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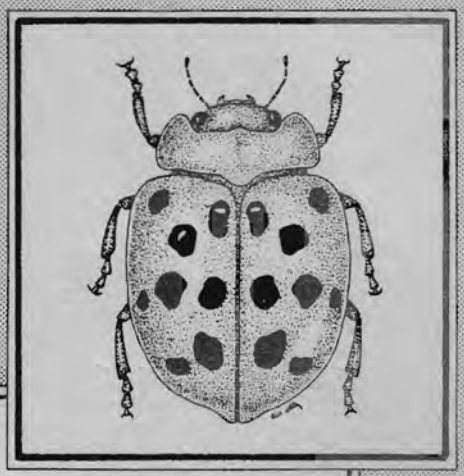
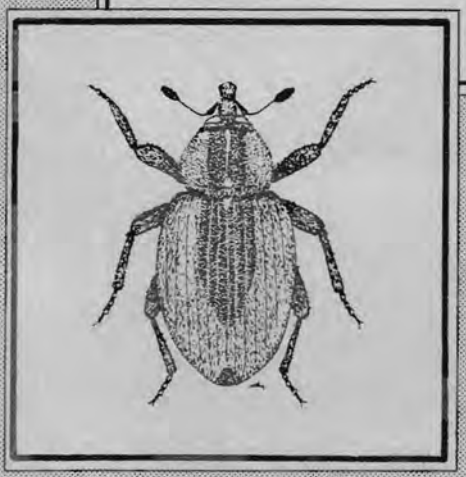


United States Department of Agriculture

Otis Methods Development Center

Progress Report

Animal and Plant Health Inspection Service



October 1, 1981 - September 30, 1982

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

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Summary

A number of products were tested for ovicidal effects on gypsy moth eggs, and, as had been observed previously, the excellent control achieved in laboratory tests was not consistently obtained in field evaluations. The products Spray and Kill (Dylox), Diazanon, Orthene, light water, linseed oil, mineral oil, pine oil, citrus oil, corn oil, soybean oil, castor oil and kerosene all prevented hatch in the laboratory. However, encouraging results were obtained with several plant oils that are presently undergoing rigorous field testing. Holding egg masses under anoxic conditions at room temperature for 1 hour also completely eliminated hatch and exposure to 50°C for 5 minutes also completely killed eggs.

A variety of synthetic pyrethroids were evaluated against larvae and were found to be very effective. These materials may have a role in the treatment of regulated items that are infested with larvae but which do not lend themselves to fumigation. Various tree wrapping or banding products were tested and were found to be marginally effective or useless.

Numerous tests concerning improving the weatherability of BT formulations with the addition of sticker were conducted. The stickers RA-1990, Rhoplex B60A, Rhoplex AC33MP and Polyco 2149 were found to be most effective. Without added sticker, Thuricide weathered better than Dipel. It was also shown that 16 BIU is more effective against large larvae than 8 or 12 BIU, suggesting that late season control can be obtained with BT by increasing the dosage. Protected from sunlight and rainfall, BT deposit on oak foliage retained its activity for at least 36 days, demonstrating the importance of protecting the deposit from rain and UV radiation in order to optimize field performance.

A number of tests were run with Avermectin B and it was determined that the LD50 is approximately 50 mg/acre. Formulations tested were not sensitive to UV radiation, but tended to wash from foliage when exposed to rain. Other screening tests were conducted with Zolone, neem extract, Methoxychlor, CGA-105623 (BT), CGA-112913 (growth regulator), BASF Lab 136733I (growth regulator), Depart RO-13-5223 (growth regulator). Various formulations of Sevin were evaluated with different stickers and it was found that Sevin 80S washes easily from foliage, but other formulations are more resistant to wash off. The stickers TS-85, TS-100, RA-1990 and Acrylocoat improved weathering characteristics.

Field tests conducted in 1981 and 1982 consistently showed that BT applied at the rate of 12 BIU's per acre prevented defoliation more effectively than lesser amounts of material. Additionally, only a single application was necessary to achieve foliage protection. Some reduction in egg mass density was also observed at the 12 BIU application rate.

Mist blower applications on 1 acre blocks demonstrated low dosages of the pyrethroid Pounce very effective in preventing defoliation. Avermectin, Sevin, SIR-8514 and Turcam were less effective when applied to small plots in areas of very heavy infestation.

Studies are underway to determine the likelihood of gypsy moth establishment and reproduction in Florida. Egg masses in various stages of development are being shipped to secure insectaries near Gainesville and hatch synchrony is being monitored along with host plant phenology and synchrony. Early indications are that reproduction can occur in Florida, but that the hatch period will be very broad.

Over the year, approximately 8,000,000 insects were reared and distributed to 15 projects and cooperators throughout the United States and abroad. Significant progress has been made in improving rearing efficiency. Supro-610 has been demonstrated to be a suitable replacement for casein and will be used in the future if casein costs become excessive. Significantly, a semi-automated dispensing machine has been developed whereby eggs are deposited directly into rearing containers. This substantially reduced labor cost from previous neonate infesting techniques, and was used successfully during the 1982 sterile male rearing program. Extensive preliminary testing and calibration tests resulted in implementation of the equipment with no compromise in production yield or insect quality.

The second full year of evaluation of the alfalfa weevil parasite redistribution program has been completed, and several interesting trends in parasite abundance are beginning to emerge. Nationally, Microctonus aethiopoides has been observed moving westward, and Bathyplectes anurus is apparently outcompeting B. curculionis for hosts in the eastern areas. Preliminary testing has been completed in the development of an artificial diet that can be used for sustaining parasitized larvae and adults during redistribution. A modified wheat germ-casein diet has proven effective and antibiotic screening has resulted in a diet formulation resistent to fungal contamination. Emphasis is now being placed upon diet substrates which are suitable for feeding large numbers of insects in small shipping containers.

Mexican bean beetle field studies were concluded which demonstrated that maximum Pediobius foveolatus production from nurse plots is attained with release ratios of 10-20 parasites per host counted in 3 row feet of beans at certain host densities. The release formula must be increased with increasing host counts for optimum parasite yield. Field tests in Ohio demonstrated that Pediobius foveolatus parasitism increases to near 100% as far as one mile away from release sites late in the season, and it is theorized that significant reduction of overwintering MBB populations results from parasitization. Accordingly, greatest benefits from parasite release may be realized the year following parasite release.

Pediobius foveolatus has been successfully used to control Mexican bean beetle in small plantings of green beans by direct field release. These trials eliminated the need for pesticide application for MBB control.

A new program has been initiated in the Center which deals with development of techniques useful for identifying plant pathogens. Initial efforts are directed towards using selective media for identifying fungal pathogens of corn. Immunological assays are to be developed for virus, fungal and bacterial diseases of corn. Finally, a system is being formulated to standardize survey procedures that are used nationally by cooperators in the National Plant Pest Survey Detection Program, such that reported data are comparable from state to state.

PESTICIDE TESTING SECTION

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APPENDICES

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Project Number: GM 6.1.5
Project Title: Regulatory Treatments
Report Period: October 1, 1981 - September 30, 1982
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 have 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.

Testing was conducted with Spray-N-Kill, a new material for treatment of gypsy moth egg masses. This formulation consists of Dylox (trichlorfon) as tne active ingredient. The material is sold in 8 ounce containers and is to be used with a hand-held or pressure-type garden sprayer. The recommended dosage is 2 ounces per gallon of water. For best results it is recommended that egg masses be sprayed in April or shortly before hatch.

Tests were conducted with 1, 2 and 4 ounces of material per gallon of water. Three rates of application were used, light, medium and heavy. Five, ready to hatch, egg masses were treated with each dosage and rate with a hand-held sprayer. Five untreated egg masses were used as a check.

Following treatment, egg masses were incubated at 27°C for 5 days. On the 5th day hatch was counted and recorded. No attempt was made to compute percentage of hatch.

Table 1. Results of gypsy moth egg mass treatments with Spray-N-Kill.

	Total number of neonates after 5 days		
Dosage	Light spray	Medium spray	Heavy spray
1 oz/gallon	290	0	0
2 oz/gallon	1	1	0
4 oz/gallon	0	0	0
Check	3,453		

Bacto-Zyme was tested against all gypsy moth larval instars by direct application from a spray can. The material used in the following tests was diluted further (as indicated) from a 1 x 30 aqueous stock provided by Mr. Gus DiRoma. A minimum of 10 laboratory reared larvae of each instar were treated with light, medium, heavy and extra heavy dosages. Mortality readings were made shortly after treatment and, again, 24 hours later. No treatment had any effect on 6th instar larvae. Mortality occurred with early instar larvae at the heavy and extra heavy dosages. Most of this mortality was a direct result of the test insects being nearly covered with material and suffocation most likely occurred. The data indicate that the material would be ineffective as a larval spray unless very large amounts were applied.

A number of egg masses were treated with Bacto-Zyme at various dilutions. Egg masses were treated with an eye dropper until each mass was completely saturated. Eggs were then incubated for hatch. Five egg masses were treated with each dilution. Five untreated masses were used as a check.

Table 2. Hatch from 5 Bacto-Zyme treated gypsy moth egg masses.

Dilution	Total hatch
1 x 1	529
1 x 5	323
1 x 10	1,412
1 x 20	1,246
1 x 30	927
1 x 40	1,838
1 x 50	1,501
Check	1,919

Some hatch reduction occurred with dilutions of 1 x 1 and 1 x 5. However, control was not sufficient to warrant additional testing.

Using an oak seedling bioassay technique, Bacto-Zyme was tested as a stomach insecticide. Seedlings were treated in a laboratory spray chamber with various dilutions of the material. Shortly after treatment, 2nd instar larvae were placed onto treated foliage and allowed to feed and move about. Five plants (20 larvae per plant) were used at each dilution. Five plants were used as a check. Mortality readings were made at 24 hour intervals after introduction of test insects to plants.

Table 3. Bioassay results of Bacto-Zyme sprayed onto foliage.

Dilution	Percent mortality				
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	168 Hrs
undiluted	12	23	28	34	47
1 x 1	10	17	22	25	27
1 x 5	6	12	19	20	23
1 x 10	6	27	27	27	31
1 x 20	4	11	16	25	24
1 x 30	14	19	25	30	54
1 x 40	12	19	22	26	44
1 x 50	24	30	32	35	54
Check	5	8	10	11	16

The laboratory sample that was used for all tests was a 1 x 30 dilution with water.

Although Bacto-Zyme prevented some hatch of egg masses and caused some mortality of larvae exposed to treated foliage, it is unlikely that this product would be useful in the control of life stages of the gypsy moth.

Tests were conducted with McLaughlin Gormley King products, TL-2163 and F-2132, to determine residual activity. Plants were treated with either light or heavy dosage, and bioassayed after various aging intervals. Results indicate that the tested materials might be effectively used on the under sides of house trailers and/or recreational vehicles.

Table 4. Mortality of larvae placed on oak foliage sprayed with TS-2163 and F-2132.

Material	Days aged	Percent mortality			
		Light dosage		Heavy dosage	
		1 Day	4 Days	1 Day	4 Days
TL-2163	-	77	92	81	100
TL-2163	4	46	91	85	100
TL-2163	11	76	89	97	100
F-2132	-	75	100	85	100
F-2132	4	48	100	80	100
F-2132	11	78	100	96	100
Check	-	1	2		

Table 5. Mortality of larvae 4 days after placement on foliage treated with TL-2163 and F-2132 and aged 0-46 days.

Treatment		Days aged 0	4	11	18	25	32	39	46
TL-2163	Light dosage	92	91	89	90	85	65	90	100
	Heavy dosage	100	100	100	99	100	99	100	100
F-2132	Light dosage	100	100	100	99	100	95	100	98
	Heavy dosage	100	100	100	100	100	100	100	100
Check		0	-	-	-	-	-	-	-

TL-2163 and F-2132 were also sprayed directly onto various gypsy moth larval instars. Those results are in Tables 6 and 7.

Table 6. Effects of TL-2163 sprayed directly onto various gypsy moth larval instars.

Instar	Percent mortality after 3 hours: Light dosage	Medium dosage	Heavy dosage
I	100	100	100
II	100	100	100
III	100	100	100
IV	100	100	100
Check	0	0	0

Table 7. Effects of F-2132 sprayed directly onto various gypsy moth instars.

Instar	Percent mortality after 3 hours: Light dosage	Medium dosage	Heavy dosage
I	88	88	96
II	88	100	100
III	88	100	100
IV	92	100	100
Check	0	0	0

Johnson Wax has provided a number of materials that have been tested as direct sprays to larvae, surface treatments, egg mass applications, foliage treatments and tree band treatments.

The first test consisted of treating plastic petri dishes and exposing laboratory reared gypsy moth larvae to the treated surfaces for various amounts of time. The material used was 4799D71-3 and it was applied at 3 dosages, fine, medium and heavy coverage. After exposure to the treated surfaces, larvae were placed onto artificial diet and held at 26°C and 50% RH. Mortality readings were made after 48 hours. Each treatment was replicated 5 times.

Table 8. Mortality of larvae placed in petri dishes treated with 4799D71-3.

Treatment dosage	Larval instar	Percent mortality after exposure for: 1 Min	3 Min	5 Min	10 Min
Fine coverage	1	5	6	0	0
Fine coverage	2	0	0	0	0
Fine coverage	4	8	12	22	30
Medium coverage	1	1	5	2	3
Medium coverage	2	0	0	0	0
Medium coverage	4	4	8	1	45
Heavy coverage	1	3	5	9	1
Heavy coverage	2	0	0	2	0
Heavy coverage	4	11	2	1	18
Check	2	-	-	-	0

The technique described for Table 8 was used to test eight additional materials. However, in this test all materials were applied to the point of run-off and only 2nd instar larvae were exposed for 5 minutes.

Table 9. Mortality of 2nd instar larvae exposed for 5 minutes to petri dishes treated with various Johnson Wax products.

Material	Percent mortality	
	24 Hr	48 Hr
5475D 6-1	100	-
5362D 67	100	-
5362D 69	3	98
4899D 89-1	100	0
4899D 90-1	53	55
4899D 92-1	100	-
4899D 94-1	100	-
Raid Yard Guard	2	2
Check	0	0

A test was also conducted in which 1-5 instar larvae were sprayed directly. Larvae were placed in a hood, and direct application was made from the aerosol can until thorough coverage of test insects was achieved. Insects were then placed on diet and held under previously described conditions. Mortality readings were made after a 24 hour period. Tests were replicated 5 times.

All materials resulted in 100 percent mortality within 24 hours. Materials tested were 4719D38-2, 5362D67, 4799D 92-1 and Raid Yard Guard. No mortality occurred in the check during the 24 hour test period.

Materials were also sprayed onto tender red oak seedlings and then bioassayed against 2nd instar larvae. Tests (5 replicates) were conducted at 3 dosages, fine (15 droplets cm^2), medium (30 droplets cm^2) and heavy coverage (to run-off). Mortality readings were made on a daily basis.

Table 10. Mortality of 2nd instar larvae on plants treated with various Johnson Wax products.

Material	Type treatment	Percent mortality 2 Day	4 Day	7 Day	Phytotoxicity
3538D 46-2	Fine	2	25	56	
3538D 46-2	Medium	12	60	89	
3538D 46-2	Heavy	28	84	93	Light
3538D 46-2	Heavy1/	19	65	74	Light
5302D 40	Fine	40	80	90	Light
5302D 40	Medium	61	98	-	Medium
5302D 40	Heavy	87	100	-	Heavy
5302D 40	Heavy1/	44	71	83	Heavy
5302D 28	Fine	100	-	-	
5302D 28	Medium	100	-	-	
5302D 28	Heavy	100	-	-	
4719D 38-2	Medium	20	69	-	Light
4719D 38-2	Heavy	26	96	-	Heavy
4799D 71-3	Medium	66	82	-	
4799D 71-3	Heavy	100	-	-	Light
4899D 92-1	Medium	85	95	-	Light
4899D 92-1	Heavy	100	-	-	Light
4899D 89-1	Medium	49	76	-	Heavy
4899D 89-1	Heavy	97	99	-	Heavy
5475D 6-1	Medium	76	90	-	
5475D 6-1	Heavy	97	99	-	Medium
4899D 90-1	Medium	72	86	-	
4899D 90-1	Heavy	97	98	-	Medium
4899D 94-1	Medium	74	88	-	
4899D 94-1	Heavy	100	-	-	Medium
5362D 69	Medium	100	-	-	Light
5362D 69	Medium	100	-	-	Light
5362D 67	Medium	66	84	-	
5362D 67	Heavy	100	-	-	
Check	-	0	0	0	-

1/ Plants received 1.0 inches of rain after treatment.

