatch in ca. 50 egg samples from gypsy moth egg masses that were subjected to various irradiation and chill treatments.

Source of eggs[a]	Chill treatment	Days in chill	n of masses sampled	Mean hatch per sample	Duration of hatch (days)	Mean Julian date of hatch (± S. D.)
wild	field	--	10	46.6	19	130.5 ± 4.2
NJS IR 10K	outside	(210)	10	18.0	32	130.9 ± 5.4
NJS IR 10K	5-6°	ca. 210	10	0.2	16	150.5 ± 10.6
NJSS	8-9°	182	5	46.8	26	129.3 ± 4.6
	8-9°	161	5	33.8	19	141.1 ± 3.8
	8-9°	137	5	34.0	23	145.0 ± 5.7
	8-9°	116	5	34.6	23	151.1 ± 3.7
	8-9°	96	5	44.6	19	153.7 ± 3.0
	8-9°	74	5	37.6	30	160.6 ± 5.2
	5-6°	272	5	1.8	29	133.7 ± 10.6
	5-6°	251	5	1.8	2	127.3 ± 0.5
	5-6°	223	5	1.0	2	127.2 ± 0.5
	5-6°	203	5	0.4	1	(143.0)
	5-6°	182	5	12.2	15	141.1 ± 3.6
	5-6°	161	5	9.0	18	147.8 ± 4.1
	5-6°	137	5	22.4	19	148.3 ± 3.7
	5-6°	116	5	18.8	20	150.7 ± 3.2
	5-6°	96	5	26.8	23	152.0 ± 2.4
	5-6°	74	5	29.0	26	153.5 ± 3.0
	2-3°	251	5	0	--	--------
	2-3°	181	5	3.2	3	151.0 ± 1.0
	2-3°	154	5	1.0	5	155.8 ± 1.6
	2-3°	119	5	3.4	13	156.5 ± 2.9
	2-3°	77	3	10.7	16	159.3 ± 4.6

a Wild egg masses were collected in Forestdale, Massachusetts; NJS-IR 10K refers to egg masses from crosses of irradiated (10 Krad) laboratory-reared males X laboratory-reared females; NJSS = New Jersey Standard Strain (F24).

Development on Black Oak Foliage. In both rearing studies, the survival of F1-sterile larvae was significantly lower than that of either of the unirradiated strains (Tables 3 and 4). Most of the "extra" mortality in the F1-sterile strain occurred during the first instar (Figure 1). From that point on, the numbers of individuals dying per stage was similar among the 3 strains; however, because there were many fewer F1-sterile individuals entering the second instar, their stage-specific mortality remained relatively high throughout development. When combined with reduced hatch, this means that an F1-sterile egg mass can be expected to produce only ca. 25 percent (optimistically) of the number of adult males that are produced by a wild egg mass. The efficacy of an F1-sterile release may be reduced further by the somewhat slower development that is typical of F1-sterile larvae (Table 4, also see Lance et al. 1982). This lag in development could cause asynchrony between the times of appearance of F1-sterile males and wild males (or wild females, for that matter). Still, the advantages of the F1-sterile egg release technique should make it a relatively cost-effective method of delivering sterile male gypsy moths. Further work needs to be done to determine the behavior, survival and synchrony of adult eclosion for F1-sterile gypsy moths under natural field conditions.

Table 3. Development and survival of gypsy moth larvae that were reared in 15-cm diameter petri dishes which were placed over black oak terminals in the field.

Source of eggs	Percent[a] survival	Male Development [b]			Female Development [b]		
		n of larvae	mean days as larvae	mean pupal weight (mg)	n of larvae	mean days as larvae	mean pupal weight (mg)
NJS-F_{23}	10.4a	39	48.9	264	9	57.0	473
Wild	14.6a	48	49.0	261	19	52.7	492
F1-sterile	6.3b	15	51.7	242	9	53.4	433

a Percentages that are not followed by the same letter are significantly different ($p < 0.05$), as determined by Chi^2 analysis.

b Within columns, there were no statistically significant differences among means (Student-Newman-Keuls test at $p < 0.05$).

Table 4. Development and survival of gypsy moth larvae that were reared on black oak foliage on screen cages in an insectary.

Source of eggs	Percent[a] survival	Male Development [b]			Female Development [b]		
		n of larvae	mean days as larvae	mean pupal weight (mg)	n of larvae	mean days as larvae	mean pupal weight (mg)
NJS-F_{23}	46.0a	110	39.1a	355a	83	45.5a	823b
Wild	48.7a	140	42.1b	353a	71	47.2b	901a
F1-sterile	16.4b	39	44.9c	296b	12	51.4c	735b

a Percentages that are followed by the same letter are not significantly different ($p < 0.01$) as determined by Chi^2 analysis.

b Within a column, means that are not followed by the same letter are significantly different ($p < 0.05$), as determined by the Student-Newman-Keuls test).

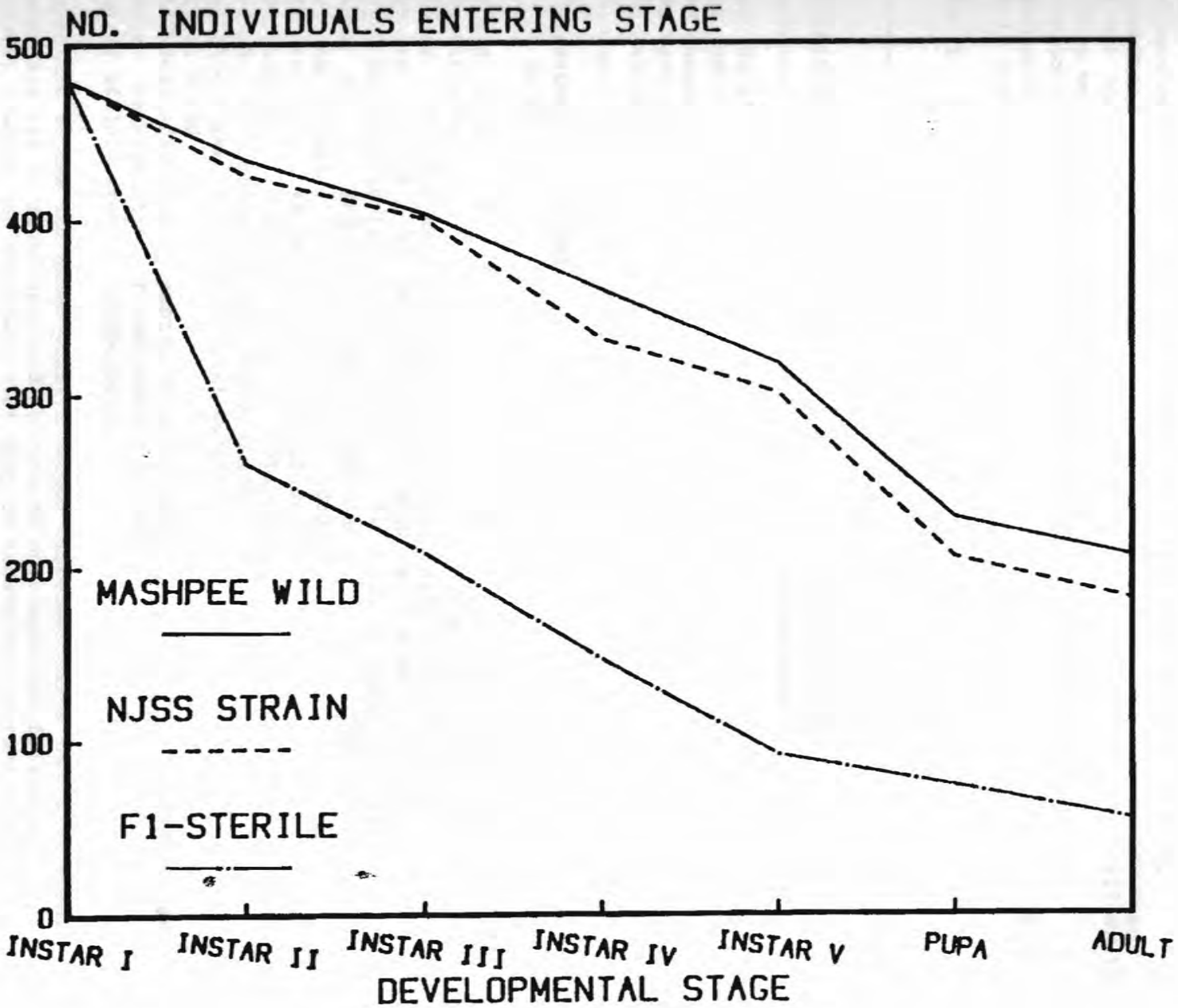

Figure 1. Survivorship of F1-sterile and untreated gypsy moth larvae on excised oak foliage in an outdoor insectary.

Project Number: GM 3.2.6
Project Title: Bioassay of 1983 and 1982 Gypsy Moth Trapping Survey Disparlure Dispensers
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leader: E. C. Paszek

Two lots of 1983 disparlure dispensers D0043 and D0053 and unused 1982 dispensers were compared with 1982 used dispensers from traps that had been set out for 6 months in Santa Barbara County, California. The used California samples were further aged outdoors for 12, 8, 4 and 1 week at the Otis laboratory. To determine if dispensers can be used for 2 seasons, seven treatments replicated 10 times were randomized 50 m apart on the points of a 7 x 10 gridded plot in a light infestation in Yarmouth, Massachusetts.

Table 1. Total number of moths captured (mean number of moths per trap in parentheses).

Treatment 1/	Weeks dispensers aged outdoors at Otis 1	4	8	12
1983 (D0043)	256 (8.5) a	-	-	-
1983 (D0053)	204 (6.8) ab	-	-	-
1982 Unused Control	124 (4.1) ab	-	-	-
1982 Used 6 months in California	79 (2.6) b	106 (3.5) b	78 (2.6) b	219 (7.3) ab

1/ Treatments followed by the same letter are not significantly different at 5% level as computed by Duncan's Multiple Range Test.

The 1983 lure Lot No. D0043 captured 8.5 moths/trap, indicating that it was slightly more effective in capturing moths than the 1983 Lot No. D0053 which captured 6.8 moths/trap and the 1982 control which captured 4.1 moths/trap (not significant). The 1982 dispensers which were used in California for 6 months and aged in traps outdoors for 1, 4, 8 and 12 weeks all captured moths. The 1, 4 and 8 week samples captured fewer moths than the 1982 control (not significant). The samples aged outdoors for 9 months, 6 months in California and 3 at Otis were as effective in capturing moths as 1983 Lot. No. D0053 and the 1982 unused controls. This sample was not refrigerated upon arrival. It immediately was placed outdoors in traps on 5/18/83. The remaining three (1, 4 and 8 week) California samples were stored in a freezer for 11, 8 and 4 weeks prior to outdoor aging. This may account for their being less effective in capturing moths than the California dispensers aged 9 months. Refrigeration of the samples may have temporarily inhibited the release of lure from the dispensers.

Disparlure in the laminated plastic Hercon dispenser has a long life span. Dispensers may be saved and used in case of emergency the following year. However, I would not recommend that they be reused for routine trapping surveys as there may be a variation in the amount of lure remaining in the dispensers which would make them less effective than new dispensers.

Project No. GM 3.2.7
Project Title: Lure Dispenser Installation Study
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: E. C. Paszek

Hercon® Gypsy Moth Lure Tape® Plus is a plastic laminated pheromone dispenser 7/8 x 1/8" in size and it is used as the standard lure dispenser in gypsy moth survey traps. This small dispenser is stapled above the trap adhesive inside the Delta survey trap or onto a twist'em plant tie which is suspended inside the large capacity milk carton trap. If these small plastic dispensers would adhere to double stick tape, it would simplify the assembling of traps as it is difficult to staple them into traps or onto the twist'em plant ties. LURE N KILL™, Integrated Pest Management Products, attaches the Hercon® Lure Tape® Plus inside a tea bag web which is suspended inside their traps. The following types of double stick tapes and dispenser holders were studied in order to seek a better method of attaching lure dispensers inside traps.

1/ Scotch® 3M Double Stick Tape (1/2" wide)
2/ Scotch® 3M 410 Flat Stock Liner Double Coated Tape (3/4" wide)
3/ Sears Self Adhesive Mounting Tape (1/2" wide, 1/16" thick polyurethane foam)
4/ Dispenser attached inside a tea bag web (2-7/8 x 1")

The dispensers used in this study were from the 1982 Lot No. D0032. When placed in the MC traps the 2 Scotch brand 3M Double Stick tapes had one side of the dispenser totally sealed on them. The Sears self adhesive mounting tape was cut into 1-1/4 x 1/4" lengths and the 7/8 x 1/8" long dispenser was attached lengthwise across the width of the tape allowing it to have a 5/16" overlap on each side of the tape. This overlap allowed for air to circulate completely around 5/8" of the dispenser. The double stick tapes with the dispensers attached were aged outdoors for intervals of 6 weeks, 2 weeks and 0-days, and were field bioassayed with 0-day controls. The dispensers attached inside the tea bag web were bioassayed at 0-day along with similar dispensers stripped from the tea bag webs. This comparison was made to determine if the tea bag affected pheromone release and, therefore, trap catch. Additionally, three methods of installing dispensers with double stick tape study were aged for various periods. Seven replicates of each were placed randomly in a 12 x 7 grid with the traps spaced 50 m apart. They were bioassayed from August 3-12, 1983 in a moderate infestation.

1/ 1/2" wide pressure sensitive transparent tape, sticky on both sides, used as a light duty glue, made by Household and Hardware Products Division/3M, 3M Center, Box 33053, St. Paul, Minnesota 55133.

2/ 3/4" wide, opaque stick on both sides drafting tape made by Scotch brand tape/3M, St. Paul, Minnesota 55144.

3/ 1/2" wide, 1/16" thick white double coated vinyl foam tape used for holding and hanging light weight paintings, plaques, charts, etc. Sold by Sears Roebuck and Co., Chicago, Illinois 60684.

4/ 1" x 2-7/8" tea bag web made by LURE N KILL™ Integrated Pest Management Health-Chem Corporation, 1107 Broadway, NY, New York 10010.

Table 1. Mean number of moths captured in traps with aged dispensers attached to 3 types of double stick tape.

Tape type	(Age of the Dispensers) 6 week	2 week	0-day
3M Double Stick	44 a[1/]	14 b	4 c
Sears Mounting Self Adhesive	42 a	34 a	3 c
3M Double coat	33 a	12 b	3 c
Control	-	-	6 bc

1/ Groups followed by same letter not significantly different at 5% level.

In the comparison of the 3 types of double stick tape, there was no significant difference in the number of moths captured between the dispensers attached to the 3 types of tape in the treatments aged 6 weeks. In the 2 week aging treatments dispensers attached to Sears Mounting Self Adhesive tape captured significantly more moths than the dispensers attached to the 3M tapes. In the 0-day treatment there was no significant difference between the dispensers attached to each of the 3 tapes. There is a slight difference between the 0-day control (free hanging dispenser) and the 3 tape attached dispensers. Since there was no control to compare with the 6 week and 2 week treatments, this study will be repeated in 1984.

A group of 5 traps from each of the three 6-week aging tape treatments were returned from the grid and hung outdoors in the rear of the laboratory. The dispensers were checked in the traps 3 times 8/19/83 (2 mo.), 10/31/83 (4-1/2 mo.) and 2/15/84 (8 mo.) to see if there was any dislodgment of the dispensers from the tapes. All of the dispensers were firmly attached to the tapes.

Another observation made in this study is that the longer the dispensers were aged in the traps outdoors the more efficient they were in capturing moths. This is consistent with results from other dispenser aging tests.

Table 2. Mean number of moths captured in traps with dispensers attached inside tea bag webs and with dispensers stripped from tea bag webs.

Type of dispenser	Number moths captured
Tea bag stripped[1/]	19
Tea bag web[1/]	12

1/ When compared using analysis of variance (split plot design), means were not significantly different at the 5 percent level.

The dispensers attached inside tea bag webs were as good as the dispensers stripped from the the tea bag webs in capturing moths. The additional cost of tea bag webs and process of making same was four cents per dispenser in 1982.

Project Number: GM 7.3.6
Project Title: Insect Production and Distribution
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: J. J. Baker and John Allen Tanner

The primary objective of the rearing facility is to produce sufficient quantities of all gypsy moth life stages for the support of projects at this laboratory, and at research institutions in the United States and abroad. The following is a summary of FY 1983 production and distribution of such material.

A. PRODUCTION

(1) The number of eggs, cups and liter of B-4 diet used to provide gypsy moth life stages for in-house and cooperative programs.

	Eggs Infested	B-4 diet liters	Number cups
Cooperative Programs	404,164	1,813	21,330
Colony	209,480	2,050	25,183
Insecticide	948,040	1,485	15,728
Sterile male behavior evaluation	847,648	4,636	49,195
Quality control	102,700	874	9,120
Maryland sterile male, red	1,670,164	8,900	105,330
Otis sterile male, red	158,240	905	10,855
North Carolina sterile male, red	2,580	1,852	448
F1 pilot study, eggs plain	1,137,260	5,825	71,337
F1 pilot study, eggs red	351,720	1,852	19,007
TOTAL	5,831,996	30,192	327,533

	Neonates Infested	B-4 diet liters	Number cups
(2) North Carolina Fl red	15,604	132	1,258

(3) The number of eggs refrigerated October 1, 1982 - September 30, 1983.