Some seedlings treated with various Johnson Wax materials were aged in the laboratory and then bioassayed again with 2nd instar larvae. Table 11 summarizes those results.

Table 11. Percent mortality of larvae 72 hr after placement on seedlings sprayed with various amounts of Johnson Wax products and aged 6 or 8 days.

Material	Days aged	Type treatment			
		Fine	Medium	Heavy	Heavy (Rain)[1/]
5302D 40	8	44	72	100	73
53024 28	8	99	99	99	99
3528D 46-2	8	-	35	56	35
5475D 6-1	6	-	56	95	-
4899D 92-1	6	-	73	100	-
4799D 71-3	6	-	44	100	-
4719D 38-2	6	-	20	-	-
5362D 67	6	-	47	100	-
5362D 69	6	-	100	-	-
4899D 94-1	6	-	49	100	-
4899D 90-1	6	-	58	100	-
Check	6		0		

1/ 1.0 inches

Four Johnson Wax products were tested on tree bark outdoors. Test materials were sprayed onto tree bark, and then laboratory reared larvae were allowed to walk on treated surfaces for various amounts of time. Larvae were then placed on artificial diet and returned to the laboratory. Mortality readings were made at 24 and 48 hours. This test was complicated by high winds, and we had trouble keeping the 2nd instar larvae on the treated tree bark.

Table 12. Percent mortality of larvae 48 hr after 1-15 minutes exposure to tree bark sprayed with varying amounts of Johnson Wax products.

Material	Type treatment	Minutes exposure			
		1	5	10	15
4719D 38-2	Fine	-	25	38	13
	Medium	0	9	0	28
4899D 92-1	Fine	-	100	65	65
	Medium	100	85	85	100
	Heavy	100	100	100	100
4799D 71-3	Fine	-	41	20	11
	Medium	25	15	5	33
	Heavy	15	10	5	10
5362D 69	Medium	-	22	63	0
Check	-	-	-	-	0

Four materials were also tested on foam wrap. Strips of thin foam wrap material were sprayed with test materials and then placed inside cardboard containers in such a manner as to form a barrier to insects in the containers. Larvae of various stages were placed below the treated band and allowed to move around at will. Most larvae moved over the treated band at least once during the test. At the end of an hour inside the containers, test insects were then removed and placed on artificial diet. Mortality readings were made at various time intervals following treatment. All tests were replicated 5 times.

Table 13. Percent mortality of larvae 5 days after 1 hour exposure to foam bands treated with Johnson Wax products and aged 0-7 days.

Material	Instar	Days aged		
		0	7	25
5475D 6-1	2	16	0	0
4899D 90-1	2	30	0	0
4899D 92-1	2	23	1	1
4899D 94-1	1	22	-	-
4899D 94-1	2	24	1	0
4899D 94-1	4	100	-	-
Check	2	0	-	-

Most materials that we tested were very effective against gypsy moth larvae when applied at heavy dosages to foliage or sprayed directly onto insects. However, when applied to foliage at medium to heavy dosages, phytotoxicity may be a problem.

When larvae were exposed to treated surfaces such as tree bark, petri dishes or foam wrap bands, mortality was relatively low. It is possible that in some tests, larval contact with the treated surfaces was inadequate. The petri dish technique is a sound method of testing as insects are confined to the treated surfaces. Mortality was high with this technique only when dishes were treated to point of run-off. We did see some knock-down of larvae and recovery a short time later.

Most materials tested would be effective larvicides if applied directly to the gypsy moth larvae. All instars should be affected by the direct spray if heavy dosages are used. Additional work should be conducted to test the materials phytotoxicity spectrum.

Four products designed to attach to the end of garden hoses were sprayed directly onto 3rd instar larvae with light, medium and heavy applications. Treated larvae were then placed onto artificial diet, and mortality readings were made after 1, 24 and 48 hours. There was some difficulty in applying material from the can containing D63, and this may account for the variable results with that material.

Table 14. Percent mortality of larvae 48 hr after treatment with varying amounts of Johnson Wax products dispensed from hose end applicators.

Material	Dosage		
	Light	Medium	Heavy
D-86-1	100	100	100
D-63 *	100	65	40
D-86-2	100	100	100
D-46-2	85	100	100
Check	0		

* Problem with spray can.

A number of materials were also tested on egg masses. Five masses were treated with each material until they were completely saturated. Eggs were then incubated for 6 days. Hatch was recorded over this period by counting the number of neonates. No attempt was made to compute percentage hatch. We found that a number of the materials would not penetrate the egg mass and just "rolled off". Because of this, some were dipped into the test material. All others were treated with a syringe. Penetration of the egg mass can normally be achieved by adding alcohol or an emulsifier to the formulation.

Table 15. Hatch from egg masses treated with various Johnson Wax compounds.

Material		Type treatment	Total hatch after 6 days
R-24-1	#	Syringe	53 1/
R-24-2	#	Syringe	0
R-24-3	#	Syringe	0
R-24-4	#	Syringe	0
R-24-5(1%)	*	Dip	2,500 +
R-24-5(10%)	+	Syringe	0
R-24-6(1%)	*	Dip	2,500 +
R-24-6(10%)	#	Syringe	16
R-25-1	#	Syringe	0
R-25-2	+	Syringe	62
R-25-3	*	Dip	2,500 +
R-25-4	*	Dip	2,500 +
R-25-5	*	Dip	24
R-25-6	*	Dip	2,500 +
R-25-7	*	Dip	2,500 +
R-25-8	*	Dip	6
R-25-9	*	Dip	2,500 +
R-25-10	*	Dip	0
R-25-11	*	Dip	2,500 +
R-25-12	+	Dip	0
Check	-		2,500 +

Penetration fair
+ Penetration excellent
* Penetration poor

1/ Total neonates from 5 egg masses.

Insecticide treated trunk wraps similar to those commonly marketed were tested during the year. Wraps were placed inside plastic containers, and 25 gypsy moth larvae were exposed to each treatment. Larvae were held inside the containers for a 30 minute period after which they were placed on artificial diet. Tables 16 and 17 present the results of these bioassays.

Table 16. Results of a bioassay of treated tree wraps from Johnson Wax. Treatments were aged for various amounts of time before bioassay.

Materials	Days after preparation that wraps were bioassayed		
	1	6	14
Sevin (3 days)1/	93	74	-
Sevin (5 days)	100	-	7
Resmethrin + Dursban (3 days)	75	85	-
Resmethrin + Dursban (5 days)	100	-	74

1/ Time in parentheses is the time after exposure that mortality readings were made.

Table 17. Bioassay results of tree wraps exposed to UV radiation.

Material	Days wrap exposed to UV				
	0	1	3	5	7
Resmethrin + B-trans allethrin	100/72[1/]	4/4	0/0	0/0	0/0
Dursban + synergized pyrethrin	40/28	28/24	8/8	0/0	0/0
Dursban + synergized pyrethrin	44/68	48/36	4/8	0/0	0/0
Check	0/0				

1/ 24 hr % knockdown/72 hr % mortality.

A series of tests was conducted with J. T. Baker candidate ovicides. Starting in November 1981, six Baker products and 2 Otis materials were used to treat egg masses in the field. Baker samples consisted of cotton seed oil, mineral oil, citrus oil, corn oil, caster oil and pine oil. Otis formulated agents were pine scent and light water. Periodically throughout the winter, egg masses (10/material/interval) were treated with a hand sprayer to the point of saturation with each material. Treated masses were allowed to stay in the field until they were collected and checked for hatch in mid April. The materials 268-C, 273-1, 273-3, 278-1, Otis 1 and Otis 2 had no effect on hatch. Egg masses treated with the Baker product 272-1, however, did not hatch. Hatch was prevented by soaking egg masses with 272-1 at each of the 14 treatments applied from November 12 - March 6.

Laboratory produced egg masses, ready to hatch, were treated with Baker products with a hand-held syringe. Five masses were treated with each material, and 5 untreated masses were used as checks. The test materials were deposited directly into the center of each egg mass. Two rates were used, 1 ml and 2 ml per mass. After treatment, eggs were incubated in an environmental chamber, and hatch was recorded on a daily basis.

Table 18. Hatch of gypsy moth egg masses treated with J. T. Baker compounds.

Formulation	1 ml	2 ml
1047-247-1	0	0
1047-247-2	0	0
1047-247-3	0	0
1047-247-4	0	0
1047-247-5	9	25
1047-247-6	0	0
1047-247-7	0	0
Check	256	645

Values are total neonates hatched from 5 egg masses.

The formulations in Table 18 were held at -15°C, and samples 1047-247-5 and 1047-247-7 were frozen solid after only hours. All other samples were more viscous than before exposure to cold temperatures which might be a problem in applying materials outdoors during cold months.

The ovicidal properties of a number of materials were evaluated by dipping intact or dehaired egg masses into test solutions. After air drying, eggs were held in an environmental chamber to determine hatch. The following formulations completely prevented hatch:

100% MOS	10% MOS in isopropyl alcohol
100% Corn oil	10% Corn oil in isopropyl alcohol
100% Castor oil	10% Castor oil in isopropyl alcohol
100% LO-R	10% LO-R in isopropyl alcohol
100% LO-B	10% LO-B in isopropyl alcohol
100% Pine oil	10% Pine oil in isopropyl alcohol
100% Citrus oil	10% Citrus oil in isopropyl alcohol
JTB 272-1	
Cottonseed oil	
Kerosene	

Hatch was reduced ca. 90% with 10% aqueous solutions of Top Job and Pine Sol, slightly reduced with isopropyl alcohol and unaffected by ethanol.

All treated eggs were examined microscopically, and in all cases the test material had coated over the eggs not allowing emergence. It appeared that some materials penetrated the eggs and caused them to dry out. Good penetration of the intact egg masses occurred with all materials.

Egg masses that had been in cold storage for 120 and 180 days were dipped in the following materials and exposed to 1, 4 or 10 inches of rain.

Light water 50%		
Linseed Oil (LO)	Citrus Oil	Caster Oil (CO)
Mineral oil	Corn Oil	TCE + PO + LO
Pine Oil (PO)	Soybean oil	Kerosene + PO + CO
Isopropyl Alcohol + PO + CO		Kerosene

In all cases, hatch was completely prevented.

It was felt that some oil formulations tested were effective because neonates were being deprived of oxygen. Dehaired eggs were held under anoxic conditions for periods of time and then checked for hatch.

Table 19. Hatchability of eggs held under anoxic conditions for varying periods at different temperatures.

Time under anoxia (hr)	Percent hatch	
	Cooled	Room temp.
.2	85	85
1	85	0
7	85	0
Check	85	85

Eight materials were tested for ovicidal properties. The interiors of one quart freezer containers were sprayed to the point of saturation with each material. Some materials were used at more than one dosage. One-hundred containers were treated with each formulation and then stored inside at 27°C. At various time intervals 25 pupae of each sex were introduced into each of 5 containers from each dosage. After adult emergence and mating, resulting egg masses were cut out and held at room temperature for 30 days for embryonation. Eggs were then held at 2-4°C for 150 days and subsequently removed to an incubation room for hatch.

Adults in a number of containers did not produce egg masses because they were killed by the insecticide as soon as they emerged and moved about the treated surface. Sevin XLR, Orthene 75S spray, Dylox 80S spray, Diazinon spray and Baygon dust were very effective adulticides.

Table 20. Total number of egg masses collected from cartons treated with each formulation and average egg mass hatch.

Material	Formulation	Total number egg masses	Average percent hatch
Baygon	10% Dust	38	5
Diazinon	4% Dust	110	14
Diazinon	1.0 lbs. AI/gal	14	0
Dimilin	0.25 lbs. AI/gal	136	14
Dimilin	0.06 lbs. AI/gal	159	19
Dimilin	10% Dust	127	21
Dylox	10% Dust	152	14
Dylox 80S	1.0 lbs. AI/gal	19	8
NC-6897	1.0% Dust	81	10
Orthene 75S	1.0 lbs. AI/gal	6	0
Orthene	10% Dust	114	12
Sevin 80S	1.0 lbs. AI/gal	108	15
Sevin XLR	1.0 lbs. AI/gal	0	-
SIR-8514	0.06 lbs. AI/gal	143	14
SIR-8514	0.25 lbs. AI/gal	108	8
SIR-8514	10% Dust	97	10
Check	-	364	11

Because of low hatch in the check, it is difficult to say how effective treatments were. Additionally, larvae from growth regulator treatments were not reared to determine survival. Previous tests have shown that these materials do not prevent hatch, but survival of neonates to 2nd instar is very low.

Twelve oak trees were treated with "Acecaps" to control gypsy moth larvae. Acecaps contain Orthene which is taken up by the tree and acts as a systemic insecticide. Holes were drilled into trees at various distances around the base, depending on tree size, and Acecaps inserted per the instructions on the label. To evaluate effects on larvae, pre and posttreatment larval counts were made on designated branches.

Table 21. Mean larval counts from red and white oak trees treated with the systemic insecticide, Acecaps. Values in parentheses are % reduction from pretreatment counts.

	n	Pretreatment	Posttreatment (days) 5	7	20
Treated	12	61.1	52.0(15)	36.6(40)	8.5(86)
Control	2	54.0	49.0 (9)	52.5 (3)	13.5(75)

Orthene dissolved from all Acecaps a short time after injection into trees. Although we did observe a small number of larvae on the ground under one tree, it appears that the treatment was ineffective in controlling gypsy moth larvae. A number of treated trees were completely defoliated, and feeding occurred up until pupation. After 20 days, there was a reduction in larvae on a number of trees. However, check trees also had a reduction in numbers. It should be noted that the 7 day count was appreciably below the corresponding control sample. The results may have been poor due to the size of the gypsy moth, 3rd instar, and the late date of tree treatment. Treatments should take place at the time of maximum upward sap flow.

Six new designs of banding designed and supplied by Harry Moore and Repel 'M tape were evaluated in the laboratory to determine their repelling properties to gypsy moth larvae. Each type of banding supplied by H. Moore was snugly wrapped around a cardboard box and held in place by masking tape to insure that the larvae could only ascend by crossing over the material. Two bands of Repel 'M tape, about 6" apart, were also wrapped around a cardboard box. On the Moore banding sites, 20 second instar and 10 fifth instar larvae were positioned about 4 inches below the banding. On the Repel 'M banding site, 20 second instar and 10 fifth were positioned between the bands. The experiment was conducted for 3 hours. Data on location of larvae was collected at 15 minute intervals. General observations were noted throughout the duration of the experiment.

Generally, it can be stated that the Moore bands were somewhat successful in containing the larvae below the band. However, second instar larvae were able to ascend past the barrier. In no cases were 5th instar larvae observed crossing the barrier.

The Repel 'M sticky tape worked the best with the larger larvae although after 120 minutes, 66% of the 2nd instar larvae and 43% of the 5th instar larvae were able to traverse the tape barriers. Because of the brevity of the experiment, it was not determined whether over a longer period of time, a natural bridge of larval bodies would result, facilitating movement of larvae over the barrier. This possibility should be investigated in the field.

A small test was conducted to determine effects of hot water on egg hatch.

Table 22. Percent hatch of gypsy moth egg masses submersed in water at various temperatures.

Temperature	Exposure time (min) 1	5
40°C	80	80
50°C	70	0
60°C	0	0
70°C	0	0
80°C	0	0
Check	80	-

A test was conducted with d-Phenothrin 10% to determine its residual on glass plates. Using an aerosol can d-Phenothrin was sprayed on glass plates at various dosages, and the plates were aged 0-8 days. Second instar gypsy moth larvae were then exposed to treated surfaces for 5 minutes and placed on artificial diet. Mortality readings were made over a period of days. In all treatments, nearly 100% mortality was observed, and effectiveness did not diminish when plates were aged up to 8 days.

A series of laboratory egg masses was treated with the commercial product "Zapper". TL-2163 was also tested on eggs at this time. Zapper is a creosote based material packaged in an aerosol can and sold exclusively for gypsy moth egg mass treatments. Both materials completely prevented hatch when applications were either light, medium or heavy.

A tree band material from Applied Research Technologies International was tested against various gypsy moth larval instars. The sticky material is formulated with a synthetic pyrethroid material for quick knockdown. The material was smeared onto glass plates, and test insects were placed onto the material for 5 seconds. Treated insects were transferred to artificial diet, and mortality readings were made. Some plates were aged outside and the rest inside. Outside plates were exposed to various amounts of rainfall and ultraviolet light.

All second instar larvae were dead 1 hr after the 5 second exposure. One hundred percent mortality of 3rd, 4th and 5th instar larvae was observed within 24 hrs of exposure. After 21 days of indoor aging, 100 percent mortality of 3rd, 4th and 5th instar larvae was observed after 48 hours post exposure time. Material aged outdoors gave 0-30 percent mortality of all larvae instars after 48 hours posttreatment.

Table 23. Percent mortality of larvae 24 hours after placement into plastic cylinders treated with Tree Ban.

	Cylinder cover	
Instar	On	Off
II	70	70
III	70	40
IV	50	60
V	100	90

Four Johnson Wax materials were tested on gypsy moth pupae and adult females. Adults and pupae were sprayed directly with a hose end spray system.

Table 24. Percent mortality of pupae and/or adults 3 days after treatment with Johnson Wax products.

Material	Adult	Pupae	Dosage Light	Medium	Heavy
D86-1	X		-	100	-
D86-1		X	24	32	16
D86-2	X		-	100	-
D86-2		X	48	48	36
R46-1	X		-	100	-
R46-1		X	24	28	4
N69-1	X		-	100	-
N69-1		X	4	20	0

Three experimental egg mass treatments were tested on car paint finishes. Color standard cards of various colors of acrylic lacquer paint were used as test surfaces. Six paint colors were used. Using an eye dropper, material was placed onto paint surfaces and allowed to dry for 48 hours. Spots were then cleaned using the various methods and results recorded. Damage was identified using the following codes:

1. Spot not visible
2. Spot partially visible
3. Completely visible but faintly
4. Distinctly visible

Table 25. Visibility of test material on car paint after washing treated surfaces with water only.

Material	Paint Colors Beige	Pastel Blue	Yellow	Light Brown	Dark Brown	Red
Light H_2O						
5%	2	2	2	1	1	2
10%	2	2	2	2	2	2
25%	3	2	4	2	2	4
50%	2	2	4	1	2	4
Pine Scent						
5%	3	3	3	1	1	3
10%	3	3	4	3	1	4
25%	3	3	4	4	4	4
50%	3	3	4	4	4	4
Top Job						
5%	1	1	1	1	1	1
10%	1	1	1	1	1	1
25%	1	2	1	1	1	1
50%	1	2	1	1	1	1

Table 26. Visibility of test material on car paint after a light washing of treated surfaces with water and Mr. Clean detergent.

Material	Paint Colors					
	Beige	Pastel Blue	Yellow	Light Brown	Dark Brown	Red
Light H_2O						
5%	2	2	2	1	1	2
10%	2	2	2	2	2	2
25%	3	2	4	2	2	4
50%	2	2	4	1	2	4
Pine Scent						
5%	2	3	3	1	1	3
10%	3	3	4	3	1	4
25%	3	3	4	4	4	4
50%	3	3	4	4	4	4
Top Job						
5%	1	1	1	1	1	1
10%	1	1	1	1	1	1
25%	1	1	1	1	1	1
50%	1	1	1	1	1	1

Table 27. Visibility of test material on car paint after a hard washing of treated surfaces with water and Mr. Clean detergent.

Material	Paint Colors					
	Beige	Pastel Blue	Yellow	Light Brown	Dark Brown	Red
Light H_2O						
5%	2	2	2	1	1	2
10%	2	1	2	2	2	2
25%	3	2	4	2	2	4
50%	2	2	4	1	2	4
Pine Scent						
5%	2	3	3	1	1	3
10%	3	3	4	2	1	4
25%	3	3	4	3	4	4
50%	3	3	4	3	4	4
Top Job						
5%	1	1	1	1	1	1
10%	1	1	1	1	1	1
25%	1	1	1	1	1	1
50%	1	1	1	1	1	1

Table 28. Visibility of test material on car paint after a 15 minute soaking and hand washing of treated surfaces with water and Mr. Clean detergent.

Material	Paint Colors					
	Beige	Pastel Blue	Yellow	Light Brown	Dark Brown	Red
Light H_2O						
5%	2	2	2	1	1	2
10%	2	1	2	2	2	2
25%	3	2	4	2	2	4
50%	2	2	4	1	2	4
Pine Scent						
5%	2	3	3	1	1	3
10%	3	3	3	2	1	4
25%	3	3	4	3	4	4
50%	3	3	4	3	4	4
Top Job						
5%	1	1	1	1	1	1
10%	1	1	1	1	1	1
25%	1	1	1	1	1	1
50%	1	1	1	1	1	1

When treating egg masses with Dylox, Dimilin and SIR-8514, there is sometimes limited hatch at low dosage. A test was conducted to determine what percentage of neonates died following hatch.

Table 29. Percent mortality of neonates that survive initial treatment of gypsy moth egg masses with insecticides. Readings made 7 days postemergence from eggs.

Material	Formulation	Mortality
Dylox	25% Dust	68
	10% Dust	34
SIR-8514	25% Dust	100
	10% Dust	100
	5% Dust	99
Dimilin	25% Dust	98
	10% Dust	99
	5% Dust	99
Check	-	0

Table 29 indicates that neonates produced by SIR-8514 and/or Dimilin treated eggs will not survive the first week following emergence.

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period: October 1, 1981 - September 10, 1982
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

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

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

During 1982 a number of State and Federal agencies used Bacillus thuringiensis to treat gypsy moth infestations. In all, more B. thuringiensis was used in 1982 than any previous year. In most cases treatments were very effective in controlling defoliation using a single application of 12 BIU/acre. This confirms our 1981 field results and recommendations for 1982.

Although results were generally good with B. thuringiensis during 1982, there were still questions about stickers: which one to use, will it affect car point, will it clog screens, how costly, what's in it, is it cleared for use and will it help stick the material to foliage. There are a number of stickers available for use, and screening continues for new ones.

During this report period work continued with B. thuringiensis and stickers. The following tables summarize that work.

Table 1. Mortality of 2nd instar gypsy moth larvae 4 or 5 days after placement on oak foliage treated with 8 BIU of Thuricide 32 LV and stickers and exposed to 0.3 inches of rain.

Sticker	1% Sticker Day 5	3% Sticker Day 4	3% Sticker Day 5
- 1/		66	100
- 1/		58	60
Acrylocoat		82	
RA-1990		84	
RA-1990	91		
Polyco-2142		70	
Polyco-2142	97		
TS-100		55	
TS-100	97		
TS-85		49	
TS-85	84		
TS-30		41	
TS-30	58		
Check		0	0

1/ No rain

Table 2. Percent mortality of larvae 3 and 4 days after placement on seedlings treated with 8 BIU/acre Thuricide 32 BX or Dipel 4L and exposed to varying amounts of rain.

Inches rain	Thuricide 32 BX		Dipel 4L
-	61 1/	95	76
0.05	40	96	52
.10	26	80	31
.20	13	28	5
.30	26	57	5
.40	4	24	0
.50	13	48	4
.60	11	38	0
.70	12	43	6
.80	12	56	4
.90	9	43	2
1.0	19	74	3
1.1	5		
1.2	3		
1.3	6		
1.4	10		
1.5	9		
Check	0	0	0

1/ 3 day mortality reading

Table 3. Percent mortality of larvae 4 days after introduction onto seedlings sprayed with 8 BIU/acre Dipel and Thuricide and exposed to 0 - 1.0 inches of rainfall.

Formulation	Inches rain 0	.25	5	1.0	Average % defoliation
Dipel 4L	55	5	6	15	97
Dipel 4L + 1% Plyac	-	18	11	39	81
2% Plyac	-	18	16	5	91
3% Plyac	-	35	7	8	88
4% Plyac	-	1	2	13	95
1% Polyco 2142	-	6	5	4	92
2% Polyco 2142	-	69	39	28	59
3% Polyco 2142	-	15	5	5	89
Thuricide 32 LV	89	35	27	6	60
Thuricide 32 LV + 1% Plyac	-	15	8	7	71
2% Plyac	-	7	15	8	80
Check	0	-	-	-	100

Table 4. Larval mortality 4 days after placement on oak seedlings treated with 8 BIU/acres of various combinations of stickers and Dipel 4L or Thuricide 32 LV and exposed to 0.5 inches rain.

Sticker	Dipel 4L	Thuricide 32 LV
Control (no sticker, no rain)	70	79
Control (no sticker)	0	50
1% Plyac	16	29
2% Plyac	22	22
1% Rhoplex B60A	9	31
2% Rhoplex B60A	20	79
1% Polyco 2142	11	16
2% Polyco 2142	13	78

Table 5. Percent mortality of 2nd instar larvae 5 days after introduction onto seedlings sprayed with 8 BIU/acre Dipel 4L and various stickers at 1% and 3% exposed to 0.3 inches rainfall.

Sticker	1%	3%
Control (no sticker, no rain)	98	97
Control (no sticker)	1	4
Acrylocoat		58
RA-1990	90	86
Polyco-2142	30	82
TS-100	52	62
TS-85	40	53
TS-30	19	57
Check	0	0

Table 6. Percent mortality of 2nd instar larvae 4 days after placement onto oak seedlings treated with 8 BIU/acre of Thuricide 32 LV and Polyco 2142 at .5, 1, 2 and 3% exposed to various amounts of rainfall.

Percent	Inches rain				
sticker	0	0.5	1.0	2.0	3.0
-	83	34	17	45	18
0.5		50	22	32	4
1.0		38	34	13	24
2.0		83	80	44	51
3.0		78	72	54	67
Check	0	0	0	0	0

Table 7. Percent mortality of 2nd instar larvae 4 days after placement onto oak seedlings treated with 8 BIU/acre of Dipel 4L and Thuricide 32 LV and various stickers at 2 and 3% exposed to 0.3 inches rain.

Sticker	Thuricide 32 LV		Dipel 4L	
	2%	3%	2%	3%
(no sticker, no rain)		77		82
(no sticker)		68		2
Acrylocoat	77	82	67	40
RA-1990	98	93	86	45
Polyco-2142	86	97	55	38
Polyco-571		57		9
Polyco-2113		89		35
Polyco-2607		90		17
Polyco-2151		69		13
NuFilm-17		68		6
Chevron		54		4
TS-100	51		38	
TS-85	73		55	
TS-30	73		40	
Check	0	0	0	0

Table 8. Percent mortality of 2nd instar larvae 3 days after placement onto seedlings treated with 8 BIU/acre of Bactospeine, Dipel 4L and Thuricide 32 LV and various stickers exposed to 1.0 inch of rain.

Sticker	Bactospeine	Dipel	Thuricide
(no sticker, no rain)	29	41	32
(no sticker)	1	0	2
1% Clear Spray	4	1	4
3% Clear Spray	22	2	8
3% Exhalt-800	0	3	15
3% Exhalt-4-10	2	1	4
3% Wilt-Pruf	8	1	22
Check	0	0	0

Table 9. Percent mortality of 2nd instar larvae 4 days after placement onto seedlings treated with 8 BIU/acre of Dipel #466 and various stickers exposed to 0.5 inches of rain.

Sticker	Mortality	Percent defoliation
-	23	81
1% Bivert	21	77
3% Bivert	18	73
1% Clear Spray	57	46
3% Clear Spray	51	50
1% C-3500	24	71
3% C-3500	81	26
1% Plyac	26	57
3% Plyac	24	76
1% TS-100	46	55
3% TS-100	41	36
1% TS-85	37	53
3% TS-85	56	40
1% TS-30	38	50
3% TS-30	60	39
1% Polyco-2142	47	44
2% Polyco-2142	53	42
3% Polyco-2142	52	35
4% Polyco-2142	44	48
1% Polyco-2149D	49	42
3% Polyco-2149D	68	23
Check	0	0

Table 10. Percent mortality of 2nd instar larvae 4 days after placement onto seedlings treated with 8 BIU/acre of Dipel 4L and Polyco-2142 exposed to various amounts of rainfall.

Percent sticker	Inches rain				Percent defoliation
	0	0.25	0.5	1.0	
-	57	9	4	5	94
1.5		5	8	3	75
2.0		5	1	2	78
2.5		1	6	5	89
Check	0	0	0	0	100

Table 11. Percent mortality of 2nd instar larvae 2 and 3 days after placement onto seedlings treated with 8 BIU/acre of Dipel 4L and various stickers and exposure to rainfall.

Sticker	Inches rain	2 day	3 day	Percent defoliation
-	0		24	26
-	1.0		1	99
3% Polyco-2142	-		52	7
3% Polyco-2142	0.1		16	42
3% Polyco-2142	0.5		16	77
3% Polyco-2142	1.0		4	88
3% Polyco-2149	-		43	11
3% Polyco-2149	0.1		25	64
3% Polyco-2149	0.5		8	93
3% Polyco-2149	1.0		3	100
1% C-3500	1.0		2	99
2% C-3500	1.0		2	98
3% C-3500	1.0		0	100
Bivert 1 x 4	-	19		36
Bivert 1 x 4	1.0	1		97
Bivert 1 x 4	- 1/	13		25
Bivert 1 x 4	1.0 1/	0		100
Check		0	0	100

1/ 4 BIU/acre

Table 12. Percent mortality of 2nd instar larvae 4 days after placement onto seedlings treated with 8 BIU/acre of Dipel 4L and various stickers and exposed to 0.5 inches of rainfall.

Sticker	Percent sticker 2%	Percent sticker 4%	Percent defoliation
-	20		100
Plyac	10	45	95
Polyco 2142	15	5	85
Plyac + Polyco 2142	45	45	90
Plyac + B60-A	25	10	100
Polyco 2142 + Acetic Acid	40	15	100
Polyco 2142 1/	100		10
Check	0	0	100

1/ Thuricide 32LV/8 BIU/acre

Tests were conducted to determine if the order of mixing B. thuringiensis formulation ingredients might have any effect on how the final mix weathers. Four mixing orders were used with Dipel 4L, and all were then tested using 8.0 BIU/acre.

Mix A - H_2O + Dipel 4L + Sticker
Mix B - Dipel 4L + H_2O + Sticker
Mix C - H_2O + Sticker + Dipel 4L
Mix D - Dipel 4L + Sticker + H_2O

Table 13. Percent mortality of 2nd instar larvae 4 days after introduction onto seedlings sprayed with Dipel and Polyco 2142 sticker and exposed to 0.25 inches of rain.

Mix	No sticker/rain	Rain only	1.5%	2.0%
A	76	9	21	21
B	74	2	13	22
C	47	3	8	17
D	98	1	14	24
Check	0	0	0	0

Table 14. Percent mortality of 2nd instar larvae 3 days after placement on seedlings treated with 8 BIU of Dipel 4L at 1 qt and 1 gal rate with and without emulsifier and exposed to natural sunlight and rain.

Inches rain	Hours sunlight			Emulsifier
	None	1.0	2.0	
- 1/	88	88	50	None
1.0 1/	85	7	2	None
- 1/	65	43	40	Yes
1.0 1/	58	2	2	Yes
- 2/	30 3/	53 3/	37 3/	Yes
1.0 2/	43 3/	10 3/	3 3/	Yes
- 2/		32	35	Yes
1.0 2/	42	5	5	Yes
Check	0	0	0	

1/ at 1 qt/rate undiluted
2/ at 1 gal/rate
3/ with Rhoplex B-60A sticker

Table 15. Percent mortality of 2nd instar larvae 3 days after placement on seedlings treated with 8 BIU of Dipel 6L at a 1 qt rate with and without emulsifier, sticker, sunlight and rain.

Sticker	Emulsifier	Hours sunlight	Inches rain	Mortality
-	-	-	-	60
-	Yes	-	-	40
-	Yes	2	-	28
-	-	-	1.0	3
-	Yes	-	1.0	0
-	-	2	-	18
2% 525	Yes	2	1.0	0
"	Yes	-	1.0	2
"	-	-	1.0	0
"	-	2	1.0	0
4% 525	Yes	-	-	33
"	Yes	-	1.0	0
2% 514	Yes	-	1.0	0
"	Yes	2	1.0	2
"	-	-	1.0	0
"	-	2	1.0	0
2% Rhoplex B60A	Yes	-	-	5
"	Yes	-	1.0	0
2% Acrylocoat	Yes	-	-	17
"	Yes	-	1.0	0
Check				0

Table 16. Percent mortality of 2nd instar larvae 3 days after placement onto seedlings treated with Dipel 6L and cottonseed oil at 0.3 gal/ac rate and exposed to natural sunlight and rain.

Hours sunlight	Emulsifier	Inches rain	Mortality
-	Yes	-	88
-	Yes	1.0	0
2	Yes	1.0	0
-	-	1.0	5
2	-	1.0	2
Check			0

It is recommended that 2 applications of B. thuringiensis be applied at 7 to 10 day intervals to control gypsy moth larvae. The first treatment should be applied when the majority of larvae are in the early 2nd instar. The 2nd application should be applied when larvae are no larger than 3rd instar. This is based on the assumption that larger larvae are not as susceptible to the B. thuringiensis spray. At this time we have not established dosage response curves for B. thuringiensis. Table 1 represents data resulting from an experiment in which larvae in various stages were fed oak seedlings treated with 8.0 BIU/ac B. thuringiensis. When plants were completely eaten by larger larvae, the insects were transferred to new, treated plants. They, therefore, fed on treated foliage for the entire test period. The test was replicated 3 times.