	30,688,420	NJ F25	October 1, 1982 - February 28, 1983
	43,094,660	NJ F26	March 7, 1983 - September 26, 1983
TOTAL	73,783,080		

B. DISTRIBUTION OF REARED INSECTS, 1983

Project	Egg Masses	L-2	L-3 L-4	L-5 L-6	Male prepupae	Male pupae	Female prepupae	Female prepupae
W. McLane (APHIS)	-	116,820	8,820	-	-	-	-	-
V. Mastro (APHIS)	-	-	-	-	116,025	-	-	12,649
R. Carde (U. Massachusetts)	-	-	-	-	-	11,400	-	5,700
P. Barbosa (U. Maryland)	490	-	6,004	-	-	-	50,485	-
J. Kennedy (England)	-	-	-	-	-	4,870	-	4,870
R. Webb (ARS)	-	-	200	-	-	-	-	-
M. Ma (U. Maryland)	-	-	-	26,250	-	-	10,500	-
C. Yin (U. Massachusetts)	-	-	17,400	-	-	-	-	-
R. Chianse (New Jersey Dept. of Agriculture)	4,017	-	-	-	-	-	-	-
R. Fusco (Pennsylvania FPM)	400	-	-	-	-	-	-	-
W. Yendol (Pennsylvania State Univ.)	110	-	-	-	-	-	-	-
F. Hanson (U. Maryland)	-	-	-	-	-	1,420	-	1,420
J. Appleby (Illinois)	50	-	-	-	-	-	-	-
G. Driver (Boston Univ.)	80	-	-	-	-	-	-	-
J. Tanner (APHIS)	-	-	-	-	-	6,600	-	6,600
TOTAL	5,147	116,820	32,424	26,250	116,025	24,290	60,985	31,239

C. SUMMARY OF STERILE MALE PRODUCTION, FY 1983

	Male Prepupae	Male Pupae	Female Prepupae	Female Pupae
Maryland sterile male, red	16,162	416,651	350	18,250
North Carolina sterile male, red	195	6,087	250	1,946
Otis sterile male, red	2,007	48,987	-	1,000
Fl Pilot study, plain	5,885	235,100	10,465	187,208
Fl Pilot study, red	2,836	92,475	9,075	63,000
TOTAL	27,085	799,300	20,140	271,404

Project Number: GM 1.3.1.
Project Title: Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: John Allen Tanner, Bernadine P. Weeks and Marjorie Palmeri.

The purpose of this project is to measure the quality of insects produced in the rearing facility, and 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.

To help identify the agent(s) which cause a change in the development of the production insects, their growth and development are compared with the growth and development of insects (internal standard) 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 variables on the production insects, and to make changes in the rearing techniques when possible.

Previously, colony and production insects were initially placed onto diet as neonates (neonate infest method). Beginning in October, 1982, both the colony and the production insects were placed onto diet while still in the egg stage (egg infest method). With the aid of a mechanical egg dispenser, this method is superior to the neonate infest method in terms of limiting the time necessary to infest gypsy moth into rearing cups (Tanner et al 1982).

Colony insects are reared on diet made from dietary ingredients that are preweighed at the beginning of each rearing year (December 1) to ensure a complete year's supply. The ingredients are stored at -15°C until needed. 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 the production insects.

Colony insects are currently serving as the internal standard. Colony insects are started once each week. Their development is compared with 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.

Egg dispensing bar No. 29 is used to dispense eggs into rearing cups containing production diet. Approximately 17 eggs are dispensed into each cup. Thirty cups are placed on each rearing tray. Three rearing trays are randomly selected and used to measure the development of the production insects. The three trays are triple-stacked in a similar fashion as the normal production insects, but are held on a separate cart for easier reading.

Egg dispensing bar No. 32 is used to dispense eggs into rearing cups containing colony diet and into 90 cups containing production diet. Approximately 12 eggs are dispensed into each cup. The cups containing colony diet are designated for colony maintenance. Thirty cups are placed on individual rearing trays. One rearing tray is placed in each rearing truck section. One week's production of colony insects fills almost an entire truck.

The 90 cups containing production diet are designated internal comparison (I.C.). The 90 cups are placed on three rearing trays (30 cups/tray) and are held on the colony rearing truck. One tray is on the top, one in the middle and one on the bottom section of the colony rearing truck. Comparing the I.C. insects with the colony and production insects enables us to observe both the effects of density and diet on the development of the gypsy moth.

SAMPLING PROCEDURES

Eclosion and Developmental Data

A brief description of the sampling procedures and the qualities measured is presented below. The ensuing procedures are followed for the colony, I.C. and production insects:

Six diet-filled cups are randomly selected shortly after the diet is dispensed and weighed to determine the amount of diet/cup. Diet batch and ingredient control numbers are also recorded. After the eggs are dispensed onto the diet, 6 cups are randomly selected and the number of eggs/cup determined. The total eggs dispensed is then estimated.

On the tenth post-egg infest day (P.E.I.D.), 6 cups are selected from specific areas of the rearing trays and rearing trucks. The colony and I.C. treatment cups are selected to represent the top, middle and bottom sections of the rearing truck, and the front-to-back sections of the rearing trays. The production treatment cups are selected to represent the top, middle and bottom tray in a triple stack level and the front-to-back sections of the rearing trays. The location of the selected rearing cups is the same for each week of rearing.

The number of unhatched embryonated and unembryonated eggs, the number of dead neonates and their location within the cups, and the number of neonates/larval stage are determined for each cup. The embryonation, percent hatch and percent establishment is then determined for the 6 cups. The mean larval stage is also determined by the following formula:

$$\frac{(\text{\# larvae/developmental stage X the development stage \#})}{\text{Total \# larvae.}}$$

On the 24th P.E.I.D., another 6 cups are selected. These cups are checked each weekday for pupation until the pupae are harvested on the 39th P.E.I.D. Male and female pupal DT_{50} are then determined. The total male and female pupae and spins harvested are also recorded. These data are used to calculate percent survival from the egg to the pupal stage.

Fifty male and 50 female pupae are randomly selected from the total pupae harvested. The selected pupae are checked for deformity and then weighed collectively by sex. The pupae are held in 16-ounce containers (25 pupae/container) until completion of adult emergence. Newly emerged adults are checked for deformity, before being discarded. Upon completion of emergence, the DT_{50}, percent emergence, percent deformity and percent survival from the egg to adult stage are determined for each sex. The sex ratio is also determined by the following computations:

male adults = (# male pupae & spins) X (percent adult male emergence)
female adults = (# female pupae & spins) X (percent adult female emergence)

$$\text{sex ratio} = \frac{\text{\# male adults}}{\text{\# female adults}}$$

Reproductive Data:

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 reproduce in our rearing facility; thus, reproductive and eclosion data are collected only on the colony insects.

Mating, fecundity and egg hatch data are taken directly from the colony. Males are mated with females from the same egg infest date. A mating container (25 matings) is selected for each egg infest date. A preweighed oviposition paper is inserted into the container and 25 pairs of male and female pupae are introduced. Twenty days thereafter, the paper is removed and the percentage of females laying egg masses, and the mean egg mass weight, are determined. The mean number of eggs/mass is determined by multiplying the mean egg mass weight by 1.33.

The oviposition paper is then sectioned into 2 equal groups of egg masses. One group is placed into a container (1 quart) holding colony egg masses that are to be chilled in an automated refrigerator (8.2°C to 4.9°C) for 170 days. In this refrigerator, all the egg masses are mechanically rotated throughout the refrigerator to insure that they are all exposed to similar conditions.

The second group of egg masses is also placed into a 1-quart container. This container holds a small portion of each week's egg mass production. These masses are stored in a standard walk-in refrigerator (5.7 - 3.8°C) and serve as a "back-up" if something happens to the egg masses located in the automated refrigerator.

All of the masses are put into their respective refrigerators 15 days after weighing. For each week of production, 5 egg masses are selected from both refrigerators after 90 days of chilling. Hatch tests are conducted on each egg mass after 120, 150 and 170 days of refrigeration. Percent hatch (based on embryonated eggs only) and HT_{50} (incubation time to 50 percent hatch) are determined for each sample period. The reproductive index is calculated for eggs refrigerated in both refrigerators by the following formula:

eggs/mass X percent mated X percent hatch.

Table 1 compares the eclosion and developmental data of colony insects started at the 12 eggs/cup density and production insects started at the 17 or 12 eggs/cup density for the 1982 rearing year.

The only major surprise was the significant reduction in hatch of eggs incubated on production diet. With the exception of the female pupal DT_{50}, colony and production insects started at the 12 eggs/cup density had similar growth and developmental data. The female pupal DT_{50} was significantly later with the production insects (12 eggs/cup) than colony insects, however, the female adult DT_{50} was similar for both groups. The mean larval stage (10 P.E.I.D.) was similar for both groups. Production insects started at the 17 egg/cup density developed at a similar rate as the colony insects but, as occurred in previous years, the pupal weights and survival was lower (Tanner and Weeks, 1981-1982). The production insects also had a slightly higher female adult emergence percentage.

Table 1. Eclosion and developmental data of colony insects started at 12 eggs/cup density, and production insects started at 17 or 12 eggs/cup density.

Parameter	Number of eggs/cup[1/]					
	Colony		Production			
	12		12		17	
	x	Sd	x	Sd	x	Sd
Eclosion data:						
% embryonation	95.2	7.1 a	95.9	6.5 a	95.2	6.3 a
% hatch	85.1	12.8 a	78.7	16.9 b	78.7	15.0 b
Developmental data:						
Mean larval stage -10 (P.E.I.D)[2/]	1.96	0.23 a	1.96	0.30 a	1.94	0.26 a
Pupal weights (gms)						
Males	0.75	0.06 a	0.77	0.05 a	0.67	0.05 b
Females	2.46	0.19 a	2.48	0.20 a	2.12	0.19 b
Pupal DT50 (P.E.I.D)						
Males	33.1	8.7 a	33.8	11.5 a	32.7	6.3 a
Females	34.6	8.5 b	36.6	14.4 a	34.4	7.5 b
% Pupal Deformity						
Males	16.6	7.6 a	15.8	9.2 a	14.9	5.8 a
Females	89.0	6.8 a	87.1	8.9 a	87.4	7.4 a
% Survival (eggs to pupae)	61.0	8.5 a	64.6	13.4 a	58.9	21.5 b
Adult DT50 (P.E.I.D.)						
Males	46.0	1.0 a	45.7	1.3 a	45.9	1.1 a
Females	45.0	1.1 a	44.6	1.1 a	44.8	0.9 a
% Adult Emergence						
Males	97.5	3.0 a	97.9	2.4 a	98.1	2.7 a
Females	95.1	5.1 b	96.2	3.2 ab	97.4	2.5 a
% Adult Deformity						
Males	1.3	1.9 a	0.7	1.2 a	0.9	1.4 a
Females	9.7	5.1 a	8.8	5.8 a	6.5	3.3 a
Sex Ratio M:F	1.3:1	0.2 a	1.2:1	0.2 A	1.2:1	0.2 a
% Contamination						
Bacteria	0.12	0.37 a	0.03	0.19 a	0.13	0.60 a
Fungus	0.22	0.33 a	0.42	0.77 a	0.42	0.91 a
Virus	0.22	0.42 a	0.13	0.45 a	0.36	1.12 a

1/ Means within a row not followed by same letters are significantly different at the .05 level (Duncan's Multiple Range Test).

2/ P.E.I.D. = post egg infest day.

Table 2 compares the 1982 and 1983 developmental data from colony insects. Female pupae tended to weigh more and female adults had less deformity and a greater emergence rate in 1983 than 1982. Male pupal deformity was higher in 1983 than 1982. No attempt was made to compare 1982 and 1983 developmental rates because different infestation methods were used.

Table 2. Comparison of the developmental data of colony insects infested in 1983 to the developmental data of colony insects infested in 1982.

	Colony Insects Infested[1/]			
	1983		1982	
	x	Sd	x	Sd
Pupal Weights (gms)				
Males	0.75	.06 a	0.74	.04 a
Females	2.46	.19 a	2.35	.13 b
% Pupal Deformity				
Males	16.6	7.6 a	10.5	7.6 b
Females	89.0	6.8 a	82.4	8.8 a
% Adult Emergence				
Males	97.5	3.0 a	98.9	1.7 a
Females	95.1	5.1 a	88.9	15.0 b
% Adult Deformity				
Males	1.3	1.9 a	1.2	1.9 a
Females	9.7	5.1 b	18.2	13.2 a
Sex Ratio				
M:F	1.3:1	0.2 a	1.2:1	0.5 a

1/ Means within a row not followed by same letter are significantly different at the .05 level (Student's T).

Table 3 compares the 1982 and 1983 reproductive and egg eclosion data from colony insects. Colony eggs tended to hatch quicker and have a greater reproductive index in 1983 than in 1982. The hatch of eggs chilled 150 days was higher in 1983 than in 1982, but similar for the two years at the 120 and 170 chill periods.

TABLE 3. Reproductive and egg eclosion data of colony insects reared in 1982 and 1983. 1/

	1983		1982	
	x	Sd	x	Sd
% of Females Depositing Egg Masses	87.6	9.9 a	83.8	13.9 a
# Eggs Deposited, Female	1052.	108 a	1017	89 a
% Hatch				
120 days chilled eggs	93.2	8.2 a	91.4	6.4 a
150 days chilled eggs	95.2	5.7 a	87.6	20.8 b
170 days chilled eggs	93.3	8.8 a	82.0	5.5 a
Time to 50% Hatch (P.E.I.D.) 2/				
120 days chilled eggs	8.7	1.8 a	8.8	0.8 a
150 days chilled eggs	3.6	1.0 a	4.0	0.6 b
170 days chilled eggs	1.5	0.5 a	2.3	0.05 b
Reproductive Index 3/				
120 day chilled eggs	909	114 a	778	150 b
150 days chilled eggs	928	108 a	779	153 b
170 days chilled eggs	907	94 a	783	118 b

1/ Mean within a row not followed by same letter is significantly different at the .05 level (Student's T).

2/ P.E.I.D. = Post Egg Infest Day.

3/ Reproductive Index = # eggs/mass x % mated x % hatch

Table 4 compares the percent hatch and egg incubation time to 50 percent hatch (HT50) of colony eggs chilled (120, 150, and 170 days) in the automated refrigerator to similar colony eggs held in the reserve walk-in refrigerator. Lengthening the chilling period resulted in a significant reduction in hatch with the eggs chilled in the walk-in refrigerator, but not with the eggs chilled in the automated refrigerator. By the 170-day chill period, the eggs held in the walk-in refrigerator averaged 13 percent less hatch than eggs held in the automated refrigerator.

Lengthening the chilling period reduced the HT50 for egg masses chilled in either refrigerator. The reduction in HT50 was more pronounced with eggs chilled in the automated refrigerator. After 120 days of chilling, the eggs stored in the walk-in refrigerator had a HT50 that was 1.6 days sooner than eggs stored in the automated refrigerator. After 150 days of chilling, the situation was reversed. Egg masses chilled in the automated refrigerator reach their HT50 1.7 days sooner than those mass-chilled in the walk-in. After 170 days of chilling, masses chilled in the automated refrigerator reach their HT50 3.2 days sooner than masses from the walk-in refrigerator.

The data show that colony eggs cannot be stored in the walk-in refrigerator for 170 days without a significant reduction in hatch, and a lengthening of the HT50 compared to eggs stored in the automated refrigerator. However, the reduction in hatch was not great enough to stop the use of the walk-in refrigerator as a back-up for holding colony egg masses.

TABLE 4. Percent hatch and egg incubation time to 50 percent hatch (HT50) in the automated refrigerator compared to similar colony eggs held in reserve within a walk-in refrigerator.

Chilling Period Days	Percent Hatch			
	Automated 1/		Walk-in 2/	
120	93.3	8.2 a	91.7	9.2 a
150	95.2	5.7 a	89.4	9.1 b
170	93.3	8.8 a	80.7	16.8 b
	HT50 (Days)			
120	8.7	1.8 b	7.1	1.0 a
150	3.6	1.0 a	5.3	0.8 b
170	1.5	0.5 a	4.7	0.9 b

1/ Temperature range: 4.9°C to 8.2°C

2/ Temperature range: 3.8°C to 5.7°C

3/ Means within a row not followed by the same letter are significantly different at the .05 level (Student's T).

REFERENCES CITED

Tanner, J. A., Tardif, J. G. R., Baker, J. J., and Weeks, B. P. 1982 Development of Mechanical Egg Infestation Procedures. APHIS Laboratory Report, October 1, 1981 - September 30, 1982: 153-168.

Project Number: GM 1.3.2
Project Title: Development of Mechanical Egg Infestation Procedures
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leaders: John Allen Tanner, J.G.R. Tardif, J.J. Baker and Bernadine P. Weeks

The egg dispenser described by Tanner et al (1982) has been successfully used during the 1982 and 1983 summer sterile male programs. The overall labor costs were reduced by 40 percent, as compared to the labor costs of a similar size program conducted in 1981 with the neonate infest method. This egg dispenser is now used to start all insect production, including those assigned for colony maintenance.

References Cited

Tanner, J.A., J.G.R. Tardif, J.J. Baker and B.P. Weeks. 1982. Development of Mechanical Egg Infestation Procedures. APHIS Laboratory Report, October 1, 1981 - September 30, 1982: 153-163.