Table 17. Percent mortality of various instar larvae 4-5 days after placement onto seedlings treated with 8 BIU/ac of Dipel 4L and Thuricide 32LV.

Material	Days following treatment	Larval instar I	II	III	IV	V
Thuricide 32LV	4	99	63	78	55	61
	5	100	94	96	85	67
Dipel 4L	4	96	40	53	53	23
	5	100	94	96	85	67
Check	4	0	0	0	0	0
	5	0	0	0	0	0

Tests were conducted in the laboratory to study B. thuringiensis residual. Twenty-five tender oak seedlings were sprayed with each of 2 dosages of Dipel 4L and Thuricide LV. Twenty 2nd instar gypsy moth larvae were then introduced onto each plant at various time intervals over the next 14 days. Following each introduction of larvae, mortality readings were made for a number of days. Aged plants were held in the laboratory and were not exposed to sunlight or rainfall. Untreated plants were used as a check.

After 14 days, all treatments continued to give good control of laboratory reared 2nd instar gypsy moth larvae.

Table 18. Percent mortality of 2nd instar larvae 4 days after placement onto oak seedlings treated with 8-12 BIU/ac of Dipel 4L and Thuricide 32LV and aged for various amounts of time.

	Days Aged 0	3	7	10	14	19	22	26	29	33	36
Dipel 8 BIU/ac	84	64	79	97	79	80	97	42	78	98	90
Dipel 12 BIU/ac	77	64	79	79	78						
Thuricide 8 BIU/ac	69	79	57	89	96	90	98	44	90	97	92
Thuricide 12 BIU/ac	73	82	90	94	83						
Check	2	0	0	0	3	1	0	0	2	1	0

Spray applications are sometimes delayed because of wet foliage due to rain or heavy dew. A test was conducted with Dipel 4L to see if there was a reduction in activity when sprayed onto wet foliage. Using 8 BIU/ac, Dipel was sprayed onto dry, lightly moist, medium moist and heavy moist seedlings and then bioassayed. After 5 days, mortality was high with all treatments indicating wet foliage can be treated successfully in the field.

Using the standard Dipel 4L formulation, 3 dosages were tested against 4 gypsy moth larval stages. All test larvae were transferred to artificial diet on the third test day.

Table 19. Percent mortality of various instar larvae 4 days after placement onto seedlings treated with various dosages of Dipel 4L.

	Instar larvae			
Dosage	II	III	IV	V
8 BIU/ac	61	81	44	50
12 BIU/ac	77	68	46	47
16 BIU/ac	84	83	70	65
Check	0	0	0	0

Laboratory screening work was conducted with Avermectin B, a streptomycete-derived macrocyclic lactone natural product discovered by Merck Sharp and Dohme. Avermectin B has been very effective in controlling a number of plant-feeding mites and insects susceptible and resistant to many of the currently used acarcides and insecticides. A representative of a new class of anthelmintics, it has also been very effective as an animal systemic insecticide. When tested against gypsy moth larvae, the material was active at extremely low dosages (Table 20-27). Because of its effectiveness, a limited field study was conducted (GM 2.1.1).

Table 20. Mortality of 2nd instar larvae 2 days after placement onto seedlings treated with various amounts of Avermectin B.

Mg/ac	Mortality
14,165	100
7,083	100
3,541	99
1,771	100
885	99
443	97
221	95
111	76
55	47
28	28
14	3
7	0
4	0
Check	0

Table 21. Mortality of 2nd instar larvae 2 days after placement onto seedlings treated with various dosages of Avermectin B and exposed to rainfall.

Mg/ac	Inches rainfall None	1.0	2.0
14,165	100	99	100
7,083	100	100	100
3,541	100	85	84
1,770	100	72	82
885	100	76	76
443	99	38	59
Check	0	0	0

Table 22. Mortality of 2nd instar larvae 2 days after placement onto seedlings treated with 4 dosages of Avermectin B and exposed to natural sunlight.

Hours sunlight	Mg/ac 1,771	885	443	221
-	100	99	98	97
0.5	100	100	100	99
1.0				94
2.0				92
3.0				90
4.0				83
Check				

Table 23. Mortality of 2nd instar larvae 2 days after placement onto seedlings treated with 3 dosages of Avermectin B and aged up to 14 days.

Mg/ac	Days aged 0	7	14
1,771	100	100	100
885	100	100	100
443	100	100	95
Check	0	0	0

Table 24. Percent mortality of various instar larvae 2 days after placement onto seedlings treated with 2 dosages of Avermectin B.

Mg/ac	Larval instar I	II	III	IV	V
443		99	92		
221	93	100	85	33	58
Check	0	0	0	0	

Dosage and weathering tests were conducted with Zolone, a product from Rhone-Poulenc Chemical Company.

Table 25. Percent mortality of 2nd instar larvae 3 days after placement onto seedlings treated with various dosages of Zolone W/P and Zolone EC and exposed to various amounts of rainfall.

lbs/ac	WP NR	1"	2"	3"	4"	EC NR	1"	2"	3"	4"
1.0	98	10				100	96			
0.5	87	21	2	11	2	100	87	76	44	85
0.25	76	31				97	84			
0.125	35	8				91	61			
Check										

Table 26. Percent mortality of 2nd instar larvae 4 days after placement onto seedlings treated with 0.5 lbs/ac of Zolone W/P and Zolone EC and exposed to various amounts of ultraviolet light.

Formulation	Minutes ultraviolet light 0	30	60	120
WP	89	90	93	93
EC	98	100	100	100
Check	0	0	0	0

A 0.5 percent Neem extract stock solution was tested for effectiveness against gypsy moth larvae. Six dilutions were made using water and sprayed onto foliage of tender red oak seedlings. Five plants were treated with each dilution at the rate of a gallon per acre. Five untreated plants were used as a check. Twenty 2nd instar gypsy moth larvae were then placed onto each treated seedling. Larvae were allowed to feed for 24 hours and then changed from treated foliage to artificial diet and reared for a period of 33 days. Mortality readings were made at various time intervals throughout the test period. At the time insects were transferred to diet, nearly all foliage had been consumed on treatment and check plants. Although final mortality figures were less than expected, there was some mortality at the higher dosages.

Table 27. Percent mortality of 2nd instar larvae placed onto seedlings treated with Neem.

Percent solution	Days following treatment 12	18	24	28	33
0.5	16	20	27	42	42
0.25	12	12	14	29	31
0.125	11	11	12	33	35
0.062	7	7	8	39	41
0.031	1	0	0	17	18
0.015	4	5	8	13	14
Check	0	0	0	6	8

A test was conducted with Dimilin to determine if limited numbers of droplets per cm^2 on foliage would effect efficacy. Oak seedling foliage was treated with Dimilin at 1-5 droplets cm^2 and exposed to 2nd instar gypsy moth larvae for 5 days. One-hundred percent mortality occurred with each treatment.

Table 28. Percent mortality of 2nd instar larvae 4 and 7 days after placement onto seedlings treated with various dosages of methoxychlor.

lbs/ac	EC 4 day	EC 7 day	W/P 4 day	W/P 7 day
2.7	51	95		
2.0			81	97
1.3	31	86		
1.0			47	88
0.6	33	93		
0.5			21	70
0.3	22	42		
0.2			8	13
Check	0	0	0	0

Studies were conducted with Dylox 1.5 oil undiluted and diluted with kerosene. A series of dosages ranging from 1.5 - 0.05 lbs/ac were tested on oak foliage using 2nd instar gypsy moth larvae as test insects. One-hundred percent mortality occurred at each dosage 3 days following treatment.

Laboratory studies were conducted to compare 2 formulations of the insect growth regulator, SIR-8514 a Mobay product. A 25WP formulation was compared to a 4 flowable at dosages of 0.06 - 0.002 lbs/acre. Each treatment was exposed to 3.0 inches of rainfall. All treatments gave 100% larval mortality after 7 days. It would, therefore, be preferable to use the 4 flowable formulation in the field.

In order to simulate a ground treatment (hydraulic) with SIR-8514, a series of dosages (0.125 - 0.007 lb/ac) were mixed per 100 gallons of H^2O. Tender oak seedlings were then dipped into each solution and bioassayed. Each treatment was also exposed to 1.0 inches of rainfall after a drying period of 2 hours. After 5 days, mortality of 2nd instar larvae was 100% for all treatments.

Laboratory studies with a new insect growth regulator proved the material to be very active against gypsy moth larvae. When tested using a number of dosages (0.073 - 0.0045 lb/ac), mortality was 100% after 8 days with all treatments. The material continued to give 100% mortality after exposure to 1.0 inches of rainfall. The experimental material (CME-13401) was supplied by EM Chemicals.

Two experimental formulations from Ciba-Geigy were studied in the laboratory. A Bacillus thuringiensis formulation (CGA-105623) was bioassayed using 3 dosages and compared to Dipel 4L. Five dosages of an insect growth regulator (CGA-112913) were bioassayed.

Table 29. Percent mortality of 2nd instar larvae 3 and 6 days after placement onto oak foliage treated with CGA-112913.

	Mortality	
lbs/ac	3 days	6 days
0.06	42	99
0.03	50	97
0.015	34	90
0.0075	29	42
0.0037	8	14
Check	0	0

Table 30. Percent mortality of 2nd instar larvae 4 days after placement onto oak seedlings treated with CGA-105623 and Dipel 4L.

	CGA-105623	Dipel 4L
BIU/ac	4 days	4 days
8.0	28	51
4.0	32	24
2.0	22	7
Check	0	0

BASF Wyandotte Corporation insect growth regulator, Lab 136733I, gave 100% mortality of 2nd instar larvae 7 days after placement onto oak seedlings at dosages of 1.0 - 0.03 lb/ac. After exposure to 1.0 inches of rain, mortality continued to be high. Dosages below 0.03 lb/ac were not as effective, especially when exposed to rain.

Depart EC and Depart EC + Sevin oil were tested on oak seedlings using 2nd instar gypsy moth larvae as test insects. After 5 days, mortality was 90% with most treatments. There was no difference in activity between formulations. After 7 days of aging, high dosages continued to give good kill. Material was furnished by Applied Research Technologies International, Inc.

Tree band material from Applied Research Technologies International, Inc. was also bioassayed using instars II - V. After an exposure time of 5 seconds, all test insects were dead 24 hours later. Activity continued to be good after aging tree band material inside and outside for 1 week.

The insect growth regulator, RO-13-5223 of Maag Agrochemicals was tested at 3 dosages, 0.5 lbs., 0.25 lbs. and 0.06 lbs. AI/acre. Two-hundred 2nd instar gypsy moth larvae were allowed to feed on treated foliage at each dosage for a period of 6 days. At that time they were removed to artificial diet and reared through to F1 generation. A check consisting of 200 test insects was also included. Little mortality occurred in the larval stage. A large number of pupae were deformed as a result of the high dosages. The final check on the F1 generation will not be made until mid 1983.

Table 31. Mortality of 2nd instar larvae 7 days after placement onto seedlings treated with RO-13-5223.

lb/ac	Mortality
0.5	6
0.25	2
0.06	0
Check	0

Table 32. Status of larvae exposed to RO-13-5223 at normal pupation time.

lb/ac	Total larval mortality	Deformed pupae	Eggs for F1 generation
0.5	153	29	6
0.25	134	26	20
0.06	90	10	50
Check	118	12	35

A number of materials were bioassayed for the New York Department of Environmental Conservation. Results are presented in tables 33 - 36.

Table 33. Percent mortality of larvae 2 days after placement onto seedlings treated with Dylox 1.5 oil.

lb/ac	Sample number 3203	3204
1.5	100	100
0.75	100	100
0.37	98	98
0.18		99
Check	0	0

Lindane sample 3210 was bioassayed at 1.6 - 0.1 lb/acre and all dosages gave 100% mortality after 2 days.

Table 34. Percent mortality of larvae 2 days after placement onto seedlings treated with Malathion sample 3209.

lb/ac	Mortality
2.5	100
1.25	100
0.625	99
0.312	98
0.156	79
Check	1

Dimilin samples A and B (NY) were tested at dosages of 0.06 - 0.3 lb/acre and gave 100% mortality after 6 days with all treatments.

When tested at 5.0 - 0.37 lb/acre, Malathion sample 3207 (NY) gave 100% mortality at all dosages, 3 days following treatment.

Table 35. Percent mortality of 2nd instar larvae 4 days after placement onto seedlings treated with NY Thuricide 16B and laboratory stock.

	Sample		
BIU/ac	NY-3201	NY-3202	Lab
12	95	98	98
8	97	91	89
4	93	86	81
2	88	70	76
Check	0	0	0

Table 36. Percent mortality of larvae 6 days after placement onto seedlings treated with 3 Methoxychlor formulations.

	Sample		
lb/ac	NY-3206	NY-3211	Lab
2.6			73
2.0	91	83	
1.3			31
1.0	55	49	
0.65			15
0.5	46	36	
0.32			9
0.25	25	6	
0.16			1
Check	0	0	0

A number of tests were conducted with various Sevin formulations. A number of dip tests with Sevin XLR and Sevin UCSF 27 were completed. Dip tests were used to simulate ground applications (hydraulic).

Table 37. Percent mortality of 2nd instar larvae 5 days after placement on seedlings treated with 0.25 lb/ac of Sevin 80S and sticker and 1.0 inches rain.

Treatment	Mortality	
	No rain	1.0" rain
Std. Target	1	
Super Target	4	
Sevin 80S	95	5
Sevin + 1% Std. Target	100	0
Sevin + 3% Std. Target	96	0
Sevin + 1% Super Target	96	7
Sevin + 3% Super Target	98	4
Check	0	0

Sevin XLR and Sevin UCSF-27 were sprayed onto oak foliage using 0.5 - 5 lbs/acre in 100 gallons of water. Treatments were then bioassayed using 2nd instar gypsy moth larvae. Treatments were exposed to 1.0 inches of rainfall. After 4 days, mortality ranged from 90-100% with all treatments.

Dip tests were conducted with Sevin XLR and Sevin UCSF-27 using oak seedlings and 2nd instar larvae. Twenty-five dilutions were tested with each formulation. Each mix was tested with and without 1.0 inches of rain. All dosages up to a (1 x 1000) mix gave 90-100% mortality after 4 days, even when exposed to rain. Percent mortality of larvae placed onto treated plants exposed to rain dropped to the 20-50% range with dosages of (1 x 1600 - 1 x 3000). Treatments not exposed to rain continued to give 80-100% mortality at (1 x 1600 - 1 x 3000).

Dip tests were also conducted with Sevin 80S, Sevin XLR, Sevimol, Sevin 5 Aqua, Sevin SL, Sevin 4 Flowable (Stauffer) and Sevin 4 flowable (Union Carbide). After 6 days, all formulations gave 98-100% mortality of 2nd instar larvae.

Table 38. Percent mortality of larvae 4 days after placement onto seedlings dipped into a solution of 1.0 lb/100 gal/ac of Sevin XLR and various stickers weathered with 1 and 3 inches of rain.

Sticker 3%	Inches rain		
	0	1.0	3.0
-	99	99	85
TS 100		69	100
TS 85			70
RA 1990		88	98
Acrylocoat		98	100
Check	0	0	0

Table 39. Percent mortality of larvae 4 days after placement onto seedlings dipped into a solution of 1.0 lb/100 gal Sevin S Aqua (Miller) and stickers and exposed to 1 and 3 inches of rain.

	Inches rain		
Sticker 3%	0	1.0	3.0
-	100	97	100
TS-85			80
TS-100		63	99
RA-1990		99	100
Acrylocoat		97	88
Check	0	0	0

Table 40. Percent mortality of larvae 4 days after placement onto seedlings dipped into a solution of 1.0 lb/100 gal of Sevin 80S and stickers and exposed to 1-3 inches or rain.

	Inches rain		
Sticker 3%	0	1.0	3.0
-	100	99	82
TS-85			82
TS-100		70	100
RA-1990		96	100
Acrylocoat		94	63
Check	0	0	0

Table 41. Percent mortality of larvae 4 days after placement onto seedlings dipped into a solution of 1.0 lb/100 gal of Sevimol and various stickers and exposed to 1-3 inches of rain.

	Inches rain		
Sticker 3%	0	1.0	3.0
-	100	96	81
TS-85			81
TS-100		71	99
RA-1990		99	93
Acrylocoat		94	52
Check	0	0	0

Table 42. Percent mortality of larvae 4 days after placement onto seedlings dipped into a solution of 1.0 lb/100 gal of Sevin SL and various stickers and exposed to 1-3 inches of rain.

Sticker 3%	Inches rain 0	1.0	3.0
-	97	97	54
TS-85			59
TS-100		68	100
RA-1990		99	94
Acrylocoat		96	100
Check	0	0	0

Table 43. Percent mortality of larvae 4 days after placement onto seedlings dipped into a solution of 1.0 lb/100 gal of Sevin 4 Flowable (Stauffer) and various stickers and exposed to 1-3 inches of rain.

Sticker 3%	Inches rain 0	1.0	3.0
-	100	83	44
TS-85			71
TS-100		75	100
RA-1990		98	98
Acrylocoat		89	60
Check	0	0	0

Table 44. Percent mortality of larvae 4 days after placement onto seedlings dipped into a solution of 1.0 lb/100 gal of Sevin 4 Flowable (Agway) and various stickers and exposed to 1-3 inches of rain.

Sticker 3%	Inches rain 0	1.0	3.0
-	98	84	66
TS-85			73
TS-100		79	100
RA-1990		93	96
Acrylocoat		94	54
Check	0	0	0

Table 45. Percent mortality of larvae 3 days after placement onto seedlings sprayed with 0.5 - 1.0 lbs/ac of Sevin FR and various stickers and exposed to rain.

		Inches rain				
Sticker 3%	lb/ac	0	1.0	2.0	3.0	4.0
-	1.0	88	78	55	60	44
TS-30						54
TS-85						62
TS-100						52
RA-1990						77
Acrylocoat						83
-	0.5	62	48	14	17	10
TS-30						11
TS-85						34
TS-100						37
RA-1990						49
Acrylocoat						40
Check		0	0	0	0	0

Project Number: GM 1.1.1
Project Title: Field Studies with Bacillus thuringiensis and SIR-8514
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leaders: W. H. McLane and J. A. Finney

SIR-8514 was studied on large scale spray plots in southcentral Pennsylvania. The test was designed to determine how this insect growth regulator would perform when used on large acreage. During the past 3 years this Mobay product has been established as very effective against gypsy moth larvae, and field studies conducted in 1980 demonstrated superior efficacy at low dosages aerially applied.

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

Dosage (lb/ac)	Pretreatment egg masses/acre	Posttreatment egg masses/acre	Percent egg mass change
0.06	117	0.5	- 99.5
0.03	153	114 1/	- 25
0.01	735	60	- 91.8
Check	285	3,426	+ 1,102

1/ Nearly all egg masses were found on 5 points in one plot, indicating a spray skip. Eliminating those points from the calculation yields a -96 percent population density change.

With the cooperation of the Pennsylvania Department of Environmental Resources we were able to locate suitable treatment areas near New Germantown, Pennsylvania, in Tuscarora State Forest, Perry County for SIR 8514 tests in 1981.

From its original registration, B. thuringiensis formulations used for gypsy moth suppression have been more costly than chemical insecticides used for the same purpose. It is recommended in the label that 2 applications of B. thuringiensis be made 7 to 10 days apart, adding to the cost of treatment. All registered chemical insecticides are applied only one time, normally resulting in good foliage protection and population control. With 2 applications of B. thuringiensis at 8.0 BIU/acre each, foliage protection is typically erratic and egg mass reduction rare. Because of these reasons, few government officials supported the use of B. thuringiensis for treating gypsy moth infestations. Such materials as Sevin®, Dylox®, Orthene® and Dimilin® were generally used.

Field work was conducted with Thuricide® (Sandoz) to determine the most effective treatments to be used against 2nd instar gypsy moth larvae. Cost could be reduced if effective control (foliage protection) would be obtained with a single application or a double application of less than the presently recommended amount of active ingredient.

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

Pennsylvania SIR-8514 Studies

Six 1,000 acre plots were established such that all 4 corners were adjacent to or near roads or pipelines. Treatment areas were rectangular in shape and were marked at each corner with orange surveyer tape. Tags containing plot information were attached to each corner tree.

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

Table 2. Prespray egg mass counts in plots to be treated with 0.6 lb AI/ac, SIR-8514 and check plots.

Plot No.	Egg masses/acre
1	1,632
2	1,521
3	843
C1	2,252
C2	213
C3	279

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

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

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

The aircraft was equipped with 18 flat fan nozzles, No. 8004. The spray boom was 90° to the aircraft slipstream and a pressure of 40 PSI was used. Application was at 120 mph in 60 foot swaths, 20 feet above the foliage. Plot corners were marked with kytoons and radios were used to communicate between ground crews and aircraft.

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

Table 3. Postspray defoliation in Pennsylvania SIR-8514 plots. Results are an average of 25 readings per plot.

Plot number	Percent defoliation
1	18.25
2	11.75
3	10.5
C1	99
C2	60.5
C3	34.5

Postspray egg mass counts were made in all plots using the same system as described earlier. However, due to limited funding, only 20 prism points were surveyed in each plot.

Table 4. Egg mass data from SIR-8514 experimental spray plots.

Plot number	Pretreatment egg masses per acre	Posttreatment egg masses per acre	Percent egg mass change
1	1632	99	- 94
2	1521	0	- 100
3	843	5	- 99
Check - 1	2252	0	- 100
Check - 2	213	3469	+ 1529
Check - 3	279	4355	+ 1461

As expected, SIR-8514 gave excellent foliage protection and population reduction. When our aerial defoliation observation flight was made in early July, the areas surrounding treated plots were near complete defoliation in contrast to treated plots which were very green. The average population reduction of 98 percent indicates that SIR-8514 is as effective as Dimilin when used in an operational program. The material handles and mixes very well and could be mixed directly in most small aircraft. Populations in check C-1 collapsed in this study, and it is interesting that little if any defoliation occurred there in 1980.

We again observed that if SIR-8514 is allowed to set in a nurse tank for one or more hours excessive separation of water and material takes place. The material will settle to a medium to hard layer on the bottom of the nurse tank and will require strong agitation before all material will go back into suspension.

Massachusetts Bacillus thuringiensis Studies

With a compass, topographical maps, rope and surveyor tape, 27 fifty acre plots were established and allocated to the treatments listed in Table 5. Boundary lines were surveyed and marked in orange tape and each corner tree was marked with double orange tape and a tag identifying corner and plot number. Minimum distance between plots was 400 feet. Plots were located in such a manner as to have a maximum number of corners on or near roadways.

Table 5. Experimental design used to evaluate Thuricide at various dosages and application frequencies. Treatments were applied in 1 gal/ac and acrylocoat was included in the mix at 3% by volume.

Material	Dosage BIU/ac	Number applications	Number plots
Thuricide 16B	4.0	1	3
"	"	2	3
Thuricide 16B	8.0	1	3
"	"	2	3
Thuricide 16B	12.0	1	3
"	"	2	3
Thuricide 32BX	8.0	2	3
Check	-	-	6

Within the center 10 acres of each plot, 20 prism points were established, 5 points on 4 parallel lines. No markers were used to identify lines or prism points within individual plots. In March, prespray egg mass counts were made on each prism tree and within each fixed radius plot (Table 6). Egg masses were collected from each plot and returned to the laboratory for hatchability tests (Table 6).

Table 6. Prespray egg mass counts in 1981 Bacillus thuringienses treatment and check plots. Average of 3 treatment plots per rate and 6 for check.

Dosage BIU/ac	Applications	Pretreatment egg masses per acre	Percent hatch
4.0	1	2,812	84
"	2	3,087	74
8.0	1	2,678	84
"	2	2,193	90
8.0 1/	2	2,852	91
12.0	1	2,135	86
"	2	1,481	87
Check	-	2,966	87

1/ Formulation used was Thuricide 32BX

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

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

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

No mixing problems were encountered with Thuricide 16B or Thuricide 32BX. We did encounter problems in using Acrylocoat as a sticking agent. When adding Acrylocoat to the Thuricide and water in the nurse tank, we observed a small number of lumps in the material. After using the first 5 gallon pail of sticker, we screened the remaining pails as they were added to the mix. Although we did not experience any clogging of nozzles or screens in the aircraft system we did experience some buildup of sticker on screens after 300 to 400 gallons of material were dispersed. However, when we tried to apply 12.0 BIU/acre we had complete clogging of 50 mesh nozzle screens after only 3 swaths of 0.25 miles each. Apparently this formulation was so thick that the sticker did not mix well. The material was applied with no further problems after the nozzle screens were removed.

If Acrylocoat is to be used, an in-line 50 mesh screen should be used on the nurse tanks and in the aircraft. All nozzle screens should be removed except for those at each end of the spray boom. After dispersing about 500 gallons, all screens should be cleaned and/or replaced. Material should be screened through a window screen as it goes into the nurse tank.

Postspray defoliation surveys were made in all spray plots and checks at the time of peak defoliation (Table 7). Defoliation estimates were made at each prism point by experienced personnel. Final estimates were based on all trees within the fixed plots.

Table 7. Postspray defoliation in Thuricide spray plots and checks. Average of 3 plots.

Dosage BIU/ac	Number applications	Percent defoliation
4.0	1	25
"	2	41
8.0	1	42
"	2	38
12.0	1	8
"	2	5
8.0 1/	2	29
Check	-	70

1/ Thuricide 32BX

Following fall foliage drop, postspray egg mass counts were made in each spray plot and check. The same technique was used as described earlier for prespray egg mass counts.

Table 8. Defoliation and percent egg mass change in 1981 Bacillus thuringiensis spray plots. Average of 3 replications.

Treatment BIU/ac	Number treatments	Percent defoliation	Percent egg mass change
Thuricide 16B 4.0	1	25	+ 55
Thuricide 16B 4.0	2	41	+ 11
Thuricide 16B 8.0	1	42	+ 31
Thuricide 16B 8.0	2	38	+ 37
Thuricide 16B 12.0	1	8	- 11
Thuricide 16B 12.0	2	5	- 38
Thuricide 32BX 8.0	2	29	+ 17
Check		70	+ 75

Bacillus thuringienses treatments in Massachusetts demonstrated clear differences between dosages and numbers of applications. Two applications at 4.0 BIU/acre were as effective as single or double treatments of 8.0 BIU/acre. There were no differences in foliage protection and/or egg mass reduction between single or double applications of 8.0 BIU/acre. We, therefore, may be accomplishing little by applying a second application of 8 BIUs. The most significant observation is the clear cut difference between both single and double applications of 12.0 BIU /acre as compared to a double treatment of 8.0 BIU/acre. For foliage protection, a single application of 12.0 BIU/acre should be recommended in the future. This would be much less costly and feasible to future users. Although good foliage protection can be achieved by a single application of 12.0 BIU/acre, population reduction (based on egg mass reduction) will normally be less than that achieved with chemical insecticides. Data indicate that if additional egg mass reduction is required 2 or more applications must be used when treating a building population. Thuricide 32BX was as effective as 16B, but since Thuricide 32BX is much more flowable, it is a superior material to work with when temperatures are cool.

Acrylocoat, a Rhom & Haas Chemical Company material, was used as a sticker with all treatments. Acrylocoat was added as the last ingredient to the final spray mix within the nurse tank. It was screened through a window screen as it entered the nurse tank. Medium agitation was taking place in the nurse tank as the sticker entered the formulation. Although we had problems with using Acrylocoat, we do feel that they can be corrected if procedures discussed earlier in this report are followed.

Laboratory data indicate that stickers should be used with all commercial B. thuringiensis formulations when used for gypsy moth suppression. Materials such as Rhoplex B60A, Rhoplex AC 33NP, Acrylocoat, RA-1990, Polyco-2142, Polyco-2149, TS-30, TS-85 and TS-100 are suitable for use with B. thuringiensis. When using such stickers, it is critical that procedures outlined earlier in this report be closely followed.

Project Number: GM 2.1.1
Project Title: 1982 Field Studies with Aerial Applications of Bacillus thuringiensis and Sevin, and Mist Blower Applications of Sevin, Turcam, SIR-8514, Pounce and Avermectin B.
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leaders: W. H. McLane and J. A. Finney

Field tests during 1981 demonstrated that single applications of Bacillus thuringiensis can be very effective when used against young gypsy moth larvae. When applied to foliage by aircraft a single 1 gallon application of 12.0 BIU/acre prevented defoliation and, to a limited extent, lowered egg mass populations. If this type of treatment is reliable, application cost would be cut in half and total active ingredient would be reduced by 4.0 BIU/acre.

Experimental aerial applications on 50 acre plots during 1982 were designed to continue the study of single and double applications of 2 Bacillus thuringiensis products, Dipel® and Thuricide®. New 48 BIU/gal formulations were also tested and two formulations of Sevin were compared. Five materials were also tested on small plots treated by mist blower (Table 2).

Table 1. Experimental materials tested using aerial application during 1982.

Material	Dosage (BIU/ac)	Rate (oz/acre)	No. plots
Dipel 4L	8.0 1/	128	3
Thuricide 32LV	8.0 1/	128	3
Dipel 4L	12.0	128	3
Dipel 4L	16.0	128	3
Dipel 6L	12.0	64	3
Thuricide 32LV	12.0	128	6
Thuricide 32LV	16.0	128	3
Thuricide 32LV	12.0	48	3
Thuricide 32LV	12.0	64	3
Thuricide 48B	12.0	64	3
Sevin FR	0.75 2/	30	3
Sevin 4-oil	0.75 2/	30	3
Check	-		6

1/ Two applications made 12 days apart.
2/ lbs per acre

Table 2. Materials and dosages applied by mist blower at a rate of 5 gal/acre.

Material	Dosage (lb/ac)
Avermectin B	400 mg
Pounce	0.005
	0.0075
	0.01
Sevin	1.0
SIR-8514	0.007
	0.015
	0.031
	0.062
	0.125
	0.25
Turcam	0.03
	0.06
	0.12

With the cooperation of the Pennsylvania Department of Environmental Resources suitable treatment areas were located at Moshannon Forest District, Clearfield, Pennsylvania. The area had a population ranging from 985 to 2,085 large egg masses per acre. Little defoliation had occurred in the area during 1981.

Forty-seven 50 acre aerial plots were established during March and April. Boundary lines were surveyed and marked with orange surveyor tape. Each corner tree was identified by 2 orange ribbons and a tag with plot information. When possible plots were located in such a manner as to have a maximum number of corners on or near roadways.

At the time of plot establishment 100 egg masses were collected each above and below the snow line for hatch tests. In general hatch was very low. In the northern area (Plots 23-48) hatch only averaged about 40 percent. Hatch of 50-60 percent was observed in egg masses collected in the southern area (Plots 1-22). It was estimated that hatch below the snow line was 60 percent and, above, 20 percent.

Within the center 10 acres of each plot 20 prism points were established, 5 points on 4 parallel lines. In plots 1-22 prism lines and points were marked with pink and blue surveyor tape. Lines and points within plots 23-48 were not identified with ribbon.

Prespray egg mass counts were made at each prism point during March and April. Prism trees were identified and egg counts made on each tree. Two hundredth acre fixed radius plots were also checked for egg masses at each of the 20 points in each plot.

Forty-two ground plots, each 200' x 200', were also established for application of experimental materials with a mist blower. Plots were separated by 200 feet and were at least 600 feet from any water. Two prism points were established near the middle point of each plot. Near each point a 100 foot line was established, marked with white ribbon and used to make postspray larval counts. Ten oak trees in each plot also had burlap bands placed around them to aid in evaluating treatment effects. During April, prespray egg mass counts were made at each prism point. Egg masses were counted on fixed radius plots and also on prism trees.

All spray plots receiving 2 aerial applications were treated first. A second application was applied 12 days later. Because of time, weather, and other logistical factors the aerial application schedule resulted in all single applications of Dipel being sprayed first followed by all single applications of Thuricide. Dipel treatments were applied May 25th when larvae were mainly 2nd instar. Single applications of Thuricide started on May 31st and were completed June 7th. At this time larvae were 3rd and 4th instar. Additionally, heavy rain followed most Thuricide applications.

Table 3. Application dates and insect stage at time of treatment.

Material	Dosage (BIU/ac)	Application date	Larval stage
Dipel 4L	8.0 1/	5/25 - 6/7	2nd - 4th
"	12.0	5/25	2nd
"	16.0	5/25	2nd
Dipel 6L	12.0	5/25	2nd
Thuricide 32LV	8.0 1/	5/24 - 6/7	2nd - 4th
"	12.0	6/2	3rd + 4th
"	16.0	6/3	3rd + 4th
"	12.0	6/3	3rd + 4th
"	12.0	6/3	3rd + 4th
Thuricide 48B	12.0	6/7	3rd + 4th
Sevin 4-oil	0.75 lbs	6/8	3rd + 4th
Sevin XLR	0.75 lbs	6/7	3rd + 4th
Check	-	-	-

1/ Applied 2 times 12 days apart.