Project Number: GM 3.3.1
Project Title: Development and Evaluation of Improved Rearing Techniques
Report Period: October 1, 1982 - September 30, 1983
Report Type: Preliminary
Project Leaders: John Allen Tanner, Bernadine P. Weeks and Marjorie Palmeri

This project is concerned with the development and evaluation of new rearing techniques and the improvement of presently used techniques. Unreliable or inefficient techniques will be modified or discarded.

B-4 diet is poured into rearing cups and allowed to cool for one hour in a clean air room before gypsy moth eggs are dispensed into the rearing cups and the cups capped. However, during a large scale production program, it is not uncommon for the diet to stand in the clean air room from 1 to over 4 hours before the eggs are dispensed. This variation in diet drying time may be contributing to some of the differences observed in the day-to-day hatch of the eggs and/or larval development. The following test was conducted to determine if the length of diet drying has any effect on the hatch of the eggs and the development of the larvae.

Methods and Materials:

B-4 diet was poured into 210 rearing cups and allowed to cool for the normal 1 hour. Beginning at this time and at hourly intervals, thirty cups were selected and infested with treated (10% formalin solution), dehaired eggs. The cups were immediately capped and transferred into an environmental chamber (25°C, 50-60% RH, 16L:8D) for egg incubation and larval growth. On the 7th post-egg infest day (P.E.I.D.), the number of unhatched embryonated and unembryonated eggs, the number of dead neonates, and the number of neonates/larval developmental stage were determined for each cup. The presence or absence of diet contamination was also recorded. A mean larval stage was calculated for each cup by the following formula:

$$\frac{(\text{\# larvae/developmental stage X the developmental stage \#})}{\text{Total \# larvae}}$$

Each cup was again examined on the 14th and 21st P.E.I.D. for diet contamination and the number of larvae/developmental stage. Mean larval stages were again calculated with the above formula.

Commencing on the 22nd P.E.I.D., each cup was checked daily and the number of male and female pupae recorded. On the 35th P.E.I.D., the test was terminated. The final number of male and female pupae was recorded, as well as all the male and female larvae and spins (pre-pupae stage), and the larvae that could not be sexed. From these data, we were able to determine male and female pupal DT_{50} (time to 50 percent pupation), percent pupation and the percentage of male larvae that completed pupation within 4 days after 10 percent male pupation had occurred. Maximizing this last percentage is important for an efficient sterile male rearing program.

Results:

Lengthening the diet drying period did not have any consistent effect on the hatch of the eggs (table 1), nor did it affect the establishment of the neonates on the diet (table 2). However, increasing the diet drying period did effect the development of the larvae (tables 3 and 4). The mean larval stages declined consistently before leveling off at around the 4 hours drying period. The male and female pupal DT_{50} also increased consistently before leveling off at around the 4 hour drying period. Both conditions characterize a reduction in the rate of larval development as diet drying increases.

The overall pupal yields decreased as the diet drying period increased (table 5). Also, in relation to the sterile male program, lengthening the diet drying period beyond 1 hour resulted in a significant reduction in the percentage of male larvae which completed pupation within four days after 10% male pupation had occurred (table 6).

Conclusion:

B-4 diet should not be allowed to dry in open rearing containers for longer than 1 hour before the eggs are dispensed and the cups capped. This 1 hour drying limit is especially critical during the sterile male program where longer drying periods causes a significant reduction in the number of male larvae pupating within the critical four day period.

Table 1. The hatch of treated[1/] gypsy moth eggs as affected by the length of the diet drying period.

	Hours of Diet Drying[2/3/]						
	1 Control	2	3	4	5	6	7
Percent hatch	84.7±11.3 b	87.1±11.0 ab	89.6±10.1 a	88.1±10.4 a	87.7±10.8 ab	90.6±9.1 a	89.1±9.4 a

1/ Egg masses treated with a 10 percent formalin solution (1 hr) rinsed (1 hr) and dehaired.
2/ ± s.d.
3/ Mean within a row not followed by the same letter are significantly different at the .05 level (Duncan's Multiple Range).

Table 2. The establishment of gypsy moth neonates on artificial diet as affected by the length of the diet drying period.

	Hours of Diet Drying[1/2/]						
	1 Control	2	3	4	5	6	7
Percent establishment	97.7±8.3 b	98.4±6.8	99.1±4.0	99.0±4.7	99.1±4.0	99.0±5.1	98.6±6.9

1/ ± s.d.
2/ Means within the row were nonsignificantly different at the .05 level.

Table 3. The mean larval stage[1/] of gypsy moth larvae on the 7, 14, and 21 PEID[2/] as affected by the length of the diet drying period.

P.E.I.D.	Hours of Diet Drying[3/4/] 1 Control	2	3	4	5	6	7
7	1.65±0.22 a	1.56±0.24 b	1.55±0.25 b	1.46±0.25 c	1.44±0.21 c	1.47±0.21 c	1.43±0.22 c
14	3.58±0.22 a	3.47±0.24 b	3.42±0.25 bc	3.28±0.27 e	3.35±0.25 ce	3.38±0.22 cd	3.33±0.26 de
21	5.20±0.28 a	5.11±0.29 b	5.09±0.28 b	4.97±0.32 c	4.95±0.29 c	5.00±0.30 c	4.94±0.30 c

1/ Mean larval stage = $\frac{[(\text{Number of larvae/instar})(\text{Instar number})]}{\text{Total number of larvae}}$

2/ P.E.I.D. = Post Egg Infest Days

3/ ± s.d.

4/ Means within a row not followed by the same letter were significantly different at the .05 level (Duncan's Multiple Range)

Table 4. Male and female pupal DT_{50}[1/] as affected by the length of the diet drying period.

Pupal sex	Hours of Diet Drying[2/3/] 1 Control	2	3	4	5	6	7
Male	29.1±2.9 a	29.5±4.1 ab	29.8±1.1 ab	29.8±4.1 ab	30.3±1.3 b	30.1±3.1 b	30.1±3.0 b
Female	31.5±1.1 a	31.7±1.0 a	31.7±1.1 a	32.3±1.2 b	32.2±1.1 b	32.2±1.0 b	32.2±1.2 b

1/ Time to 50% pupation.

2/ ± s.d.

3/ Means within a row not followed by the same letter are significantly different at the .05 level (Duncan's Multiple Range).

Table 5. The percentage of larvae pupating as affected by the length of the diet drying period.

	Hours of Diet Drying[1/]						
	1 Control	2	3	4	5	6	7
Percent Pupation[2/]	86.8±11.8 a	84.5±14.7 ab	84.5±13.9 ab	82.0±14.6 bc	82.0±12.3 bc	78.6±14.9 c	78.9±16.2 c

1/ s.d.
2/ Means within a row not followed by the same letter were significantly different at the .05 level (Duncan's Multiple Range).

Table 6. The percentage of male larvae which completed pupation within four days after 10 percent male pupation had occurred as affected by the length of the diet drying period.

	Hours of Diet Drying[1/2/]						
	1 Control	2	3	4	5	6	7
Percentage[3/]	73.0±25.4 a	59.4±30.0 b	64.9±26.9 b	63.0±25.8 b	58.6±24.7 b	62.4±26.7 b	61.9+26.1 b

1/ ± s.d.
2/ Means within a row not followed by the same letter were significantly different at the .05 level (Duncan's Multiple Range).
3/ The percentage of male larvae which completed pupation within four days after 10 percent male pupation had occurred.

Project Number: NPPSD 2.1.1
Project Title: Summary of Methods Development Support of CNPPSDP
Report Period: October 1, 1982 - Septemeber 30, 1983
Report Type: Interim
Project Leader: A. S. Foudin

I. Large scale production of viable and morphologically normal conidia of Drechslera carbonum and Drechslera maydis.

The main objective of our current methods development work is the creation of new immunological, biochemical, physiological or selective media methods of identifying and distinguishing between the two important corn pathogens, Drechslera maydis (Southern corn leaf blight) and Drechslera carbonum (Helminthosporium leaf blight). In order to pursue the development work on either an immunological or biochemical identification technique, we have found it necessary to find a method of producing gram quantities of viable and morphologically normal conidia of both fungi to use in the production of highly specific antibodies, or to examine for unique biochemical properties.

Based on preliminary work in our laboratory from 1982, we had determined that Difco lima bean agar ($70/500 g) gave superior growth and conidial production when compared to the usual growth substrate, which is potato-dextrose-agar (PDA). We therefore set as one of our short-term goals the development of an inexpensive and effective conidial production substrate based on our observations with commercial lima bean agar. Tests were performed on 11 different laboratory-produced lima bean agar (LBa) media, comparing them against PDA and commercial LBa (Table 1).

TABLE 1. Conidial production on different substrates.

	Product	Conidial Production*
1.	Ground frozen lima beans	++
2.	Ground frozen lima beans + dextrose	++
3.	Ground frozen lima beans + yeast extract (YE)	++
4.	Ground frozen lima beans + dextrose + YE	++
5.	Ground dried baby lima beans	++1/2
6.	Ground dried baby lima beans + dextrose	++1/2
7.	Ground dried baby lima beans + yeast extract	++
8.	Ground dried baby lima beans + dextrose + YE	++
9.	Ground dried baby lima beans, rehydrated overnight	++++
10.	Ground dried baby lima beans, autoclaved 1 hour	+++1/2
11.	Lima bean agar, made from baby food	+
12.	Commercial or laboratory-made PDA	++
13.	Difco lima bean agar	++++

* Plates were incubated 14 days at 26°C prior to counting of conidia.

Based on this work, we proceeded to develop a lima bean medium using ordinary ground dried lima beans. We next tested both the effects of time and method of rehydration of the LB powder upon conidial production (Table 2).

TABLE 2. Effect of the time and method of rehydration of lima bean powder on conidial production in the resulting media.

Medium	Conidial production
1. No rehydration + 20 minutes autoclaved	++
2. No rehydration + 60 minutes autoclaved	+++
3. Rehydrated overnight + 60 minutes autoclaved	++++
4. Autoclaved 60 minutes, rehydrated overnight	++++
5. Difco LBa - per instructions	++++

To determine if it was necessary to have the rehydrated lima bean powder particles remain in the substrate in order to obtain maximum conidial production, we either filtered or centrifuged the material just prior to the addition of agar. We found that removing the lima bean particles did not negatively affect the production of conidia, and it did improve the handling and pouring qualities of the final lima bean agar product. It can be assumed that the components responsible for the superior conidial production are solubilized during the extended rehydration and autoclaving process and are probably complex starches. Four rates of lima bean powder - 5g/liter, 10g/liter, 15g/liter and 20g/liter - were autoclaved, rehydrated and filtered to determine the optimum level of LB powder to incorporate. Levels above 20g/liter of LB powder were not employed, due to the difficulty of working with the highly viscous solution which resulted. The 5g/liter and 10g/liter levels produced marginal conidial production. The 15 g/liter level gave conidial production equal to the costly commercial lima bean agar and the 20g/liter level gave results equal to, or slightly superior to either the commercial product or the 15g/liter level. Considering the negligible cost of the lima bean powder (less than $1/lb), and the slightly superior performance at the higher level, 20g/liter was selected as our standard lima bean medium. Later tests have shown that, as long as the lima bean powder solution stays sterile following its initial one-hour autoclaving, an extended period of rehydration greater than 18 hours has no effect.

The agar, Difco-Bacto agar ($37/lb), was the only component we had not yet tested to determine if a superior and less expensive substitute could be found. Numerous agar products were evaluated in our lima bean medium and we found that a USP-commercial grade of agar from the Moore Company, which costs $9.25/lb, used at a rate of only 7.5g/liter as compared to 16g/liter for Difco-Bacto agar, gave consistently superior results.

Seventeen different types of beans (Table 3) were tested to determine if any would yield a medium that was easier to make, less costly to produce, or gave measurably greater quantities of conidia. The same method of medium production was employed with all bean products.

TABLE 3. Conidia production of D. maydis and D. carbonum on different bean agars.

Bean product	Relative* conidial production carbonum	maydis
1. Baby lima	control/S	control/S
2. Large lima	S	S
3. Michigan navy	S-	S-
4. Great northern	S	S-
5. Split pea	S-	S-
6. Garbanzo	S-	S-
7. Lentils	L	L
8. Kidney	S-	S
9. Fava	H	H
10. Azuki	H	H
11. Mung	S-	S-
12. Pinto	L	L
13. Turtle	L	L
14. Oriental black	L-	L-
15. Red	S	S-
16. Black eye pea	S	S-
17. Soybean	L	L

* S = standard/equal to control
L = lower than control
H = higher than control.

The two bean substrates which yielded the greatest overall conidial production were fava and azuki. The bean substrates which produced the most dramatic differences in growth characteristics between D. carbonum and D. maydis were lima beans (large or baby), split pea and navy.

Quantitative comparisons in conidial production were made by washing the conidia off of 14-day old plates, using a solution of "Dawn" liquid dishwashing detergent. The solution containing the conidia was brought up to 100 ml in a volumetric flask, and 10 individual counts were made using a hemocytometer. Four measured fields in the hemocytometer were counted for each of the 10 samples per volumetric. With the assistance of Dr. Gary Krause, agriculture experiment station statistician, we designed the appropriate statistical analysis of our data to determine the statistical significance and reliability of our counting procedure. As expected, we were generally counting too many plates per experiment and more than double the number of counts per sample in order to have statistical significance at the 0.5 percent level.

Based upon microscopic examination of conidial morphology and size, the viability of the conidia (percent germination), the quantity of conidia produced, the ease of media preparation, the cost of the bean substrate, and the ready availability of the beans used in the medium, we have selected lima bean agar at the 20g/liter level for our conidial production.

Personal communications with other plant pathologists led us to test the effect of UV light on the quality and quantity of conidia produced on our lima bean medium. Both lima bean and commercial PDA plates were grown at 25-28°C under UV lights. One-third of the plastic petri plates were wrapped in aluminum foil following inoculation; one-third were placed under glass, and one-third were left uncovered. After repeated experiments using both D. carbonum and D. maydis, which also included the use of glass petri plates, we determined that there was a critical requirement for light for the production of conidia. The foil-covered plates yielded few, if any, conidia. The plastic petri plates (UV transparent), grown under UV light, did not yield as great a quantity of conidia as those grown under glass (UV opaque). A large percentage of the conidia grown under UV in plastic plates appeared to be abnormal. Petri plates, both plastic and glass, grown under fluorescent light, produced normal conidia in quantities equal to the best results obtained under UV or any other condition. The percentage of conidia which germinated was identical, both for those produced under white and those produced under UV light. This observation may be a result of the fact that normal conidia are multicellular and can germinate from more than one cell; whereas the conidia produced under UV light had few cells and therefore may have had fewer absolute numbers of germ tubes produced with equal numbers of conidia.

Dr. Arthur Karr, a plant pathologist in the Department at the University, suggested that the sterol, lanosterol, may improve the production of conidia of D. maydis. We have tried repeatedly, but have failed to increase conidial production of D. maydis with this sterol.

During this period of test and development of a conidial production medium, we have also built and tested other instruments that we need for harvesting the conidia from the petri plates. We designed, built and tested a petri plate media drier. This device, which resenbles a fruit drier, allows us to dry down the spent agar substrate in fifty 15cm plates. To facilitate the physical removal of the conidia from the dried agar medium, we designed and tested a small cyclone collector which uses glass scintillation vials as the collection chamber. This device allows us to aseptically vacuum the conidia off of the dried agar surface. The final cyclone collectors were built for us by the University of Missouri instrument shop.

Current trial runs with D. carbonum have demonstrated that growing the fungus for 14 days at 26-28°C under white light in 15cm glass plates, with the lima bean agar medium, yielded approximately 0.4-0.45 grams of conidia per four plates. This yield represents an approximate 60-fold increase in conidial production over the medium currently being used for the growth and conidial production in other laboratories. Cultures of D. maydis have consistently yielded fewer conidia during all of our development work, and, at present, yield approximately 1/6 the quantity of conidia as compared to D. carbonum.

II. Selective media for the differentiation of Drechslera maydis from Drechslera carbonum.

As a result of our development work in 1982, we had found that a multi-well petri plate differential media identification system may be a valid short-term answer to the problem of a clinical/field technique for distinguishing between these two related fungi. In February, Dr. Oscar Calvert and I were able to distinguish, with 100 percent accuracy, between 6 different biotypes of D. carbonum and 6 biotypes of D. maydis. We employed a 6-well culture plate with the following substrates to identify the two fungi: lima bean agar, potato dextrose agar, Bacon's medium, Harold's medium, Sabouraud maltose agar, and tryptone glucose extract agar. Even though we had 100 percent success in distinguishing between the two fungi, we did not feel this method was currently usable as a clinical or field technique, because it was too difficult, took too much time for us to read, and an individual with considerably less expertise would find it impractical to use.

Other substrates evaluated in the multi-well plate system were fava bean agar, azuki bean agar, split pea agar, navy bean agar and V-8 Juice agar. None of the above substrates or substrates in combination produced a system which we felt was sufficiently easy and accurate for field personnel to use.

We are currently producing and evaluating a new substrate made by incorporating the toxin produced by the "T" race of D. maydis into our different bean agar media. There is research literature to support the observation that the toxin from race "T" can inhibit the growth of D. maydis, but not D. carbonum, when it is incorporated back into the growth substrate.

ADDENDUM

This has been the first complete year of operation of the survey methods development facility, located on the University of Missouri campus, and of the Cooperative National Plant Pest Survey and Detection Program (CNPPSDP) itself. The CNPPSDP is still in its infancy, from an operational standpoint, and is yet developing both the definitive direction drawn from past years of experience, and the adequate technical support and expertise so necessary to reach its goals. Therefore, in response to these critical technical needs, survey methods development has temporarily adopted the policy of directly supporting more applied and operationally technical needs of the CNPPSDP and postponing some of our ongoing basic biologically-oriented work. The financial, physical and manpower constraints survey methods development is operating under has necessitated that we carefully reevaluate our priorities in view of the current critical operational needs of the CNPPSDP. We have identified four major areas of survey and technical development.

The first is the continuation of the work on D. carbonum and D. maydis. We feel that the work has progressed to the point where significant results can be obtained in the forseeable future on the development of a clinical/field immunological method of identifying and separating these two important fungal corn pathogens. Our short term goal for FY-84 will be to increase our production of conidia and use these conidia for the initial development of the immunological assay.

The other three new major technical projects will directly address immediate critical needs of CNPPSDP and fall under the heading of computer/data/survey technical needs. The first project is PESMID, which is a pest survey standardization methods information data base for use as part of the CNPPSDP computer network. Dr. Paul Teng, University of Minnesota, and I have begun a cooperative development program for creating, testing and putting into place the PESMID system. This database will contain information dealing with survey and detection methodology as it relates to plant pests. PESMID will have two subsystems: user-generated unpublished methods, and published literature on methods. The approach Dr. Teng and I have adopted is to set up a pilot system with corn as the host in order to pinpoint and correct potential faults in the system. Implementation of the pilot system is scheduled for FY-84. In its final form, PESMID will permit a system user to examine the current literature on survey and detection methods and compare the actual survey techniques employed by any state as they relate to any host-pest combination. We hope PESMID will be the foundation upon which survey methods standardization will be built by the states and will lead to better vis-a-vis computer-telecommunications of survey data between different locations.

The second project is the creation of a coding system for host and pest name entry into the national computer system. Following the report by Wilson-Hill, independent consultants, on recommendations as to the type and acquisition of a database management system for use by the CNPPSDP, I will generate a technical position paper. This paper will permit the program director and his staff to examine different options prior to initiation of construction of the total coding system.

The third project for FY-84 is to supply information and guidance to the CNPPSDP staff on the needs for and types of historical data bases for the national survey program. I will be producing a position paper early in FY-84 describing the categories of historical data bases, the rationale for their use, and the critical needs the historical data bases can serve. If it is decided to initiate any or all of the historical data bases described in the position paper, the survey methods development facilities will undertake the initial development of this project.

Project Number: AW 1.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasitoid Redistribution Program
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: Philip C. Kingsley

INTRODUCTION

During this, the third year of a planned 5-year project, a fifth area, Nebraska (6 sites, 5 fields/site) was added to the evaluation survey (figure 1). Field samples collected in Nebraska were processed at the Kansas State Board of Agriculture Laboratory in Topeka, where subsamples were then sent to the APHIS laboratory in Niles, Michigan for dissections.

Samples from all 5 areas were collected and processed in accordance with those procedures described in the 1983 Evaluation Handbook and the first two AW 1.1.1 interim reports.

RESULTS AND DISCUSSION

Personnel at the Niles Laboratory dissected 41,418 AW adults and larvae from this year's evaluation samples, compared with 33,076 in 1981 and 20,155 in 1982. These weevils were dissected in order to determine the percentage of adults and larvae parasitized and, where possible, make parasitoid species determinations. In many cases, however, identification of parasitoid larvae is impossible at the time of dissection. For this reason, and where adequate numbers allowed, we reared adult parasitoids from the field-collected weevils. For example, 13,992 Bathyplectes anurus and B. curculionis were reared from field-collected AW larvae in 1983.

Because of a relatively dry spring (in most areas) and more persistant surveying, far fewer missing samples were recorded this year and may account in part for the increased number of dissections. In Nebraska, however, a wet spring resulted in a late start and numerous missing samples. Due to this late start and an excess number of days (normally 7) between sampling periods, all 30 fields were either cut and/or sprayed by the 6th survey week, as can be seen in figure 2. In addition, errors in sampling procedures may have led to an underestimate of AW populations in some sites. This would occur if larval densities were in excess of 300/100 sweeps and the surveyor discontinued counting at 300 (the maximum to be shipped), rather than continuing to count all larvae collected in those 100 sweeps. With better weather, better communication channels and a year's experience, these problems should be cleared up next year in Nebraska.

Ten new county records for AW parasitoids were recovered from evaluation samples, including new State records in Nebraska for B. anurus and Microctonus aethiopoides.

The phenological trends of AW adult and larval populations are presented for each area in figures 2-5. Clearly, in Missouri, Iowa and Illinois (areas I and II), populations have remained low compared to the numbers recovered in 1981. This can also be seen in table 1, where the mean peak larval densities for each area are given, along with the number of AW parasitoid species per site, averaged by area, and the adult and larval parasitism rates (calculated from all weevils dissected from each area).

Two species of parasitoids have shown a clear westward movement in terms of increasing parasitism rates (figure 7) and field recoveries (figures 8-12), and may account in part for the population declines mentioned above. The AW adult parasitoid, M. aethiopoides, has become far more common in Missouri and Iowa. This is indicated by an increase in the percentage of fields where the parasitoid was recovered during the first 3 survey years - 35.3 percent, 65.0 percent and 92.9 percent, respectively. This correlates with a similar increase in the percent parasitism by M. aethiopoides in area I, from 2.3 percent in 1981 to 13.2 percent in 1982 and 16.5 percent in 1983.

Bathyplectes anurus is also showing clear westward movement, but differs from M. aethiopoides in that it is apparently displacing a previously established congener, B. curculionis. Where both species occur sympatrically, parasitism rates eventually favor B. anurus. This is the case in Pennsylvania and New Jersey (area IV, figures 7,12), where both species are present in nearly all fields; but parasitism by B. anurus is more than 20 times that of B. curculionis. In Ohio (area III), where B. curculionis has been established since the early 1970's, B. anurus has steadily increased its presence over the 3 survey years, from occurring in 37.9 percent of the evaluation fields in 1981 to 43.3 percent in 1982 and 56.7 percent in 1983 (figure 11). This westward movement can also be seen on a per site basis in figure 13, where the relative number of each Bathyplectes species reared is indicated. Similarly, parasitism by B. anurus has increased from 3.3 percent to 7.1 percent and 9.3 percent during the first three years, respectively, with a corresponding change in B. curculionis rates of 7.7 percent, 15.9 percent and 6.4 percent (figure 7).

Bathyplectes anurus has also begun to move into Illinois (area II). Although parasitism rates are still very low (.08 percent in 1983), B. anurus now occurs in 18.5 percent of the Illinois evaluation fields, compared with no recoveries in 1981 and 3.6 percent in 1982 (figure 10).

Along with weevil populations, the number of evaluation fields sprayed for AW control has also declined over the last 3 years (figure 14). No fields were treated for AW in the 2 eastern areas in 1983.

Further detailed information regarding parasitism rates, AW population trends and parasitoid species distribution on a per site and weekly basis, can be found in 3 annual data reports compiled here at the Otis Methods Development Center. In addition, 3 years of economic data such as pesticide and control costs, management practices and grower attitudes have been collected from the evaluation cooperators. These data are presently being analyzed by Michael Duffy of the Economic Research Service.

TABLE 1. Three-year area summary for peak larval densities of the alfalfa weevil, percent parasitism, and parasite species diversity.

Area	Mean (SE) Peak Larval Density (#/100 Sweeps)			Mean (SE) Number of Parasite Species/Site		
	1981	1982	1983	1981	1982	1983
O NB			262.3ab (26.5)			1.5a (0.22)
I MO, IA	619.5b[1] (105.0)	193.3ab (36.2)	351.7b (56.1)	1.7a (0.21)	2.3a (0.21)	2.3a (0.33)
II IA, IL	846.3b (199.3)	251.5ab (59.0)	207.0a (33.8)	2.2a (0.17)	2.5a (0.33)	2.5a (0.34)
III OH	92.1a (22.3)	97.5a (22.9)	161.8a (8.4)	3.5b (0.22)	3.8b (0.31)	3.5b (0.34)
IV NJ, PA	218.1a (39.9)	381.7b (87.9)	171.1a (34.8)	4.5c (0.29)	4.0b (0.26)	3.8b (0.17)

Area	Adult Parasitism (Percentage)			Larval Parasitism (Percentage)		
	1981	1982	1983	1981	1982	1983
O NB			0.04c			24.c
I MO, IA	2.3a	13.2a	16.5a	14.4c	18.1a	13.8c
II IA, IL	39.1b	35.9c	35.1b	8.1a	40.0c	29.0d
III OH	32.0b	26.6b	18.3a	11.6b	26.0b	15.7b
IV NJ, PA	26.2b	32.6bc	19.1a	30.9d	26.3b	27.1cd

1. Values in columns followed by the same letter are not significantly different at the 95 percent 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.

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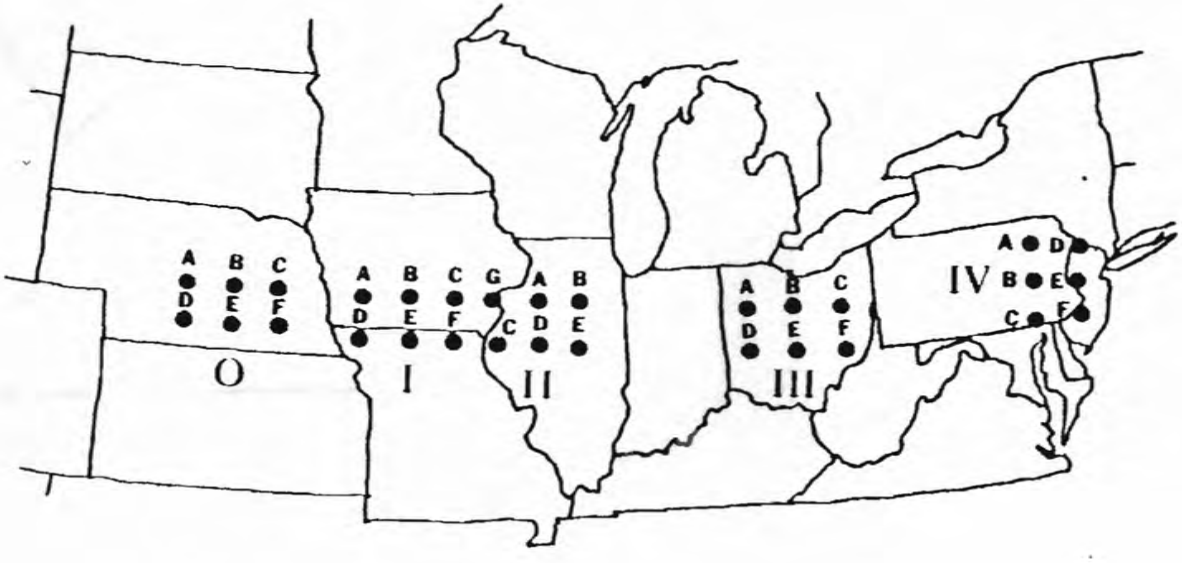

AW Evaluation Sites

Figure 1.

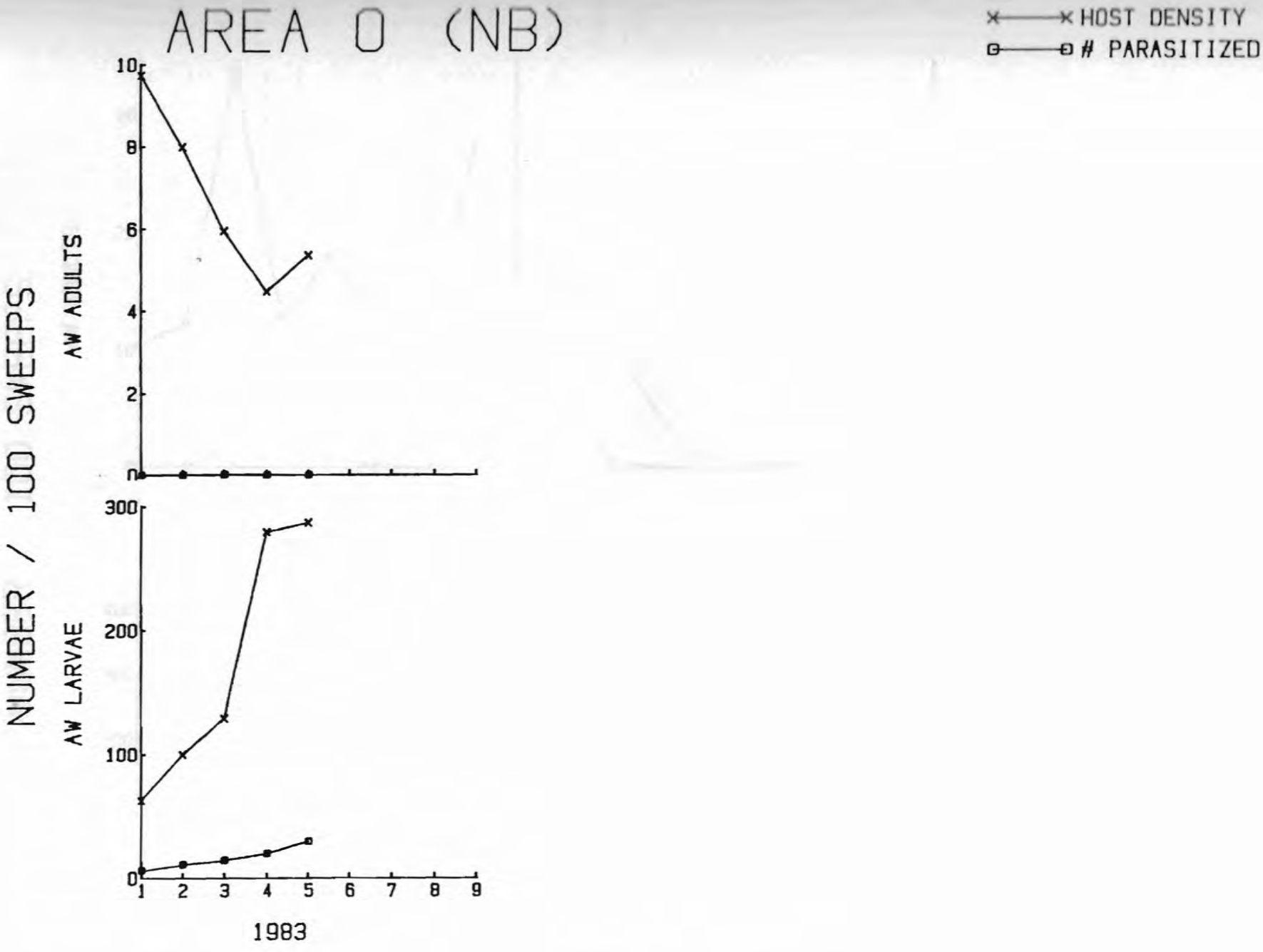
AREA O (NB)
HOST DENSITY
PARASITIZED
NUMBER / 100 SWEEPS
AW ADULTS
AW LARVAE
0
2
4
6
8
10
100
200
300
1
2
3
4
5
6
7
8
9
1983
SAMPLE WEEK

Figure 2.

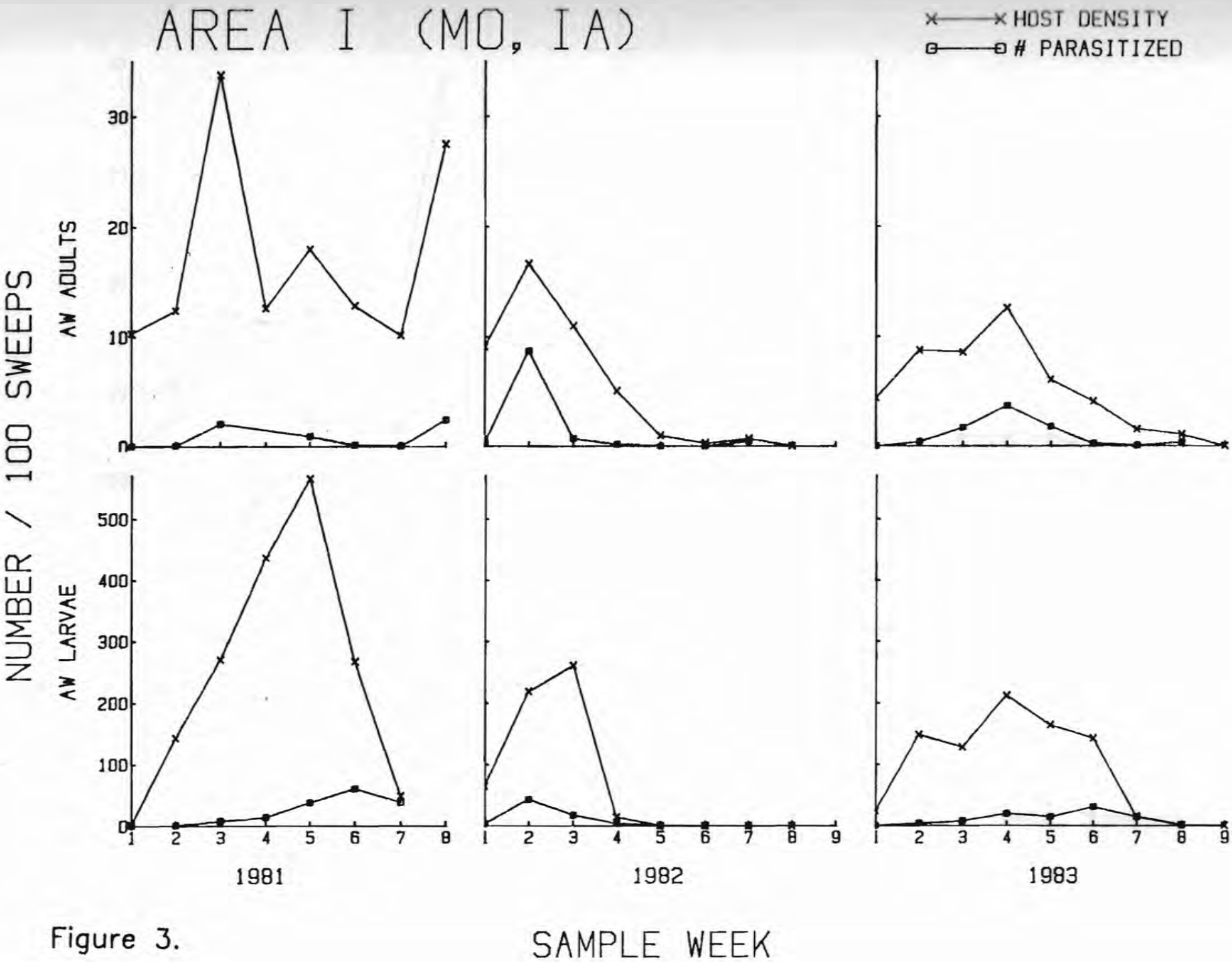
AREA I (MO, IA)
HOST DENSITY
PARASITIZED
NUMBER / 100 SWEEPS
AW ADULTS
AW LARVAE
1981
1982
1983
SAMPLE WEEK

Figure 3.