Table 4. Larval stages at various times during treatment.

Date	PERCENT LARVAL INSTAR			
	I	II	III	IV
5/28	2	46	49	3
6/1	5	15	75	5
6/3	1	4	85	10

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

Spray materials were mixed in a nurse tank before loaded into the spray aircraft. The nurse tank was thoroughly cleaned between formulation changes. The sticker Polyco 2142, from Borden, was added through window screen to the tank mix last. All mixes were blended by agitation for at least 10 minutes before loading.

Ground plots were treated with a John Bean Roto Mist truck mounted blower. Treatments were made when larvae were in 2nd - 4th instar. Following ground treatments, larval counts were made on a weekly basis along a 100 foot line and under burlaps in each plot.

At the time of peak defoliation (June 28 - July 2) defoliation estimates were made in all plots. Ten estimates were made at prism points in each aerial plot and 2 in each ground plot. All plots were also observed and photographed by air.

Table 5. Aircraft and boom characteristics.

Material	Dosage (BIU/ac)	Rate (oz/ac)	Nozzle size	Number nozzles	Screens
Dipel 4L	8.0	128	8006	24	50 mesh
Dipel 4L	12.0	128	8006	24	None used
Dipel 4L	16.0	128	8006	24	In end nozzles only
Dipel 6L	12.0	64	8006	11	In end nozzles only
Thuricide 32LV	8.0	128	8006	24	In end nozzles only
Thuricide 32LV	12.0	128	8006	24	In end nozzles only
Thuricide 32LV	16.0	128	8006	22-24	In end nozzles only
Thuricide 32LV	12.0	48	8006	9	In end nozzles only
Thuricide 32LV	12.0	64	8006	11	In end nozzles only
Thuricide 48B	12.0	64	8006	12	In end nozzles only
Sevin FR	0.75 lbs	30	8004 8006	5	None used
Sevin 4-oil	0.75 lbs	30	8004	8	50 mesh

Table 6. Percent defoliation in aerial spray plots. Values are the average of 10 observations per plot with 3-6 plots per treatment.

Material	Dosage BIU/ac	Percent defoliation
Dipel 4L	8 1/	10
"	12	12
"	16	8
Dipel 6L	12	8
Thuricide 32LV	8 1/	8
"	12	14
"	16	34
"	12	29
"	12	28
Thuricide 48B	12	30
Sevin 4-oil	.75 lb	13
Sevin FR	.75 lb	15
Check	-	51

1/ Two applications 12 days apart.

Table 7. Percent defoliation in plots treated by mist blower.

Material	Dosage (lb/ac)	Percent defoliation
Avermectin	400 mg	58
Pounce	0.01	5
	0.007	5
	0.005	5
Sevin	1.0	15
SIR 8514	0.25	32
	0.125	12
	0.062	18
	0.031	15
	0.015	12
	0.007	25
Turcam	0.12	28
	0.06	12
	0.03	28
Check		70

Following the ground applications, post spray larval counts were made under burlaps and on 100 foot lines running parallel to the road. Results are summarized in the following tables.

Table 8. Larval string counts in plots at intervals following treatment.

Material	Dosage (lb/ac)	5/26	5/28	6/1	6/4	6/8	6/14	6/21	6/23	6/28
Avermectin B	400 mg		51 1/			142	94	82	90	99
Pounce	0.01		18 1/	0	0	0	0	0	0	0
	0.0075		24 1/	4	1	0	0	0	0	3
	0.005		70 1/	2	4	2	1	3	2	6
Sevin	1.0		118 1/	38	30	70	81	89	48	34
SIR-8514	0.25	99 1/	59	51	27	26	38	17	11	1
	0.125	148 1/	102	110	49	33	15	29	23	8
	0.0625	108 1/	80	39	23	33	18	20	17	8
	0.031	144 1/	78	43	46	56	74	85	66	55
	0.015	158 1/	80	70	22	75	83	97	55	
	0.007	156 1/	51	41	29	55	10	9	7	
Turcam	0.12		72 1/	71	89	106	76	104	110	
	0.06		53 1/	125	80	141	100	117	87	
	0.03		120 1/	61	55	87	66	168	151	
Check		160	143	345	255	1249	907	1310	1084	

1/ Prespray larval counts.

Table 9. Larval counts under burlap bands in plots treated with ground equipment.

Material	Dosage (lb/ac)	6/4	6/11	6/15	6/21	6/25	6/28
Avermectin	400 mg		129	221	309	210	1034
Pounce	0.01	3	3	1	4	6	5
	0.0075	3	4	6	23	13	30
	0.005	6	4	9	7	6	12
Sevin	1.0	61	47	65	71	52	116
SIR-8514	0.25	19	18	26	14	8	6
	0.125	36	23	39	20	12	17
	0.0625	28	17	43	16	12	9
	0.031	30	17	64	37	42	120
	0.015	45	14	103	42	66	48
	0.007	31	24	55	43	36	38
Turcam	0.12	56	51	193	105	96	234
	0.06	57	25	63	105	50	143
	0.03	87	9	30	83	68	180
Check	-	106	490	663	648	780	2322

Post spray egg mass counts were conducted using the prism point method in all plots during October, 1982. Two points were done in ground treated plots and either 10 or 20 in aerially-treated plots. Results are given in Table 10.

Table 10. 1982 experimental spray plot egg mass data from aerially treated plots. Twenty points were sampled in each plot except as indicated.

Material	Dosage (BIU/ac)	Rate (oz)	Prespray EM/ac	Postspray EM/ac	Percent change
Dipel 4L	8.0	128	1540	126	- 92
"	12.0	128	1784	120	- 93
"	16.0	128	2085	188	- 91
Dipel 6L	12.0	64	1171	116 1/	- 90
Thuricide 32LV	8.0	128	1580	310	- 80
"	12.0	128	1779	631	- 65
"	16.0	128	2277	801	- 65
"	12.0	48	1004	391 1/	- 61
"	12.0	64	760	592 1/	- 22
Thuricide 48B	12.0	64	985	388 1/	- 61
Sevin 4-oil	0.75 lbs	30	886	135 1/	- 85
Sevin FR	0.75 lbs	30	1577	473 1/	- 70
Check	-		1693	775	- 54

1/ Post spray egg mass counts made at 10 points.

Table 11. 1982 experimental spray plot egg mass data (ground plots).

Material	Dosage (lb/ac)	Prespray EM/ac	Postspray EM/ac	Percent change
Avermectin B	400 mg	4070	2886	- 29
Pounce	0.01	148	190	+ 28
	0.0075	217	116	- 47
	0.005	257	103	- 60
Sevin 80S	1.0	667	473	- 29
SIR-8514	0.25	2770	378	- 86
	0.125	3007	70	- 98
	0.062	1312	93	- 93
	0.031	4160	295	- 93
	0.015	798	354	- 56
	0.007	917	114	- 88
Turcam	0.12	1504	1270	- 16
	0.06	1575	1016	- 35
	0.03	730	1013	+ 39
Check		2229	2143	- 4

Aerial Plots

When applied to the proper insect stage B. thuringiensis demonstrated once again that good foliage protection can be achieved using single or double applications. Results show single applications at 12.0 BIU/acre are as effective as double treatments of 8.0 BIU/acre. When dosage was increased to 16.0 BIU/acre little additional foliage protection was achieved. Properly timed applications of B. thuringiensis, regardless of formulation used, gave as good foliage protection as Sevin formulations that were tested. Dipel 6L resulted in good foliage protection when applied at the volume of 0.5 gallons per acre. Most treatments with Thuricide 32LV resulted in slightly more defoliation presumably because of advanced insect development at the time of application. When applied at the same time as Dipel, results were similar, as evidenced by the double application of Thuricide 32LV at 8.0 BIU/acre.

Sevin formulations resulted in similar degrees of foliage protection. It is to be noted, however that tests were conducted at reduced dosage and rates of application. Sevin 4-oil gave somewhat better population reduction than Sevin FR. However, prespray counts were also lower in those plots.

Because of the limited amount of spring egg hatch in most spray plots it is difficult to evaluate treatments based on egg mass counts along. We can say that postspray egg mass counts reflect defoliation data. Egg mass reduction was much greater with treatments that were applied at the proper time and control declined as insects became larger. It is extremely interesting to note that properly timed B. thuringiensis application caused a reduction in egg mass density greater than Sevin. This is most likely due to the reduced dosage of Sevin and the fact that insects were larger at the time of application. Lower rates of B. thuringiensis also resulted in encouraging egg mass reduction.

Ground Plots

All ground treatments except Avermectin resulted in acceptable foliage protection. All materials except Avermectin were applied to 2nd and 3rd instar gypsy moth larvae whereas Avermectin was applied to 3rd and 4th instar insects. However, when tested in the laboratory against large larvae, Avermectin was very effective. Results may have been due to a poor formulation and heavy rainfall shortly after application.

String counts in most cases do not support defoliation findings. Pounce gave good larval reduction. Turcam and Sevin string counts did not drop during the days following treatment. Avermectin counts also stayed high following treatment. SIR 8514 counts decreased with the 3 high dosages.

Burlap band counts were high and increased in Avermectin, Sevin, Turcam and low doasage SIR-8514 plots. Pounce treatments and high dosages of SIR-8514 maintained low larval counts.

Handling Characteristics of Materials

All formulations tested handled well in preparing them for application. Forty-eight BIU formulations of Dipel and Thuricide were very flowable and easy to work with. Calibration should be checked very closely as most B. thuringiensis mixes will flow faster than water. It would be advisable to use a flowable or EC formulation of Turcam.

Sticker

Two percent Polyco 2142 was used with all B. thuringiensis treatments. When pouring from 5 gallon cans we found large pieces of hard material in each container. We also found that due to pump shear action, we had a fast buildup of sticker on nozzle screens. After 300 to 400 gallons were dispensed, buildup was such that calibration could be affected and/or complete blockage could take place.

Summary

Based on 1982 work we can recommend that single applications of 12 BIU/acre applied at the proper time in 1 gallon be used for foliage protection in building populations. Although good foliage protection is achieved, only limited population reduction should be expected. Work with lower rates look promising enough to continue this work in 1983 and effectiveness against different larval stages should be evaluated. Because 1982 results demonstrated some population reduction with B. thuringiensis, multiple application tests should be conducted on isolated infestations to determine how effective it can be as an eradication tool.

Sevin FR gave good foliage protection but less population reduction than Sevin 4-oil. Both should result in acceptable egg mass reduction at 1 lb/acre. SIR-8514 is an effective material when applied by ground equipment and should be registered for that use along with aerial use. Pounce is a very effective contact poison and should be tested at low dosages on aerial plots where migration will not be a factor. Its development should be continued for use from ground equipment.

When using recommended stickers with B. thuringiensis, the material should be added last and poured into the nurse tank through a window screen. The nurse tank and aircraft should have 50 mesh in-line screens. Nozzles should have screens only on each end nozzle of the boom. All other nozzles should be free of screens. After each 300 to 400 gallons of material dispersed, all screens should be replaced and/or cleaned with the proper cleaning agent.

Project Number: GM 8.2.2
Project Title: Radiological Sterilization of Male Gypsy Moth
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: V. C. Mastro and J. A. Argentine

The dose-age optimization test described in the last annual report has carried all treatments through three generations. F-3 egg masses are now being hatched and evaluated. Upon completion of this evaluation, data will be summarized and a final report completed. A few selected treatments will be reared through to the F-4 egg mass stage to determine if fertility is less or greater than in preceding generations.

Project Number: GM 8.2.6
Project Title: Comparison of Several Milk Carton and Delta Type Traps
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leader: E. C. Paszek

Objective

Two traps made by the Klearfold Company, a clear plastic delta type survey trap and clear plastic carton type trap were evaluated in field study. These traps are made of a sturdy plastic and should weather better than the traps made of plasticized paper.

The clear plastic delta trap tested was identical to the USDA standard delta trap. The clear plastic carton type trap was 5-3/4" x 7-5/16" x 1-5/16" in size with 2 entrance ports 1" x 1/4" in size, one above the other spaced 1/2" apart in the center of the 5-3/4" x 7-5/16" panel of the trap. The capacity of this trap was 55.1 in^3.

Method

Test traps were placed on a 6 x 3 grid with the traps spaced 50 m apart in a natural infestation. Traps were baited with 500 ug (+) disparlure and a 3.2" (3g) piece of dog flea collar (DDVP) was used as a killing agent. Delta survey traps were used as controls. They had a 58 in^2 surface area on the inside of the traps smeared with tack trap adhesive.

Traps were observed August 18-19, 1982, and moths were counted at 3:00 p.m. each day of the test.

Average Number of Moths Captured/Trap				
Date	Clear plastic delta DDVP	Clear plastic delta tack trap	Delta survey tack trap	Clear plastic carton DDVP
8/18	2	9	8	5
8/19	4	15	13	8

Results

In this limited test there was no significant difference in moth captures between the clear plastic delta with tack trap adhesive and the standard delta survey trap. The assembly of the clear plastic delta traps was more difficult than the standard delta. The storage of these traps (with the tack trap adhesive) in knocked down position would have to be studied to see if the adhesive would have a tendency to run and smear the traps. If there is no problem with the adhesion of the tack trap to the plastic trap, these traps should be studied in a large scale test to fully evaluate them as a substitute for the standard delta survey trap. They are impervious to water and should outlast the plasticized paper standard delta trap in the field, especially in a humid climate. An opaque colored plastic delta should be compared to the clear plastic delta to see if there is any difference in moth captures due to a build up of heat in the clear traps.

The clear plastic carton type trap does not have a hood; many of the moths approached the trap, flew around the entrance ports and then flew away. The hood on the standard milk carton trap aids the moths to orient to the trap's entrances. Clear carton type traps may be more efficient if they had a hood over the trap entrances.

Project Number: GM 0.2.7
Project Title: Sterile Male Trial, Berrien County, Michigan
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leader: V. C. Mastro

Introduction:

This report summarizes the results of releasing sterile male gypsy moths in a sparse isolated population over a three year period. A small amount of material reported in the last annual report is included in order to provide continuity in the description of the project and the results. The objectives of the project were to determine the degree of interaction between released sterile males and feral insects in an isolated sparse infestation and to determine the problems associated with an operational program. The following is a description of the project from initiation to the present time.

Pilot Study Areas

I. A population was detected near Benton Harbor, MI (trap density 1/mi^2) in 1978. Delimitation trapping (32 traps/mi^2 over 9 mi^2) in 1979 confirmed the existence of the population (total of 90 males were trapped) and indicated the population was isolated (all males were trapped within the center square mile). An extensive egg mass survey during the winter was negative.

In the spring of 1980 burlap bands were placed on trees in a uniform grid pattern (47 m between lines) over an area of 162 ha. One hundred and ten larvae were found under burlap bands in the area where 1979 trap catches had been the highest. The first prepupa was found under a burlap band on July 8, which triggered accurate initiation of the sterile male release. Using the larval distribution data, a weighted strategy for sterile male release was planned. In addition, to provide a buffer zone, smaller numbers of sterile males were released in the area surrounding the area of larval finds.

A grid of traps (high capacity milk carton traps) was established over the infested area for monitoring sterile:feral (S:F) male overflooding ratios and for determining feral male distribution and density. A grid of delta traps (32/mi^2) was placed around the site to determine the degree of spread, if any, from 1979 and to confirm that the infestation was isolated.

Twenty monitor females were placed daily within the area in which native insects had been found. On the day following placement, females and any resulting egg masses were collected and returned to the laboratory for determination of mating type (i.e. sterile or fertile). As a control, 25 untreated and 25 irradiated males were selected from rearing and irradiation production lots each day and mated with untreated females in the laboratory. At the end of the field season the area was searched for native egg masses. Forty-seven were found and were collected for mating-type determinations.

Trapping results indicated that a three-fold increase in the native density had occurred. In 1979, 90 feral males were captured and in 1980, 274 were captured. Overflooding ratios calculated from trap catches were lower than optimal, and on several days S:F ratios as low as 7S:1F were recorded. During the period when most native flight occurred (July 31-August 13), the average ratio was ca. 14S:1F. Data obtained from monitor females parallels trapping data. Of the 305 egg masses recovered from monitor females, 261 were from sterile and 44 were from fertile matings: ca. 85% of the mating were sterile (ca. 5.9S:1F mating ratio). Of the 47 feral egg masses found, 38 were sterile male matings and 9 were from feral male matings: ca. 81% of these matings were sterile (ca. 4.3S:1F mating ratio).