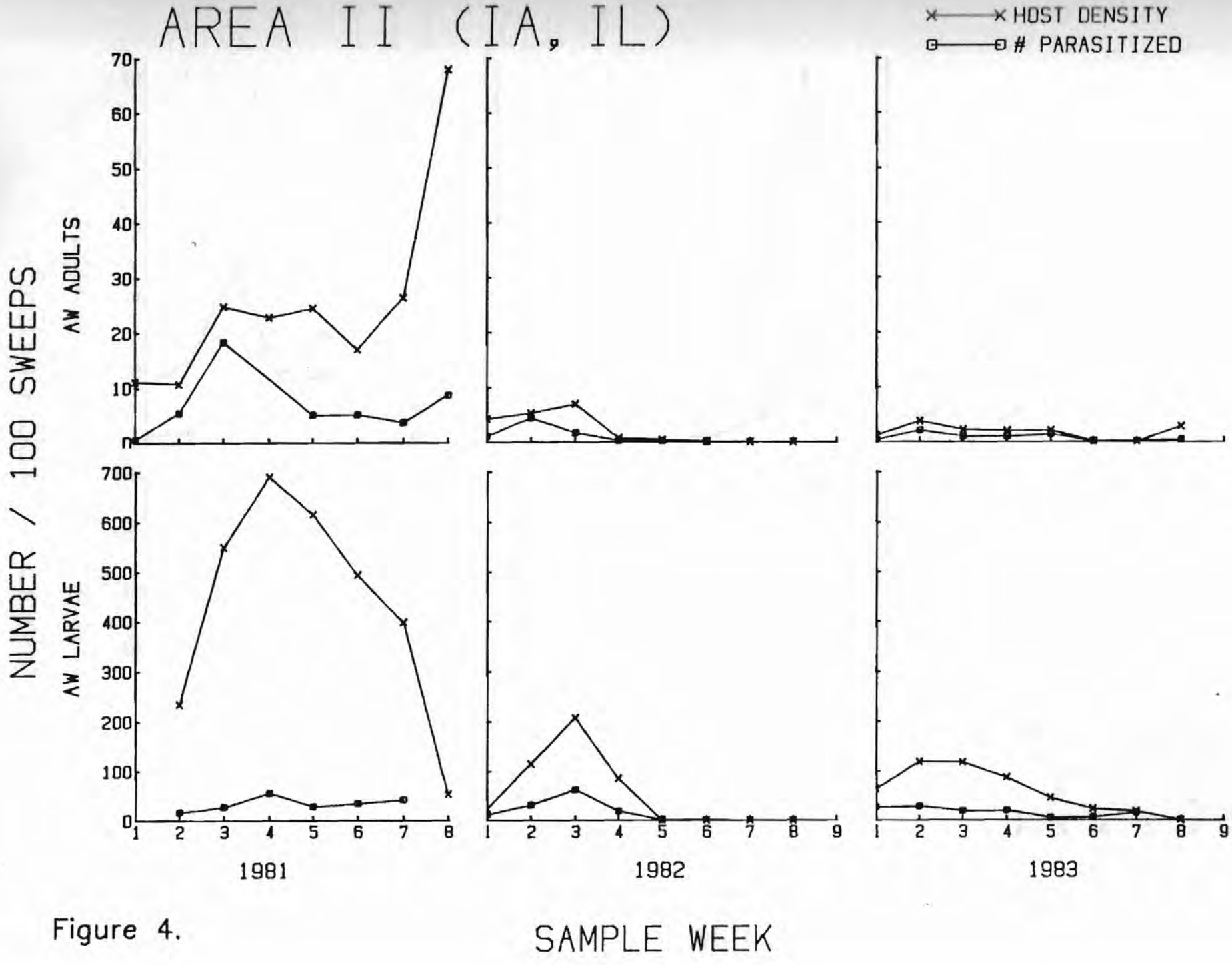
AREA II (IA, IL)
HOST DENSITY
PARASITIZED
NUMBER / 100 SWEEPS
AW ADULTS
AW LARVAE
1981
1982
1983
SAMPLE WEEK

Figure 4.

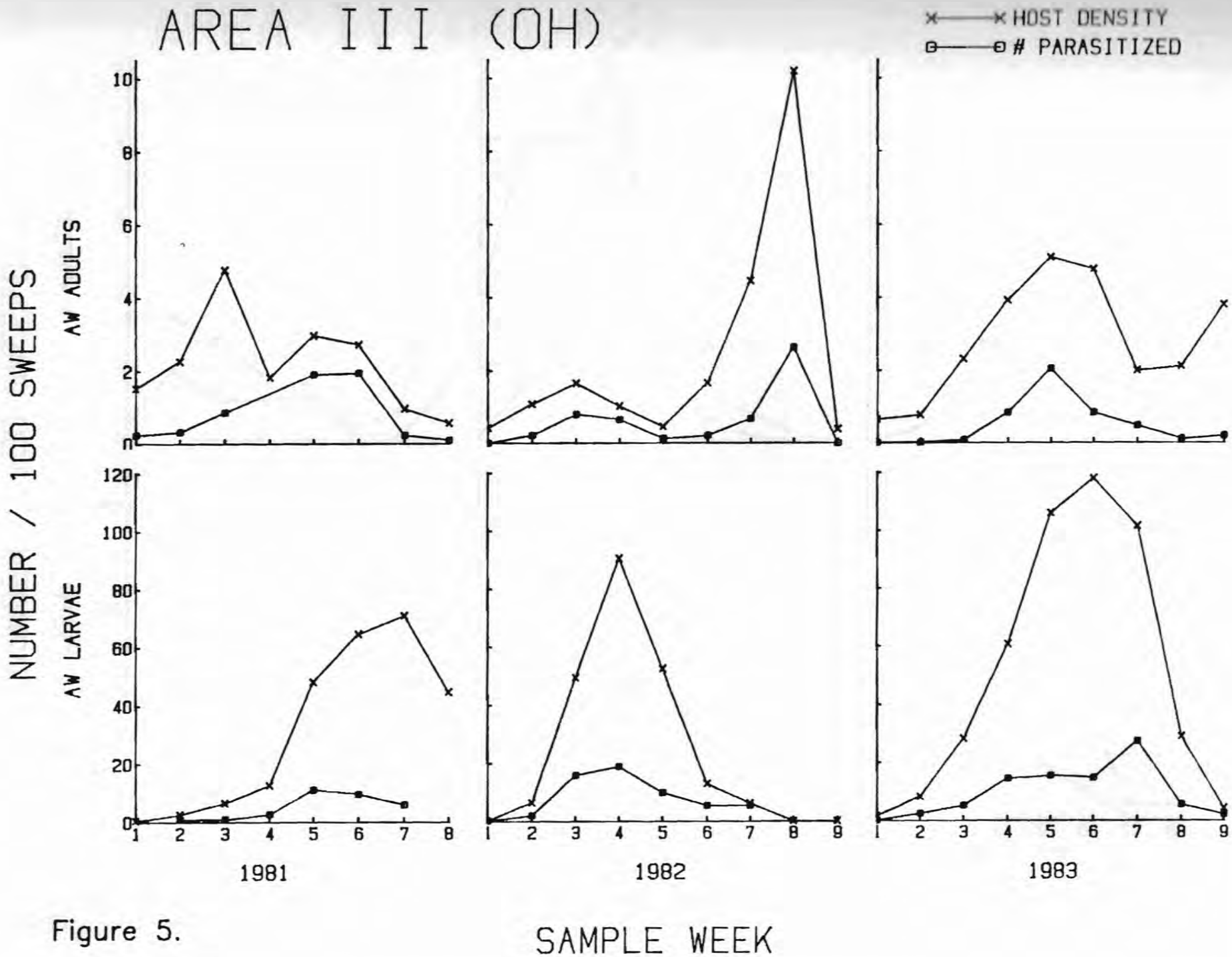
AREA III (OH)
HOST DENSITY
PARASITIZED
NUMBER / 100 SWEEPS
AW ADULTS
AW LARVAE
1981
1982
1983
SAMPLE WEEK

Figure 5.

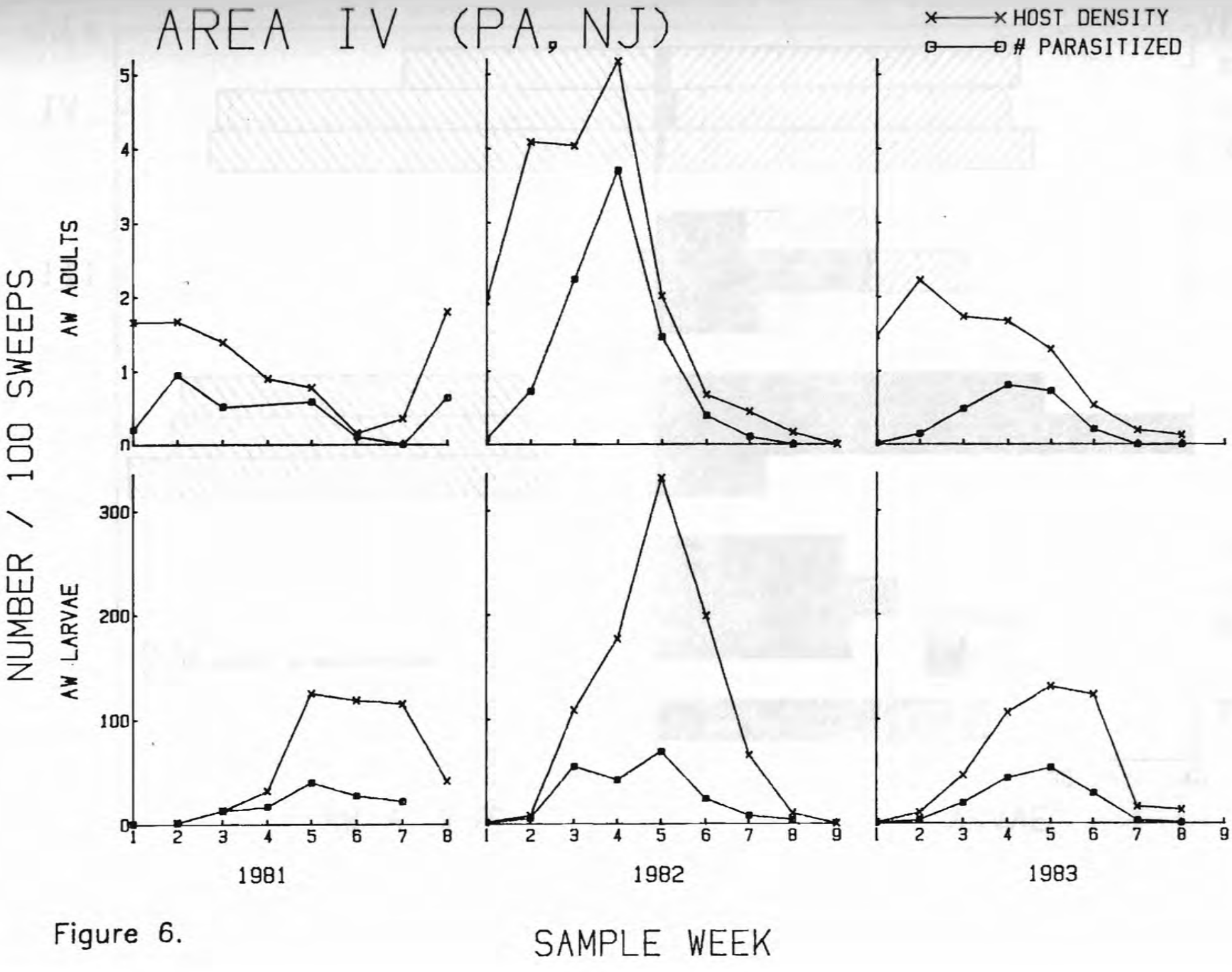
AREA IV (PA, NJ)
HOST DENSITY
PARASITIZED
NUMBER / 100 SWEEPS
AW ADULTS
AW LARVAE
1981
1982
1983
SAMPLE WEEK

Figure 6.

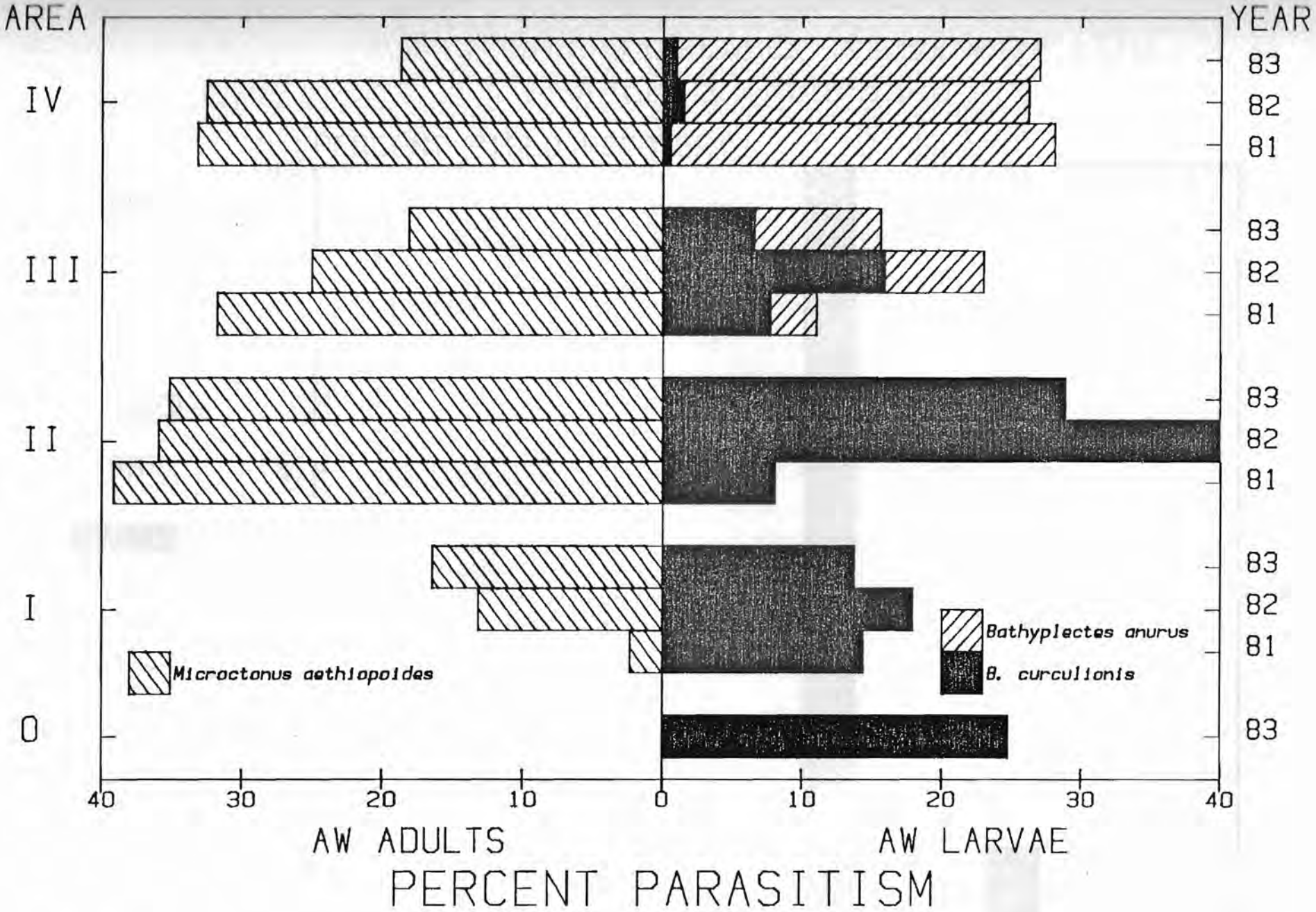
AREA
YEAR
IV
III
II
I
O
83
82
81
Microctonus aethiopoides
Bathyplectes anurus
B. curculionis
40
30
20
10
0
AW ADULTS
AW LARVAE
PERCENT PARASITISM

Figure 7.

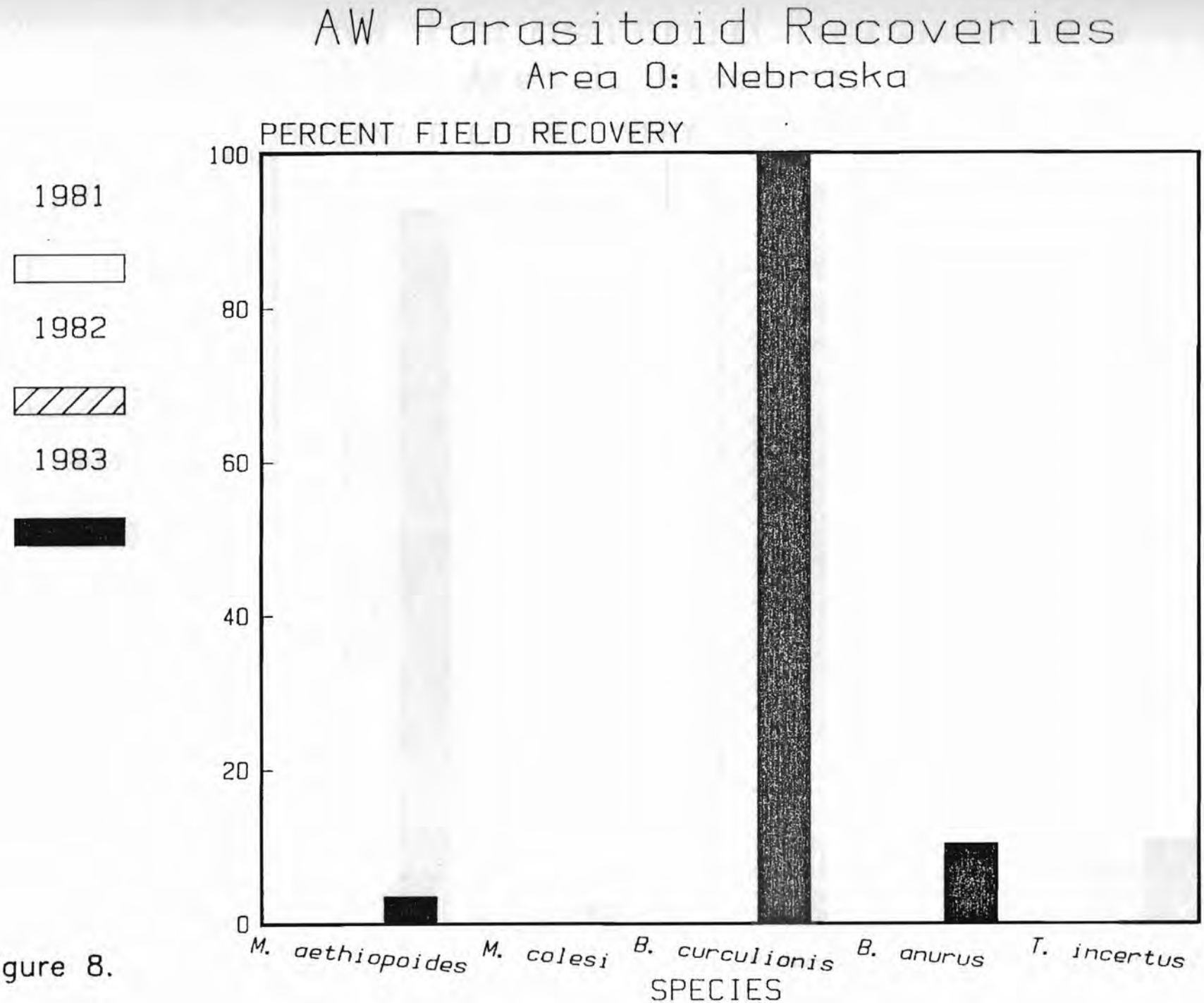
AW Parasitoid Recoveries
Area O: Nebraska
PERCENT FIELD RECOVERY
100
80
60
40
20
0
1981
1982
1983
M. aethiopoides
M. colesi
B. curculionis
B. anurus
T. incertus
SPECIES

Figure 8.

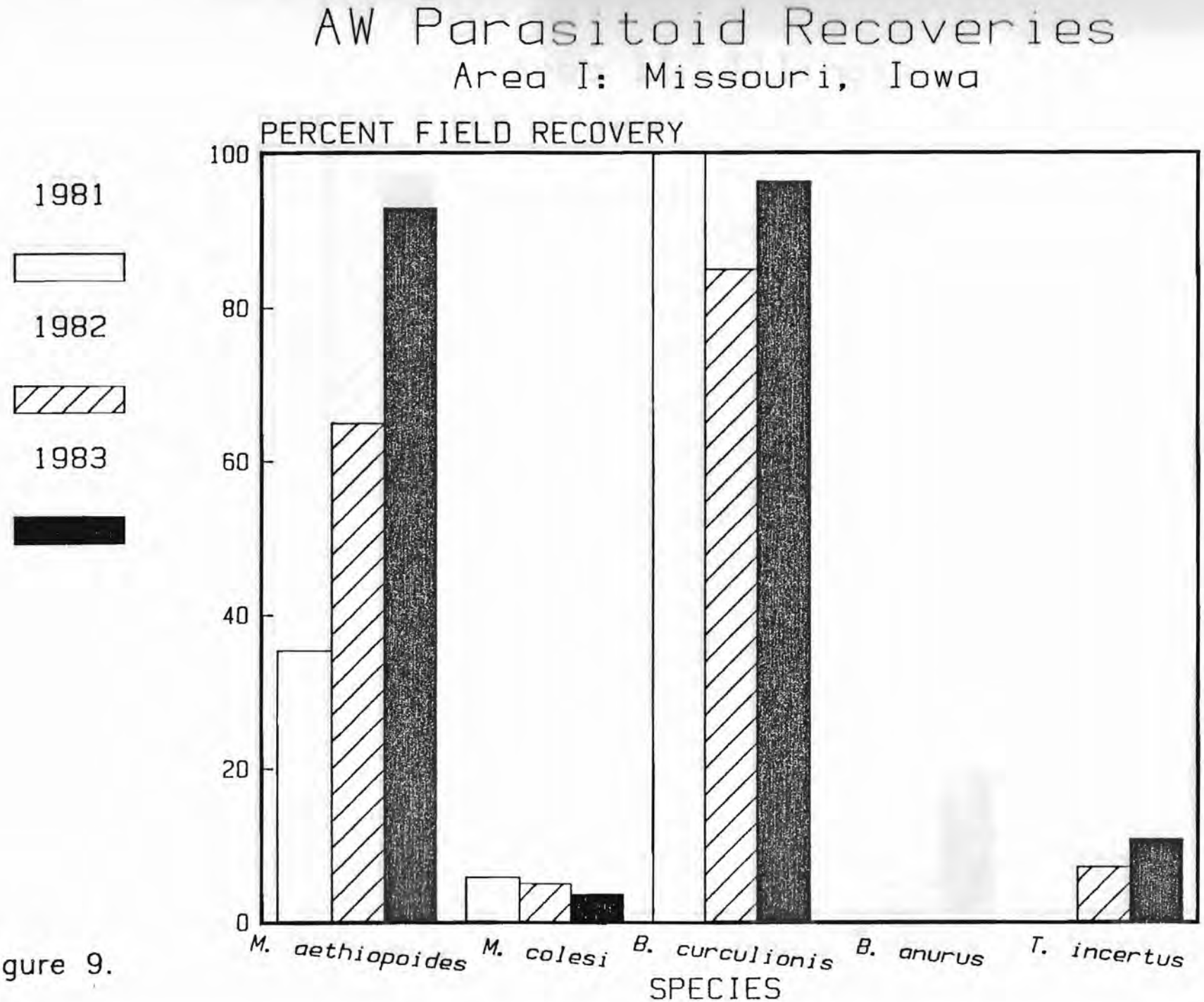
AW Parasitoid Recoveries
Area I: Missouri, Iowa
PERCENT FIELD RECOVERY
100
80
60
40
20
0
1981
1982
1983
M. aethiopoides
M. colesi
B. curculionis
B. anurus
T. incertus
SPECIES

Figure 9.

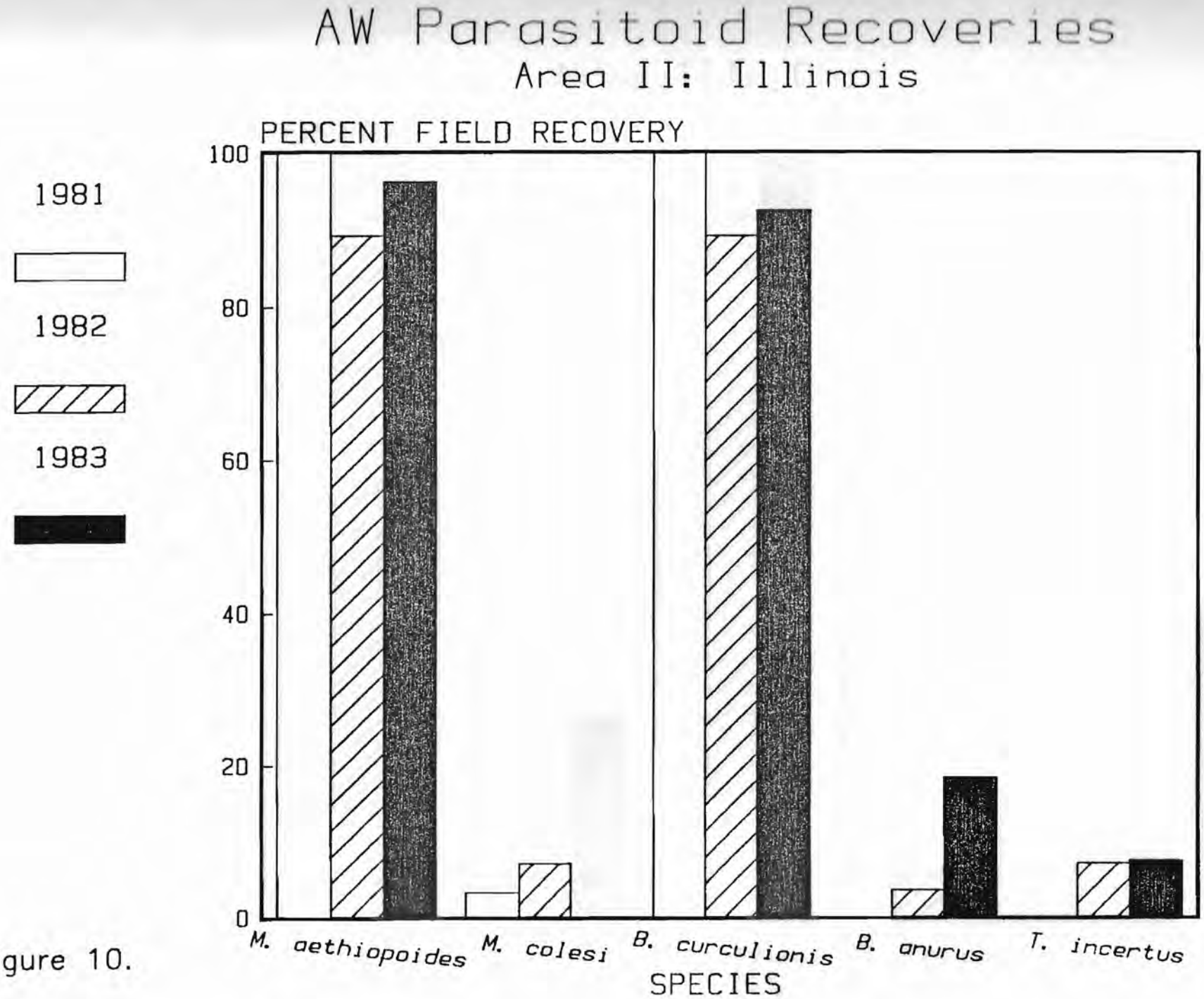
AW Parasitoid Recoveries
Area II: Illinois
PERCENT FIELD RECOVERY
100
80
60
40
20
0
1981
1982
1983
M. aethiopoides
M. colesi
B. curculionis
B. anurus
T. incertus
SPECIES

Figure 10.

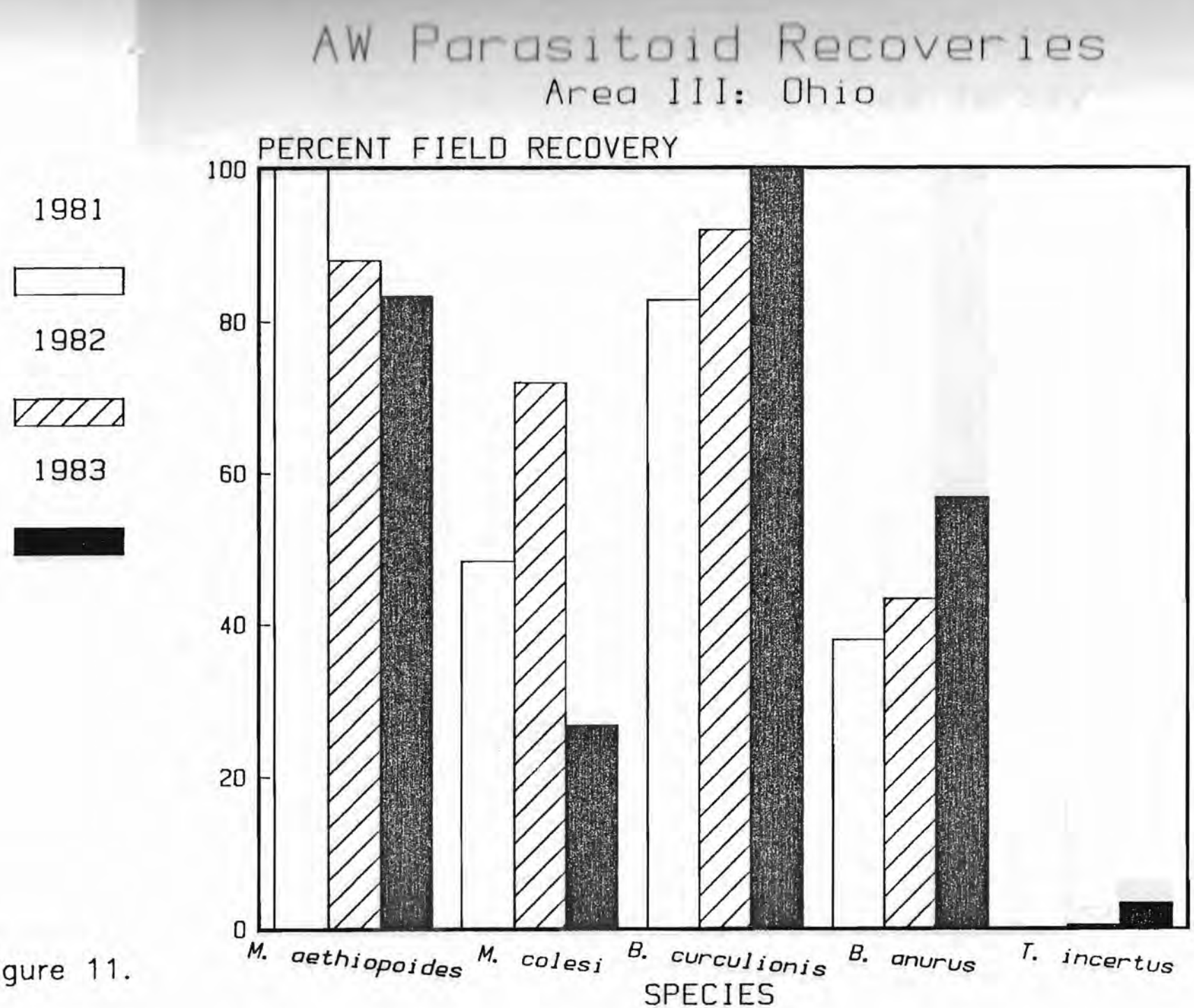
AW Parasitoid Recoveries
Area III: Ohio
PERCENT FIELD RECOVERY
100
80
60
40
20
0
1981
1982
1983
M. aethiopoides
M. colesi
B. curculionis
B. anurus
T. incertus
SPECIES

Figure 11.