In 1980 several problems were identified, among them: smaller proportions of sterile males were recovered in monitor traps than expected (ca. 2.9% of the pupae placed were recovered as adults). At least partially contributing to this low sterile male recovery was predation by birds and vespids and a lower than expected eclosion rate from release containers. It was found that fewer pupae (maximum = 450) placed in cages on a longer rotation schedule (8 days) resulted in higher eclosion rates.

Several changes were made in the 1981 field release protocol. Density of the banding was increased in the core area from 1 to 6 bands/site to maintain adequate numbers of native insect finds with the expected reduced population density. The release container design was changed to deter bird predation, and rotation period of release containers in the core area was lengthened to 8 days. In addition, the 9 surrounding square miles were surveyed with milk carton traps instead of delta traps. This change was made because sterile males are more easily and reliably identified when they are not saturated with tangle trap. Types of control matings for monitoring females were increased and included the following crosses:

1) NJ ♂ (15 krads) x NJ ♀
2) NJ ♂ x NJ ♀
3) NJ ♂ (15 krads) x feral ♀
4) feral ♂ x feral ♀
5) NJ ♂ x feral ♀
6) feral ♂ x NJ ♀

Despite the more intensive larval survey, only 14 larvae (an 87% reduction) were located in 1981. Fifty feral males were trapped during 1981 (all in the central core area) compared to 274 in 1980; this was an 82% reduction. The overflooding ratios calculated from the trap captures in the core area averaged 162S:1F for the period of male flight between July 31 and August 13. The lowest capture ratio was 35S:1F on July 22, the first day of native male flight. Results of monitor female egg mass data have not been fully analyzed.

The burlap band larval survey (6 bands/site) in the spring of 1982 failed to locate any native insects. Release of sterile males was initiated on July 12 and continued until August 20. The daily release was decreased from ca. 10,000 to ca. 6,000 males. The recovery of sterile males in monitor traps during the 1982 release was greater than expected, 10.5%. Proportions recovered in 1980 and 1981 were lower, 2.9% and 3.5% respectively. In 1982, only 1 wild male was found. It was captured on August 5 within the core area. On the same day, 124 sterile males were captured in that trap, and the overflooding ratio for the inner trap zone (or core area) was 588S:1F. The following table presents a summary of the program.

Evaluation

Year	Activity	Monitoring Population		
		Stage	Technique	Sample Size
1978	Detection	Larva	-	-
		Adult (♂)	1 trap/mi^2	1
		Egg	inspection	0
1979	Delimitation	Larva	-	-
		Adult (♂)	32 traps/mi^2	90
		Egg	inspection	0
1980	Delimitation-Intervention	Larva	banding	110
		Adult (♂)	32 traps/mi^2	274
		Egg	inspection	47
1981	Delimitation-Intervention	Larva	banding	14
		Adult (♂)	32 traps/mi^2	50
		Egg	inspection	1
1982	Delimitation-Intervention	Larva	banding	0
		Adult (♂)	32 traps/mi^2	1
		Egg	inspection	0

The population in the study area has decreased in a predictable manner given the overflooding ratios achieved in 1980-81.

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

Year	Mean S:F Ratios	No. ♂ captured ITZ 1/ during peak flight		Estimated No. Feral ♀ 2/	Estimated No. Feral ♀♀ Reproducing
		Sterile	Feral		
1980					
observed	14.4:1	3193	237	237	
expected					15.4 3/
1981					
observed	128.6:1	3673	50	50	
expected			46.2 3/	46.2	0.36 3/
1982					
observed	5750:1	5750	1	1	
expected			1.08 4/	1.08	0.0

1/ ITZ = Inner trap zone or the area considered to be the core of the infestation.

2/ Assuming 1F:1M ratio for every feral male trapped

3/ Estimated No. of feral females X proportion of feral males = estimated No. of females reproducing

4/ Estimated No. of females reproducing X 3 (intrinsic rate of natural increase or Rm) = estimated No. of males and females the following year.

Given the results from the 1982 field program elimination of the native population should have been achieved. Plans for 1983 include only monitoring the area using standard 2 quart milk carton traps placed in the same manner as in previous three years. At the completion of the 1983 field season a final report will be submitted.

Project Number: GM 0.2.8
Project Titles: Factors Affecting Sexual Periodicity in Male Gypsy Moths
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: D. R. Lance, V. C. Mastro and T. M. ODell

In 1977-1978 field trials, laboratory-reared (LR) male gypsy moths tended to arrive at pheromone-baited traps 1-2 h later than feral males. In 1980 actograph and field trials, we discovered that it was possible to advance the phase of male sexual rhythms by holding the males at cool temperatures as pupae (e.g. 20°C vs. the normal 25°). Thus, we now have a potential method for synchronizing the activity rhythms of sterile males with those of feral males in target populations.

A number of problems remain to be solved before this technique can be integrated into a sterile male program:

1. Sterile males in target populations are likely to show variation in sexual periodicity due to site-to-site variation in ambient temperature. For complete synchrony, a unique rearing (holding) temperature regime may be required for each release site.

2. Exposure to cooler temperatures results in a lengthening of the pupal stage. It may be necessary to rework age/dose irradiation schedules to fit the different pupal temperature regimes.

T. ODell of the U.S. Forest Service, Hamden, CT has continued to work on the problem of male sexual periodicity. In the Benton Harbor, MI pilot study, most males eclosed ca. 6 days after being placed into the field. This 6-day "acclimation" to local temperatures appears to have eliminated some of the difference in periodicity between sterile vs. wild males. While a minor difference in periodicity was still detectable, it appears to have had only a minimal effect on the competitiveness of the sterile males. Preliminary actograph tests indicate that 8-10 days of acclimation are necessary to negate inter-strain differences in sexual periodicity. An 8-10 day field acclimation period is not practical; pupae would have to be irradiated earlier, which would decrease competitiveness and sterility in the released males. In the South Carolina pilot study, the acclimation period was even shorter (ca. 4 days) due to the warm ambient temperatures at the site which promoted rapid adult emergence. However, since South Carolina wild males also were exposed to warmer temperatures during the pupal stage, their sexual periodicity may have been similar to males which were held at 25°C (data on periodicity is not available for the S.C. study).

In summary, inter-strain differences in sexual periodicity may not pose as serious a problem as was initially suspected. In cooler SM release areas, an extended release-to-eclosion acclimation period may advance the phase of sterile male sexual rhythms, bringing them almost into synchrony with the sexual rhythms of wild males. In warmer areas, acclimation periods will be shorter, but the need for acclimation may be greatly reduced.

In other actograph tests, Hoy non-diapause males were flown against NJS males. Quantitative analysis of the data indicated that Hoy males showed lower overall activity when compared to NJS males. During selection against diapause and/or inbreeding, Hoy males appear to have acquired a reduced tendency and/or a reduced ability to fly.

A computer program to statistically compare the periodicity profiles of moths exposed to various treatments has recently been developed by Gerry Walton, U.S. Forest Service, Hamden, CT. The program affords rapid assessment of differences within and between treatments, and provides a quantitative evaluation from which specifications and tolerances for SM flight periodicities could be set.

In other tests, the timing and competitiveness of mating was measured for multiple-mating males from NJS, NJS-IR, and wild-type males. Male and female gypsy moths (24 h old) were released into field cages. After mating, both members of each pair were marked with fluorescent dust (color was dependent upon the number of times the male had mated previously). Then, the male was allowed to remate, and the female was allowed to deposit an egg mass. As of this writing, the eggs are being held for completion of diapause and subsequent eclosion.

Preliminary examination of data indicates that moths eclosing from LR pupae which have been irradiated (10k or 15k), packaged, and shipped, were fully competitive with their wild counterparts. On the average, males searched for and mated with 3 females on cool days and 4 on warm days. Some of the males mated with 5 or more females (maximum 8); their behavior was characterized primarily by relatively short rest periods, (or none at all) between matings, rather than by shorter mating periods. This "macho" behavior is a highly desirable trait and should be further characterized for possible genetic selection. The results of these tests will be covered in more detail in subsequent reports.

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, 1981 - September 10, 1982
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 environmental factors such 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 intrinsic 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 have a 2-fold purpose for investigating population quality:

(1) to quantify and explain the reasons for density-correlated shifts in population quality, and

(2) to assess the quality and competitiveness of F1-sterile larvae (treatment of male pupae with a proper dose of gamma radiation results in total sterility in the F1 generation).

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

For successful application of the F1-sterility technique, F1-sterile adults must be present when wild moths are mating. This requires synchrony in development between F1-sterile and wild individuals. Initially, F1-sterile first instars must eclose at the time when wild eggs are hatching and when young, tender foliage is available. In this study, the timing of hatch is examined in egg masses which were exposed to irradiation, rearing and chill treatments which are relevant to the development of an F1-sterile insect program.

Materials and Methods

In the spring of 1982, egg masses were rubber-cemented onto plywood boards and were held in an outdoor insectary. Each egg mass was surrounded by double-sided sticky tape to deter inter-mass movement of first instars. Newly eclosed larvae were counted and removed on (Julian dates) 90 and 105, and then daily from 109 to 152. Egg mass treatments are summarized in Table 1.

Results and Discussion

Effects of parental irradiation. Hatch of F1-sterile eggs started in synchrony with hatch in NJS x wild and wild x wild eggs. However, for F1-irradiated egg masses, the hatch period was extended and peak hatch occurred 4-7 days after the control peaks (Fig. 1). This is in contrast to a previous study (Lance et al, 1981) in which the timing of hatch was similar for NJSS egg masses vs. [IRR - 8 Krad male x NJS female] egg masses. The degree of asynchrony here is unlikely to have a serious effect upon the quality of food available to 1st instar larvae, but, when combined with similar extensions in larval development (Study #4), it could push the eclosion of F1-sterile adults past the time when most wild adults are mating.

Diapause in the NJSS strain. The timing of hatch was similar for NJI-F1 (NJS-F22) eggs vs. CSF-F1 eggs (Fig. 2). Recently, there has been some concern that the NJS strain has been aquiring abnormal diapause characteristics. If so, the abnormalities did not show up in this study. Still, in the CSF-F1 and NJI egg masses, peak hatch occurred 7 days later than peak hatch in the NJS x wild eggs.

All 3 groups were exposed to similar chill treatments (see Table 1), but CSF-P1 and NJI-P1 larvae were reared individually on B-4 diet in 180 ml cups, rather than being collected as pupae from the field. Perhaps parental nutrition and/or rearing environment also can influence the timing of hatch.

Length of the chill period. Using F1-sterility, it would be possible to release sterilized insects as eggs rather than as pupae or adults. If this were done, the timing of hatch would be greatly affected by the length of the chill period (Fig. 3). Eggs which were exposed to long or short chill periods hatched unacceptably early or unacceptably late, respectively. For the gypsy moth, the timing of hatch can be predicted (in large part) by degree-day accumulation models. The eggs appear to "accumulate" degree-days at 6-8°C; perhaps synchrony in older eggs would be improved if colder chill temperatures were used (e.g. 0 or -4°C). Also, it may be possible to improve synchrony by placing the eggs into the field as early as possible (e.g., in autumn). When eggs are placed into the field in the spring, there is a very narrow range of chill periods which result in properly timed hatch. This eliminates one of the potential benefits of releasing sterile insects as eggs (i.e., being able to produce sterile insects throughout a large portion of the year).

Study #2 - Dispersal of first instar gypsy moth larvae from F1-irradiated and normal egg masses.

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, in part, to see if first instar larvae disperse more or less frequently from trees which were or were not defoliated in the previous year. Also, F1-sterile larvae were tested against wild-type and NJS larvae to see if irradiation of the male parent affects the activity and dispersal of first instar larvae.

Table 1. For gypsy moth egg hatch synchrony tests, the egg mass treatments, larvae per egg mass and date at which 50% of the larvae had hatched.

Source of Eggs[1/]		Chill Treatment					
Male Parent	Female Parent	Chill Regime	Approximate Date Placed Into Regime (1981)	Date Placed On Board (1982)	n of Egg Masses	Mean Larvae Per Mass	Julian Date of 50% Hatch (1982)
IR 8-9D					6	260	133
IR 10-11D	wild	insectary	214	74	6	224	135
NJS					6	450	129
wild	wild	(field-collected 1982)		74	10	458	130
NJI	NJI	insectary	204	74	3	1395	136
CSF-P1	CSF-P1	insectary	210	74	3	1394	136
			244	74	4	709	114
NJS	NJS	6-8°C	244	91	4	679	115
			244	105	4	564	115
			304	74	4	794	136
NJS	NJS	6-8°C	304	91	4	827	136
			304	105	4	709	129
			364	74	4	961	147
NJS	NJS	6-8°C	364	91	4	927	147
			364	105	4	904	145

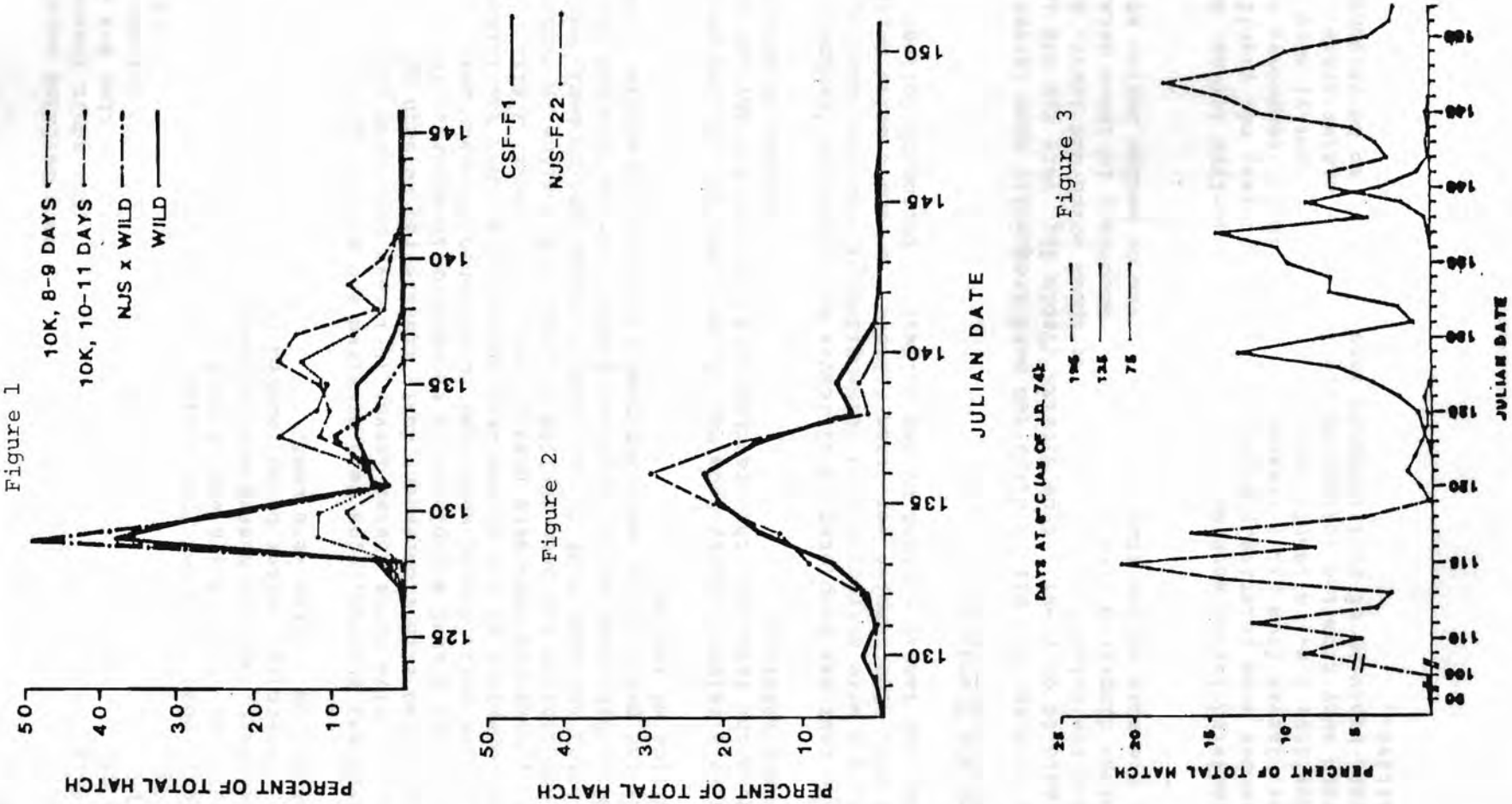
1/ IR 8-9D and IR 10-11D represent New Jersey Standard strain (NJS) males which were exposed to 10 krads of gamma radiation at 8-9 days or 10-11 days after pupation, respectively. All wild insects were collected from a healthy, building population in Forestdale, MA. NJI and CSF-P1 individuals were reared at 1 per 360 ml plastic cup on B4 diet under SLRC (25°C, 50-60% RH, 0500-2100P_D). NJI moths came from NJS-F21 eggs; CSF-P1 were reared from eggs which were field-collected 4/81 in Cadwell State Forest, Pelham, MA.