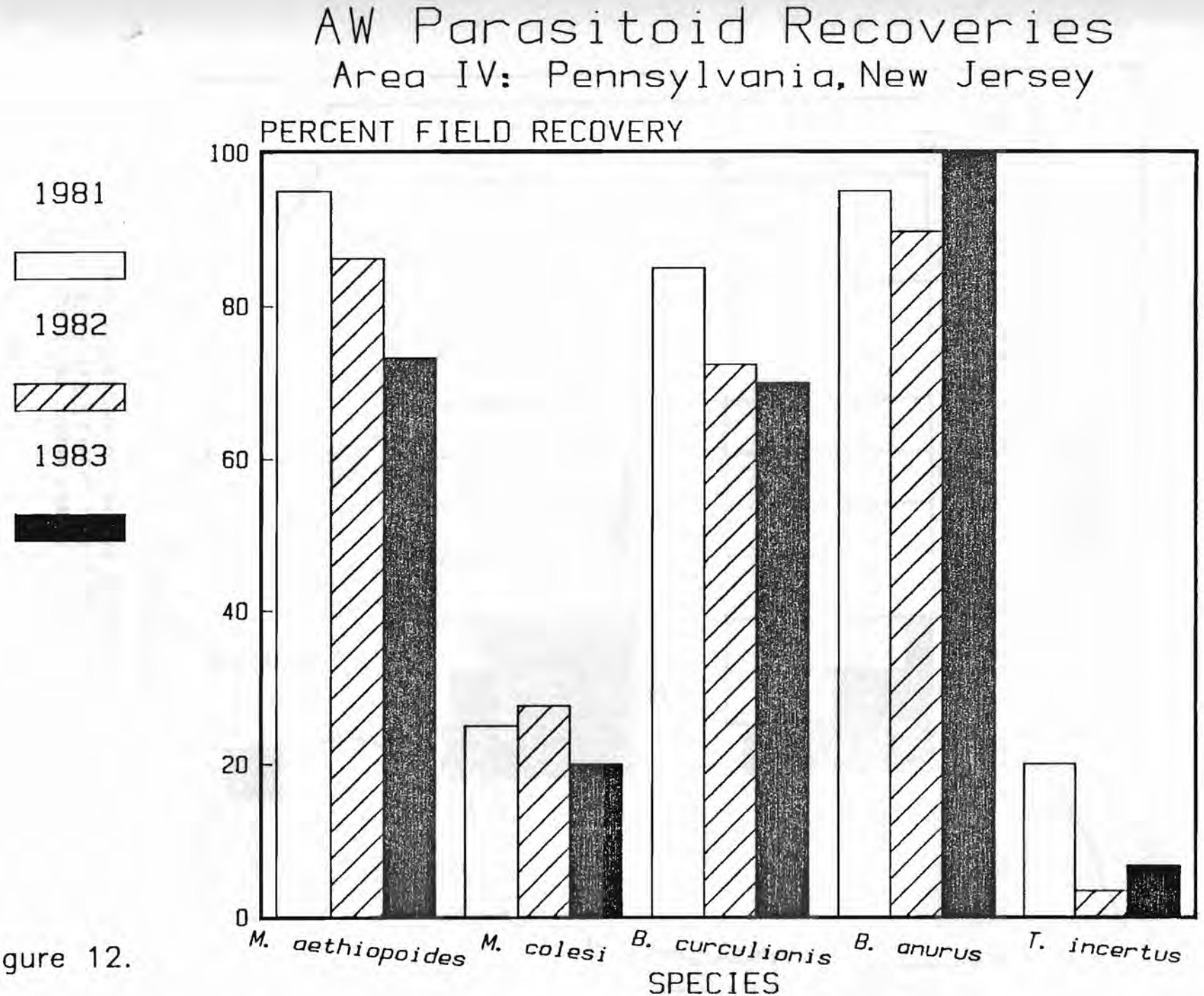
AW Parasitoid Recoveries
Area IV: Pennsylvania, New Jersey
PERCENT FIELD RECOVERY
100
80
60
40
20
0
1981
1982
1983
M. aethiopoides
M. colesi
B. curculionis
B. anurus
T. incertus
SPECIES

Figure 12.

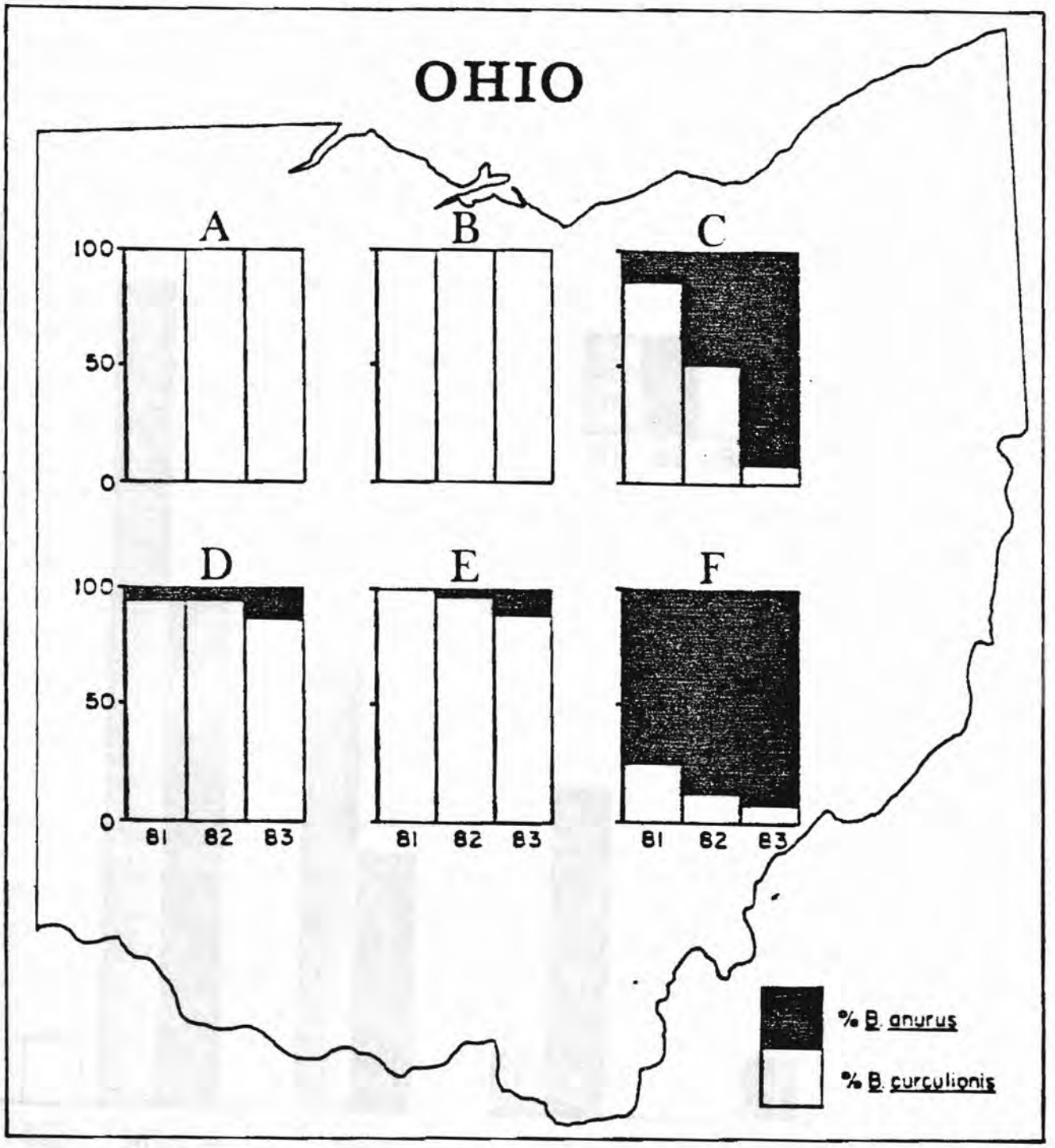

Figure 13. Relative frequencies of the two AW parasitoids Bathyplectes anurus and B. curculionis reared in six AW evaluation sites in Ohio, by year. The total number reared in 1981, 1982 and 1983 was 1495, 2432, and 2426, respectively.

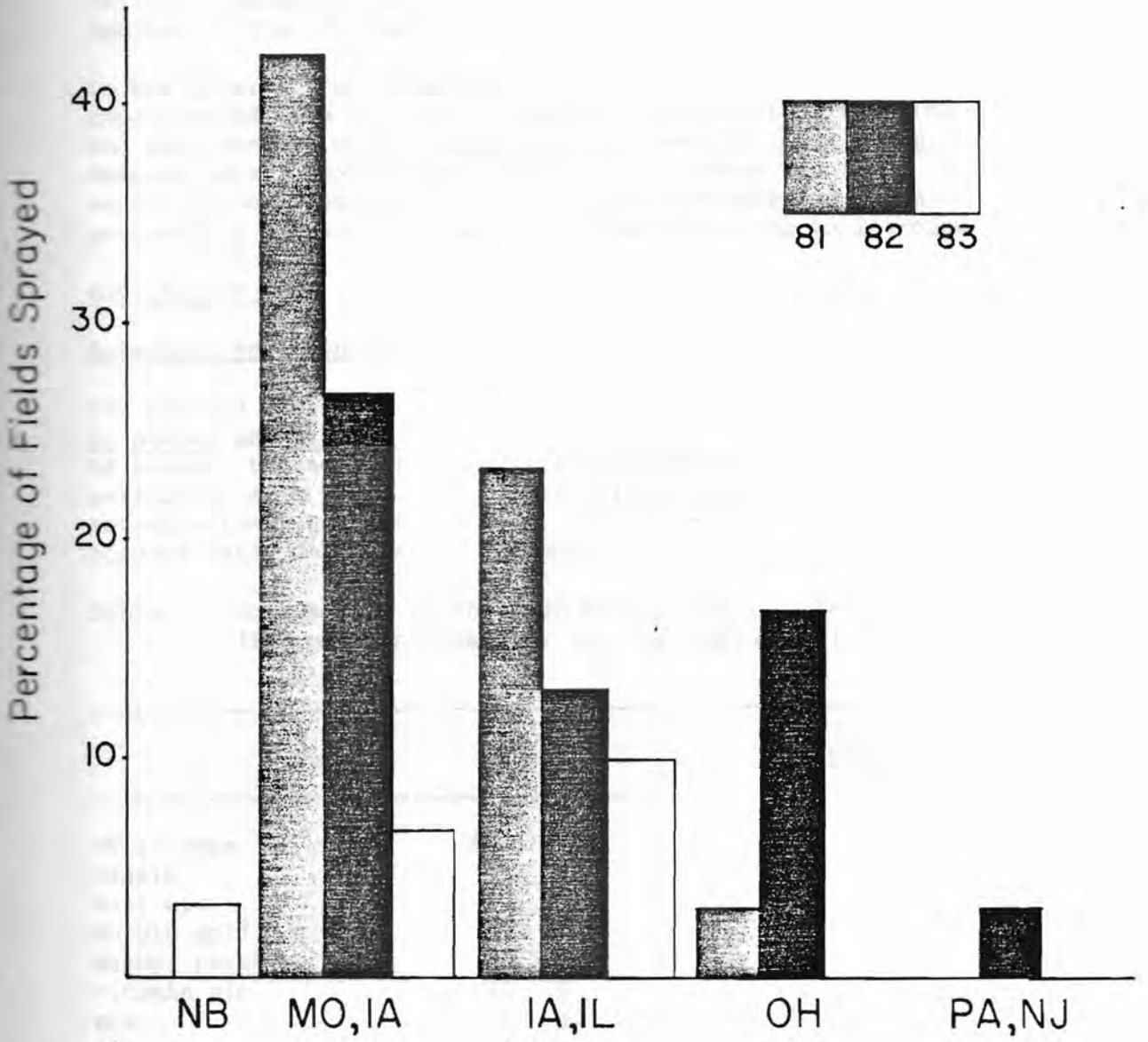

Figure 14. AW EVALUATION Percentage of fields sprayed for alfalfa weevil control by year and area.

Project Number: AW 2.1.1
Project Title: Alfalfa Weevil Rearing
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: P. C. Kingsley

Introduction

As a continuation of our work in developing an artificial diet and diet containerization, to be utilized in the mass distribution and recovery monitoring of parasitized AW larvae (see AW Rearing Interim Report AW 2.1.1., 1982), we conducted several experiments studying the effects on two parasitoid species of diet-fed hosts.

In the first of two experiments to be described here, we tested an antibiotic, incorporated into the diet to reduce contamination, in terms of development and survivorship of *Bathyplectes anurus* and *B. curculionis* (Hymenoptera:Ichneumonidae) reared on AW larvae fed diet. The second experiment was designed to test a diet substrate and container in the mass movement of parasitized field-collected AW larvae to introduction sites.

Antiobiotic Test

Materials and Methods

Six day old (third instar) alfalfa-fed AW larvae were exposed to mated female *B. anurus* and *B. curculionis* parasitoids at a host:parasite ratio of 10:1 for 24 hours. Larvae were then distributed among three diet treatments: alfalfa, artificial diet (Table 1), and artificial diet plus .00125 percent (.039g) tetracycline. After three days, all larvae were transferred to alfalfa and checked daily for parasite cocoons.

Table 1. Composition of the high wheat germ diet for the alfalfa weevil. (For more information, see the 1982 AW 2.1.1 interim report.)

			Percent of total diet
Wheat germ	420	g	13.6
Casein	87	g	2.8
Salt mix	28	g	0.9
Sorbic acid	7	g	0.2
Methyl paraben	3.5	g	0.1
Vitamin mix	35	g	1.1
Agar	53	g	1.7
Alfalfa powder	63	g	2.0
Water	2400	ml	77.5

Results:

Overall survival, (that is, AW pupae plus parasitoid cocoons divided by AW larvae set-up) was 28.1 percent (24 + 93/417), whereas overall parasitism, or parasite cocoons divided by cocoons plus AW pupae, was 79.5 percent (93/117). This low survival might be due to the daily disturbance of larvae when checking for parasite cocoons, as survival after three days on the diet treatments was 87.5 percent (365/417).

Although there were no significant differences between treatments in terms of parasite cocoon yield, developmental time to cocoon, or length of cocoon (Table 2), a similar trend indicating a detrimental effect of artificial diet on yield is worth noting. Additional information will be collected from the F1 adult parasites, including sex ratio, fecundity and survival.

TABLE 2. Percent yield, developmental time, and length of parasite cocoons resulting from host larvae fed three diets[1].

Parasite Species	Treatment	Percent Yield[4/]	Mean (SE) Development[5/] (Days)	Mean (SE) cocoon length (mm)
Bathyplectes anurus	Alfalfa	33.8 (23/68)	11.3 (0.37)	3.13 (.042)
	Diet[2/]	25.4 (18/71)	12.4 (0.46)	2.98 (.054)
	Diet plus tetracycline[3/]	19.4 (14/72)	12.2 (0.37)	3.00 (.054)
Bathyplectes curculionis	Alfalfa	22.1 (15/68)	11.7 (0.30)	3.05 (.082)
	Diet	18.3 (13/71)	12.5 (0.37)	3.07 (.052)
	Diet plus tetracycline	14.9 (10/67)	12.3 (0.45)	3.05 (.056)

1/ No significant differences were found between treatments at the 95 percent level of confidence. (Chi-square, Duncan's multiple range test).
2/ See Table 1 for diet ingredients.
3/ .00125 percent of diet ingredients.
4/ # of cocoons/# of larvae set up on treatments.
5/ # of days from parasite oviposition to cocoon at 23° C.

Field Test

Materials and Methods

On May 25, in Cass County, Michigan, we initiated an experiment designed to test the use of an artificial diet in the mass movement of field-collected, parasitized AW larvae to introduction sites. In the field, larvae were mass collected by APHIS personnel at the Niles laboratory and placed on an artificial diet (modified high wheat germ diet with .00125 percent tetracycline incorporated, see Table 1) in one-gallon waxed milk cartons. The diet had been sparingly applied to an excelsior substrate in order to allow adequate circulation. Two densities were tested: 1,000 and 3,000 larvae per gallon carton, and alfalfa was used as a control. Two cartons were set up for each density and diet, except for the high density artificial diet treatment which had an additional replicate. The nine cartons were placed in a large "freezer-safe" box with three blue ice "freeze-pacs". The following day, May 26, the larvae were flown to Otis where we took two subsamples of 200 larvae from each carton for rearing. These larvae were placed in 1/6 bushel paper bags and supplied with indoor-grown alfalfa until pupation. The number of resulting adult weevils and parasite cocoons was recorded. On June 7, in Barrien County, Michigan, a second test at a higher density of 9,000 larvae per gallon was run. In addition to the artificial diet and alfalfa treatments, a blank (escelsior only) treatment was also included. Three cartons were set up for each treatment and outdoor alfalfa was used for rearing. It should also be noted that the larvae collected on June 7 were almost entirely in the fifth stadium, whereas those from May 25 were fourth and fifth instars. Results are given below in Table 3.

Table 3.

Density	Treatment	Percent Parasitism	Percent Survival
May 25, Cass County, Michigan			
1000	Diet	48.0 (190/396)a[1]	49.5 (396/800)b
1000	Alfalfa	49.2 (191/388)a	48.5 (388/800)b
3000	Diet	45.6 (306/675)a	56.3 (675/1200)a
3000	Alfalfa	46.5 (174/374)a	46.8 (374/800)b
June 7, Barrien County, Michigan			
9000	Diet	22.3 (204/916)x[1]	76.3 (916/1200)x
9000	Alfalfa	20.3 (155/763)x	63.6 (763/1200)y
9000	Blank	23.6 (219/928)x	77.3 (928/1200)x

1 Those frequencies followed by the same letter are not significantly different at the .05 percent level (Chi-square test).

At the time of each collection, a subsample of 50 larvae was taken to determine, by dissection, the percent parasitism in the field. These rates were 46 percent and 36 percent for May 25 and June 7, respectively. When these values are compared to those determined by rearing, the high density (9000/gallon) parasitism rates were significantly lower.

Parasitism was not affected by diet, although the total number of parasites produced was. At the two higher densities, overall survival was significantly better on the artificial diet treatments. Survival was also higher on the blank than on the alfalfa, suggesting that, in the ultimate instar at least, there may be no benefit derived from the diet. A detrimental effect of the alfalfa diet seems clear, however. This was apparent when the cartons were received at the lab. Those larvae on alfalfa were wet and still, most probably due to inadequate ventilation, whereas those on the other two treatments were dry and active. Additional data will be collected from the parasites as they break diapause.

Conclusions

Under the conditions of the laboratory test, artificial diet was apparently detrimental to parasitoid survival to cocoons. Diet in this experiment was poured in diet cups, whereas diet in the field test was applied to an excelsior substrate and may be a major factor in increasing the suitability of the diet to the AW larvae. Also, exposure to diet in the first experiment was during the third and early fourth stadia, whereas in the field test, exposure to the fourth and fifth AW larval instars.

Project Number: AW 3.1.1
Project Title: AW Strain Identification
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leaders: P. C. Kingsley, J. Romero-Andreas

Introduction

The Animal and Plant Health Inspection Service is presently conducting a large scale biological control program against the alfalfa weevil, *Hypera postica*. Parasites are being distributed from eastern states to states further west where the parasites do not occur. The preferred method for parasite redistribution, based on efficiency and cost, is to move mass numbers of field collected parasitized hosts to various locations in the release state. An alternate method is to establish insectaries in the release states, using reared parasites, and subsequently distribute parasites from these insectaries.

Two strains (eastern and western) of the alfalfa weevil, *H. postica*, occur in the United States. In addition to *H. postica*, the Egyptian alfalfa weevil, *H. brunnipennis* has also become established in western areas of the United States. The ranges of these populations, to a large extent, are not well defined. This is primarily due to the fact that these strains and species, although biologically different, cannot be separated morphologically. Electrophoresis techniques may provide a practical means for their identification.

The fact that there are three closely related weevil biotypes established in the United States complicates the parasitized host method of redistribution. The ramifications of moving parasitized, and a small percentage of unparasitized, eastern strain weevils into areas where only western or Egyptian populations occur are unknown. Understandably, some western states have been reluctant to release potentially unparasitized host material into their areas.

Methods

At the onset of this project (February, 1983) the electrophoresis techniques for the identification of AW strains were not as yet perfected, although several researchers were optomistic that such isozymes would soon be identified.

Under this assumption, and because of the urgent need for the resulting information, we initiated a sample collection program. Up to 200 "new" (not overwintered) AW adults were collected from each of several sites in states west of the Mississippi by PPQ personnel and their cooperators. The number and location of these sites were based on the states' alfalfa acreage, varying climatic zones or cropping practices, and possible geographically isolated weevil populations.

Results

Over 10,000 weevils were collected from 94 western and south-central region sites (Figure 1). In addition 2400 weevils were collected from 18 eastern sites. All weevils were sent to the University of Wisconsin where they were immediately frozen and stored for electrophoresis.

Through a cooperative agreement with the University of Wisconsin we are developing electropheretic methods to differentiate these three weevil groups.

After testing numberous enzyme stains on weevils from the Northwest and East, it was apparent that certain stains (Lactate dehydrogenase and Glucose-6-phosphate dehydrogenase) may be effective in separating strains. Upon further studies, however, it has become clear that the physiological state of these adult weevils may play an important role in the observed differences. Investigations were continued using stock cultures from Wisconsin where reproductive and non-reproductive weevils were screened. These results indicate that earlier stains, which look promising as strain identifiers, may be unreliable because of the unknown physiological state of these field collected adults.

Work is continuing in an effort to find enzyme stains useful in the identification of biotypes.

1983 Collection Sites for the Alfalfa Weevil Strain Identification Project

Figure 1.

Project Number: MBB 1.1.1
Project Title: Determining the Optimum Temperature for Storing Larval *Pediobius foveolatus*
Report Period: October 1, 1982 - September 30, 1983
Report Type: Final
Project Leaders: Patricia J. Coughlin, Othel T. Forrester

Final experiments in this project have been concluded and will be reported in the future.

Project Number: MBB 1.1.4
Project Title: Direct Release of Pediobius foveolatus into Soybean Fields for Control of Mexican Bean Beetle
Report Period: October 1, 1982 - September 30, 1983
Report Type: Interim
Project Leader: O. T. Forrester

No work was undertaken on this project because the availability of Pediobius foveolatus could not be assured at the proper release time.

Project Number: MBB 2.1.1
Project Title: Dispersal of Pediobius foveolatus from Standard Nurse Plots
Report Period October 1, 1982 - September 30, 1983
Report Type: Final
Project Leader: Othel T. Forrester

Introduction

The growing season of 1983, the second and final season of this study, was marked by the worst drought in 15-20 years. Soybean yields were reduced by 35 to 40 percent in most areas, and many of the study fields appeared more typical of late fall than mid-summer. Generally, Mexican bean beetle populations were low and bean leaf beetle populations were high.

The objective of this study is to obtain information on the dispersal of Pf from standard nurse plots (50' x 100') into surrounding soybean fields. The data obtained from these tests will be used to estimate the soybean acreage protected by a standard nurse plot.

Materials and Methods

Standard nurse plots planted with a mixture of 1/3 soybeans and 2/3 snap beans were established in Fayette, Highland, Pickaway, Pike and Ross Counties through late April and early June. Nurse plots were checked at weekly intervals until 50 percent or more of the MBB larvae observed were second instar or older. A single Pf release was made in each plot one or two days after release criteria were met. Release ratios were determined by use of the formula in the release ratio test of 1980. Pf used in the study were supplied by the Niles, Michigan, satellite facility and the New Jersey Department of Agriculture.

Two weeks after Pf release, the first 25 mummies encountered in each nurse plot were collected and individually held for eclosion in #000 Lilly gelatine capsules. Yield and sex ratio for each of the collected mummies were recorded. Nurse plot production was estimated by multiplying the number of row feet in the plot times the average number of mummies in one row foot times the average number of Pf per mummy.

Dispersal of Pf from the nurse plot to the adjacent soybean field was determined by random sampling of two 3 row foot sections of soybeans at 1/16, 1/8, 3/16 and 1/4 mile intervals.

Six concentric zones were established at 1/4 mile intervals from the center of the nurse plot to determine Pf dispersal to surrounding soybean fields (Figure 1). Zones 2 through 5 were divided into quarters on the north-south and east-west axes. Zone 6 was divided into eighths, and one soybean field in each eighth was selected for sampling. All sample sites were intersected by or very close to the 1/4 mile radii lines. Sample selection was the same as that used in the field adjacent to the nurse plot. Distance from the nurse plot was determined with a Model 400 Rototape measuring wheel and with map locations. All sites were sampled weekly.

Results and Discussion

Nurse plot planting began the last week in April and was completed the first week of June. Cool, wet weather caused the extended planting period. Poor stands and abundant weeds were the norm this season. Weed control is not essential to a successful nurse plot but it is important as a source of weed seeds for several years and not acceptable to most farmers.

Pediobius release criteria were met in Pike, Pickaway I, Ross, Pickaway II and III, Fayette and Highland on June 28, July 2, July 7, July 7, July 19, July 23, and July 23, respectively.

Host counts on trigger date for Pike, Pickaway I, Ross, Pickaweay II, Pickaway III, Fayette and Highland were 341, 261, 297, 176, 238, 95 and 43 larvae per 15 row feet, respectively.

Estimated Pf production for the Pike, Ross, Pickaway I, II and III nurse plots was 227,000, 83,300, 207,600, 88,560 and 198,100, respectively. Pf production was not estimated for Fayette and Highland because for some reason poor Pf establishment was experienced there.

Dispersal from the Ross County nurse plot was observed at 1/4 mile after two weeks, 3/4 mile after 8 weeks, 1 mile after nine weeks and 1 1/2 miles after eleven weeks (Table 1A).

Dispersal from the Pike County nurse plot was observed at 1/8 mile after two weeks, 1/4 mile after three weeks, 3/4 mile after seven weeks and 1 1/2 miles after eight weeks (Table 1B).

Dispersal from Pickaway County nurse plot I was observed at 1/2 mile after two weeks and 1 1/2 miles after eight weeks (Table 1C). Dispersal from Pickaway County nurse plot II was observed at 3/4 mile after three weeks, and 1 1/2 miles after ten weeks (Table 1D). Dispersal from Pickaway County nurse plot III was observed at 3/16 mile after two weeks, and 1/4 mile after ten weeks (Table 1E).

The Ross County nurse plot was planted in late May and partially replanted in mid-June because of groundhog damage. Estimated Pf production was 83,300; this is nearly six times lower than the 1982 estimated production. The probably cause for this difference is the lower host population at trigger date (297 larvae per 15 row feet in 1983, and 639 larvae in 1982). Release ratio was 80X in both cases.

Dispersal rates in 1982 were compared with only two data points, four and eight weeks after Pf release. Dispersal was observed at 3/4 mile after four weeks and 1 1/2 miles after eight weeks. In 1983, dispersal was checked weekly. Dispersal was observed at 1/4 mile after two weeks, 3/4 mile after 8 weeks, 1 mile after 9 weeks, and 1 1/2 miles after 11 weeks.

Mexican bean beetle populations were low during the five weeks after Pf release; this was a major factor, I think, in the slower rate of dispersal. MBB populations increased eight to ten weeks after Pf release, but only in localized areas that remained green. The final sample was a destructive sample, collected on September 22 at 3/4 mile northeast and northwest, and 1 1/2 miles southwest of the nurse plot. All soybeans in two sections of three row feet each were cut. All larvae, pre-pupae and pupae were collected and held individually in #000 gelatine capsules for three weeks. The percent parasitism for northeast and northwest was 30 and 67.6 percent, respectively; the percent parasitized at 1 1/2 miles southwest was 87.7.

Defoliation levels did not exceed the economic threshold in any of the fields checked in the 1983 season. In 1982, all fields in this area were above the economic threshold and all were sprayed for MBB by August 4.

The Pike County nurse plot was planted in late April. The stand was poor and partial re-planting was required. Estimated Pf production in 1983 was 227,000; this is 7.5 times lower than the 1982 estimated pf production. Population density in 1982 was 709 larvae per 15 row feet, and 341 larvae per 15 row feet in 1983. The lower Pf production in 1983 was probably caused by a lower host density . Release ratio was 80X in both cases.

Dispersal from the nurse plot was slower in 1983 than in 1982. Dispersal was not observed beyond 1/4 mile until eight weeks in 1983. In 1982, dispersal was observed at 3/4 mile after four weeks, and 1 1/2 mile eight weeks after release. The slower dispersal rate may be caused by the lower host population in 1983.

MBB populations were low the first week after Pf release with very few stages observed outside of the nurse plot. Populations increased in the western half of the study the second week after Pf release, and were lower the third week after release. Populations also increased 7 through 9 weeks after release, also in the western half. The tenth week after release, was marked by a population collapse. Field scouting was terminated ten weeks after release.

Defoliation was higher in 1982 than in 1983. In 1982, the economic threshold was exceeded throughout the study area by early August. In 1983, the economic threshhold was exceeded only in the southwest quadrant in four fields 3/4, 1, 1 1/4 and 1 1/2 miles from the nurse plot. The soybeans in this area were planted ten ~~to~~ twelve days earlier than the remainder of the study area; this may be the reason for the increased defoliation.

The primary pest in this area in 1983 was the bean leaf beetle. Three hundred acres of soybeans were sprayed to reduce pod feeding.

All three nurse plots in Pickaway County were planted during the last week of May. Estimated Pf production for Plots I, II and III were 207,600, 88,500 and 198,000, respectively. The estimated Pf production in 1982 was 113,000. This cannot be compared to 1983 production because all plots were well removed from the 1982 plot; however, the mean production of 1983 plots is reasonably close to the 1982 estimated production (137,800).

Dispersal from the 1982 nurse plot was not observed at four weeks. Dispersal was observed from 1/2 through 1 1/2 miles eight weeks after Pf release. Dispersal from Plot I was observed at 1/2 mile after 2 weeks, 1/1/2 miles after 8 weeks, was observed after Pf release. Plot II dispersal was observed at 3/4 mile after three weeks, and at 1 1/2 miles after ten weeks. Plot III dispersal was observed at 3/16 mile after two weeks, and at 1/4 mile after ten weeks.

Economic levels of defoliation were not observed in any of the Pickaway County study areas during the 1983 growing season. The major pest of this season was the bean leaf beetle. Late season pod-feeding went largely unnoticed by most growers because of the drought symptoms.

Estimated Pediobius production in the 1983 nurse plots was substantially lower than the estimated Pf production in the 1982 nurse plots. The mean number of Pf produced per mummy in 1982 and 1983 was essentially the same, 20.8 and 19.4, respectively. The major difference in nurse plot productivity appears to be availability of host material. The mean MBB larval population per 15-row feet in 1982 was 523, compared to 263 in 1983. The mean estimated Pf production in 1982 nurse plots was 780,945, 4.8 times higher than the mean estimated Pf production of 1983. Factors influencing the nurse plot population differences are: cool wet spring in 1983, later planting dates, and some population suppression from the 1982 releases. These results support the results of the release ratio study. Nurse plots with high host counts are most efficient with respect to Pf production.

The late season parasitism observed in 1982 was not as obvious in 1983. High percent parasitism (87.7) was observed 1 1/2 miles from the nurse plot in only one area, Ross County. The most important factor appears to be the weather. Weather conditions in 1983 were not favorable for development of high MBB populations. The cool wet spring delayed nurse plot and soybean planting, and the hot dry summer lowered MBB survival. The reduced availability of host material also appears to have slowed dispersal and parasitism.

The 1983 season was not a favorable year for Mexican bean beeetle or Pediobius foveolatus.

Table 1A. Percent MBB parasitized in Ross County.

Weeks after Pf release	Nurse Plot	Distance from nurse plot (mi)								
		1/16	1/8	3/16	1/4	1/2	3/4	1	1-1/4	1-1/2
2	19.9	0.0	*	*	0.0	0.0	0.0	0.0	*	0.0
3	32.1	*	*	*	100	0.0	0.0	0.0	*	0.0
4	100	-	-	-	*	*	*	0.0	*	*
5	100	*	*	*	*	*	*	*	*	*
6	*	-	-	-	0.0	*	*	0.0	0.0	0.0
7	*	-	*	-	32.6	*	0	0.0	*	*
8	14.3	-	10.2	-	6.7	*	5.9	*	*	*
9	14.3	-	0.0	-	4.5	0.0	10.0	2.4	*	*
10	0.0	-	-	-	7.1	5.0	0.0	0.0	-	-
11	-	-	-	-	71.4	-	52.0	-	-	87.7

* No suitable hosts observed.

Table 1B. Percent MBB parasitized in Pike County.

Weeks after Pf release	Nurse Plot	Distance from nurse plot (mi) 1/16	1/8	3/16	1/4	1/2	3/4	1	1-1/4	1-1/2
2	48.5	5.9	7.6	0.0	0.0	0.0	0.0	0.0	0.0	*
3	50.0	6.4	0.0	14.2	4.4	0.0	0.0	0.0	0.0	0.0
4	100	40.0	25.0	13.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	100	0.0	*	0.0	0.0	0.0	7.7	0.0	0.0	0.0
7	*	0.0	0.0	0.0	0.0	*	8.8	4.0	1.3	3.4
8	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
9	*	0.0	0.0	0.0	0.0	*	0.0	0.0	0.0	0.0

* No suitable hosts observed.

Table 1C. Percent MBB parasitized in Pickaway I.

Weeks after Pf release	Nurse Plot	Distance from nurse plot (mi) 1/16	1/8	3/16	1/4	1/2	3/4	1	1-1/4	1-1/2
2	44.0	*	*	*	*	*	*	*	0.0	0.0
3	78.6	*	*	87.5	57.0	43.0	*	*	0.0	0.0
4	100	*	*	*	0.0	*	*	*	*	*
5	*	*	*	*	*	*	*	*	*	*
6	*	*	*	*	0.0	*	*	*	*	*
7	100	*	*	*	*	*	*	*	*	*
8	*	*	*	*	*	*	*	*	*	*
10				Drougnt						

* No suitable hosts observed.

Table 1D. Percent MBB parasitized in Pickaway County II.

Weeks after Pf release	Nurse Plot	Distance from nurse plot (mi) 1/16	1/8	3/16	1/4	1/2	3/4	1	1-1/4	1-1/2
2	64.3	*	*	*	*	*	*	*	*	*
3	100	*	*	*	*	*	50.0	*	*	*
4	100	*	*	*	*	*	*	*	*	*
5	*	*	*	*	*	*	*	*	0	*
6	100	*	*	*	*	*	*	*	*	*
7			Drought							
8										
9										
11	-	-	-	-	-	-	-	-	-	42.0

* No suitable hosts observed.

Table 1E. Percent MBB parasitized in Pickaway County III.

Weeks after Pf release	Nurse Plot	Distance from nurse plot (mi)								
		1/16	1/8	3/16	1/4	1/2	3/4	1	1-1/4	1-1/2
2	60.9	100	100	25.0	*	-	-	-	-	-
3	42.9	-	*	*	*	*	*	*	*	*
4	71.4	-	0	*	*	*	*	0	*	*
5	0	-	0	*	*	*	*	*	*	*
6	-	-	0	-	*	*	0	0	*	*
7	*	-	1.2	*	0	*	0	*	*	0
8	-	-	-	-	21.0	-	0	-	-	-
9				Drought						

* No suitable hosts observed.