Figures 1-3. Timing of hatch for gypsy moth eggs in an outdoor insectary.

Fig. 1. Effect of parental irradiation upon the timing of hatch.

Fig. 2. Hatch of NJS-F22 eggs vs. the eggs of an F1 strain.

Fig. 3. Hatch of eggs which had been held at 6°C for 195, 135 or 75 days prior to being placed outside on J.D.74.



Materials and Methods

In August 1981, female pupae were collected from a building population in Forestdale, MA (ca. 200 egg masses/ha in 1982). The adult females were mated to NJS males which had been irradiated (10 krads) at either 8-9 or 10-11 days after pupation; the resulting egg masses were held in an outdoor insectary until April 1982. Also in April, wild egg masses were collected from both the Forestdale population and from a high density population (ca. 30,000 egg masses/ha) at the northern end of Otis Air National Guard Base. For a fifth egg treatment, NJS egg masses were allowed to overwinter (9/81-4/82) in an outdoor insectary. After being removed from the insectary, egg masses were held at 4°C until they were used.

For dispersal studies, eggs were dehaired and held at 25° until hatch commenced. Five first instar larvae (0-1 days old) from each treatment were lightly marked with fluorescent dust, transferred to each of 12 80 mm diameter plastic petri dishes and placed in an outdoor insectary. The following morning, the petri dishes were tied to branches of black oak trees, and 0.5 cm diameter openings in the dishes were uncovered so that the larvae could escape. Three branches were used in each of 4 sites: 2 sites in Sandwich where defoliation had been 90-100% in 1981, and 2 sites in Falmouth where defoliation had been <20% for at least 5 years (and probably many more). A band of double-sided sticky tape was placed 1.5-3 m from the tip of each branch; the petri dishes were attached to the branch within 10 cm of the distal side of the tape.

Larvae were released daily from May 16 until May 28. On the mornings following the releases, the branches were examined and any larvae that were found were returned to the laboratory for determination of marking.

When the test was completed, 8-12 leaves were selected "randomly" from each branch, and were extracted in a 50%MeOH:50%H_2O solution under N_2 at 80°C for 60 min. The samples were then filtered to remove solid matter and were analyzed for tannin content by Ian Baldwin at Dartmouth College.

Results and Discussion

Larvae from the NJS strain dispersed more frequently than larvae from the F1-irradiated or P1-wild type strains (Table 2). Very few NJS larvae failed to leave the release container, and, of those which did leave, a high percentage dispersed from the branches. According to these data, larvae either are more active than the other larvae or become active at an earlier age.

F1-irradiated larvae were more sluggish than wild-type larvae, but the differences were relatively minor and, in most cases, not significant. Further, larvae from all 5 treatments showed similar responses to variation in host quality; i.e., slightly (but again not significantly) more larvae dispersed away from the foliage of previously defoliated trees. In total, dispersal behavior of F1-irradiated larvae appears to be reasonably competitive.

Table 2. Percentages of 1st instar gypsy moth larvae which remained on black oak branches for 24 hours.

Strain	n of Larvae Released	Percent Failing To Leave Release Container[1]	Percent of Larvae Remaining on Branch[2]		
			Control	Previously Defoliated	Total[1]
NJSS	378	4.8a	40.8	35.6	38.7a
10 Krad, 8-9 days (F1)	248	33.1c	59.5	54.0	56.0b
10 Krad, 10-11 days (F1)	279	28.7bc	60.6	46.5	52.7b
Low density wild (P1)	301	25.3bc	51.2	41.7	45.2ab
High density wild (P1)	249	24.0b	48.9	42.4	47.4b

1/ Percentages which are not followed by the same letter are significantly different ($p < 0.05$), as determined by contingency table analysis.

2/ Percentages do not include larvae which did not leave the release container.

Table 3. Percentage of gypsy moth larvae which remained on black oak branches for 24 hours.

Site	Site Index[1]	Tree No.	Tanning (% TAE)[2]	n of Larvae Released	Percent of Larvae Remaining On Branch: By Branch	Percent of Larvae Remaining On Branch: By Site[3]
Sandwich High School	40	1	0.262	160	33.1	
(defoliated in 1981)		2	0.341	152	36.8	34.9a
		3	0.454	166	34.9	
Quaker Meeting House Road	44	1	0.478	163	31.9	
(defoliated in 1981)		2	0.500	147	40.1	34.5a
		3	0.307	157	31.8	
Crane Quail Management Area	37	1	0.455	159	47.2	
(not previously defoliated)		2	0.240	147	44.2	47.0b
		3	0.316	162	49.4	
Deer Horn Hill	33	1	0.541	158	43.0	
(not previously defoliated)		2	0.514	158	31.0	37.8a
		3	0.364	149	39.6	

1/ Height of trees in feet, based on 50-year growth of upland oaks.

2/ Hemoglobin binding capacity in Tannic Acid Equivalents.

3/ Percentages followed by the same letter are not significantly different ($p < 0.01$), as determined by contingency table analysis.

Total larval dispersal at the Crane site was significantly less frequent than it was at any of the other sites. The reasons for this are somewhat unclear. If the variation among the 4 sites was due strictly to defoliation history, then dispersal would be expected to be similar at the Crane and Deer Horn Hill sites. However, many factors are likely to affect foliage quality. Poor soil or harsh climate can stress trees, and this type of stress possibly may induce changes in the foliage similar to those induced by defoliation stress. In this study, tannin analysis showed high (i.e., stress-induced) levels of secondary chemicals in foliage from all 4 sites (Table 3; compare to Schultz and Baldwin, 1982). Also, site indices are low, indicating suboptimal growing conditions (Table 3). Thus, these results may be atypical of what would happen in sites where trees were under less stress from edaphic or climatic factors.

Study #3 - 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 necessary for developing 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.

Materials and Methods

The behavior of late instar larvae was observed in Cotuit, MA, and in an endemic site in the Ashumet area of East Falmouth, MA. At the high density site, small (7-10 mh) black oaks (*Quercus vetutina* Lam.) and white oaks (*Q. alba* L.) accounted for ca. 90% 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 completely defoliated by the time pupation was occurring. The trees at the endemic site suffered little or no noticable defoliation.

At both sites, 8-10 m high black oaks were surrounded by a 4 x 4 x 8 m h observation tower of galvanized steel pipe. At the high density site, late instar behavior was observed during the photophase, and, with the aid of a portable red light, for 1 - 1.5 hr into either end of the scotophase. In the endemic study site, larvae were observed using ambient light and a (borrowed) binocular light amplifier. The endemic larvae rested during the photophase and so observations were made primarily during the scotophase and crepuscular periods.

To assess daily patterns of feeding, resting and locomotor behavior, larvae at the high density site were observed at regular intervals throughout 2 days (every 2 hours during the photophase, and hourly during the crepuscular periods and into the scotophase). Observations were made on all larvae on the terminal 2 m of 3 branches (52-60 larvae per observation period on the first day; 66-86 per period on the second). Also, dispersal activity was measured by taking 15 minute counts of larvae which crawled past a point 4 m h on the boles of the study trees. At the endemic site, there were too few larvae to obtain meaningful data in this manner. Instead, individual larvae were observed every 5 minutes over a total of 17 larva-nights. These observations were used to estimate the percentage of time that each larva was engaged in various activities.

Results and Discussion

Description of Food-seeking and Feeding Behaviors. Basic food-seeking and feeding behaviors were fairly similar in the two populations. When a larva was searching for food, it went through the following sequence of behaviors:

1. It crawled out along a twig and came either to a leaf cluster or to the end of the twig (in the latter case, the larva turned around and went to another twig, or, on rare occasions, it dropped).

2. The larva went through a behavior which we have termed "petiole-sampling". Using its prolegs, the larva would hold onto the twig just below the base of a leaf cluster. It then would move its head back and forth, grasping individual leaf petioles with its mouthparts and thoracic legs. Normally, a larva "sampled" 1/2 - 3/4 of a cluster's petioles in this manner, spending ca. 0.5 to 3.0 seconds in contact with each. Occasionally, individual petioles were contacted 2 or more times. The reasons for this behavior are not clear, but the petiole-sampling appears to provide larvae with some sort of information, e.g. an indication of the chemical composition of the foliage or, perhaps, whether other larvae crawled out onto the leaves. Once larvae petiole-sampled a cluster, they either rejected the cluster (go back to 1) or crawled out onto one of the leaves.

3. After a larva had climbed out onto a leaf, one of a number of different things happened:

   a. The larva crawled back off of the leaf, or onto an adjoining leaf, (apparently) without sampling the foliage.

   b. More commonly, the larva would bite-test the leaf. The larva then either rejected the leaf, bit a different portion of the leaf, or commenced feeding. In both sites, larvae rarely settled to feed on the first cluster they encountered and seldom settled on the second. Some larvae consecutively sampled 10 or more leaf clusters. Recent evidence suggests that there is a good deal of intra-plant variation in leaf quality. Perhaps the first leaf cluster is rejected "automatically" but affects larval perception of (i.e., "provides a basis for comparison" with) subsequently encountered leaves.

c. Some larvae appeared to settle in to feed on leaves but then left the leaves after short periods (several seconds to several minutes). This behavior was more common in the high density site than in the endemic site. Finally, the larvae might feed on individual leaves for extended periods (up to several hours). This scheme of food-seeking certainly was not invariable; for example, larvae occasionally skipped petiole sampling or they went from cluster to cluster by crawling leaf-to-leaf rather than down one twig and up another. However, these steps provide a basic framework which all of the larvae followed.

Certain aspects of feeding behavior did vary between the 2 sites. In the endemic site, larvae fed on individual leaves for relatively long periods of time; i.e., they would feed on only 1 or 2 leaves during the course of a night. At the high-density site, extended observations (1/2 to several hours) on 16 larvae indicated that many high-density larvae also fed on individual leaves for long periods of time. However, half of these 16 larvae switched feeding sites frequently; these larvae fed on an average of 5.3 $\pm$ 2.6 different leaves per hour and rarely spent over 15 minutes feeding on any one leaf. Typically, they finally left the branch rather than settling to feed for a long period on an individual leaf. This behavior may have resulted from an inate "restlessness" in these larvae, or may have been caused by interactions between the larvae and the foliage. When feeding, larvae were rarely contacted by other larvae (in fact, this never occurred during the observations on the 16 larvae). Thus "feeding interference" from other larvae does not appear to be the cause of either the frequent changes in feeding sites or the shift to diurnal feeding behavior. Also, even as defoliation approached 70-80%, relatively few leaves were completely eaten; frequent leaf-switching may have been partially responsible for this. No attempt was made (at either site) to see if larvae preferred to feed on leaves which either had or had not been fed upon previously.

When feeding, larvae typically held onto an edge of the leaf. They repeatedly extended their heads forward, and then while chewing, slowly pulled their heads back along the edge of the leaf. Larvae (especially 6th instars) on black oak often clasped their posterior prolegs onto the petioles and fed in the 1st sinus from the base of the leaf. In the high-density site, leaves were irregularly shaped, and feeding positions were more variable. Also, when a larva did crawl onto a leaf to feed, it often constructed a very loose feeding "mat" of 2-6 silk threads strung in a zig-zag fashion across the surface of the leaf. These mats apparently aid the larvae in gripping the leaf.

Comparisons of behavior in the 2 populations. There were dramatic differences between the behavior of the larvae at the two sites. The high-density larvae remained in the foliage and were active during the day; the endemic larvae spent the photophase resting in the litter, on the boles of trees, or on the underside of the bases of large limbs. These differences agree with prior observations and indicate that the larvae in these two sites behaved fairly typically with respect to the type of population they constituted.

Our observations indicate that larvae in high density populations feed sporadically throughout the photophase (Fig. 4). The percentage of larvae which were feeding increased sharply during the crepuscular periods and then sharply dropped off again afterwards. However, this crepuscular feeding activity may have been affected by weather. On a rainy afternoon, feeding activity started to increase much earlier (1500 hours as compared to 1900 hours). Also, the feeding peak was less distinct and dropped off an hour earlier than normal (Fig. 4). During the scotophase, there appeared to be only a small amount of feeding activity; it is uncertain whether this was caused by feeding rhythms or (more likely) by the cooler night time temperatures.

Almost no high-density larvae crawled up and down the trees during the scotophase, and very few were seen crawling during the crepuscular periods. Most inter-tree dispersal occurred during the hottest parts of the day (Fig. 4).

In contrast, larvae at the endemic site were inactive during the day. Most of the travel up and down trees occurred during the crepuscular periods when larvae were crawling to or from their feeding sites. Some intra-tree dispersal also occurred during the night, since the larvae occasionally switched feeding sites.

Larger larvae spent more time feeding than did smaller larvae. At the endemic site, sixth instar larvae fed almost continuously throughout the night, spending 32.9 $\pm$ 3.0 percent of the entire day in feeding (Fig. 5). Fifth instar larvae fed only sporadically during most of the scotophase, but they did feed more or less continuously during the hour immediately before they returned to their daytime resting sites. Also, fifth instars left their feeding sites an average of 37 minutes ahead of the sixth instars. In total, the fifth instar larvae spent only 14.9 $\pm$ 5.2 percent of their day feeding. This size-dependent effect lends uncertainty to comparisons of time spent feeding between larvae in high-density and low-density populations. This uncertainty is compounded by both size gradation within instars and extranumerary molts in the high density larvae (but, being a fool, I will make a comparison anyway). Few if any high-density larvae were as large as the sixth instars from the endemic population, but many of them appeared to be at least as large as the endemic fifth instars. Using the percentages of non-molting larvae which were feeding during various times of the day, we have estimated that an average high-density late instar spent about 16% of its time in feeding. Thus, despite the fact that high density larvae remain in the foliage and do not restrict their feeding to the scotophase, an overtly high proportion of their time does not appear to be spent in actual feeding.

At least one anomaly has surfaced through research on gypsy moth population quality: in laboratory tests, most density-correlated stress factors either don't affect or prolong larval development, but gypsy moths in high density field populations reach adulthood 1-3 weeks earlier than individuals in endemic populations. Since high-density larvae spend most of their time in the foliage, one might have suspected that they are also spending a relatively high proportion of their time feeding. The larvae then could be utilizing this extra food for the rapid production of biomass. In reality, consumption rates do not appear to vary dramatically with population density, and so other factors must be involved in the observed differences in developmental rates.

Figures 4-6. Feeding rhythms of gypsy moth larvae.

Fig. 4. Patterns of feeding and activity in a high-density population.

Fig. 5. Feeding rhythms in an endemic population.

Fig. 6. Feeding rhythm of larvae which were reared in the laboratory on the foliage of previously defoliated trees vs. control trees.

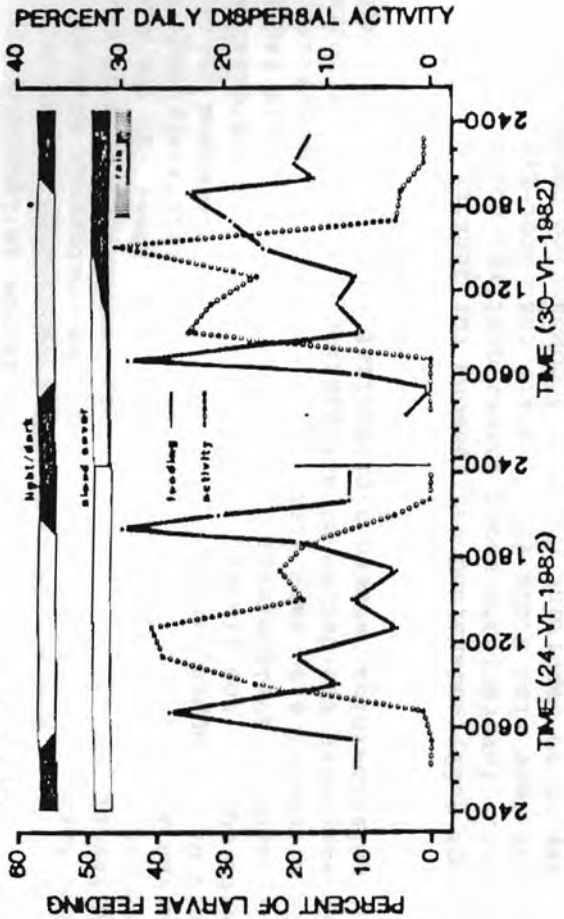


Figure 4

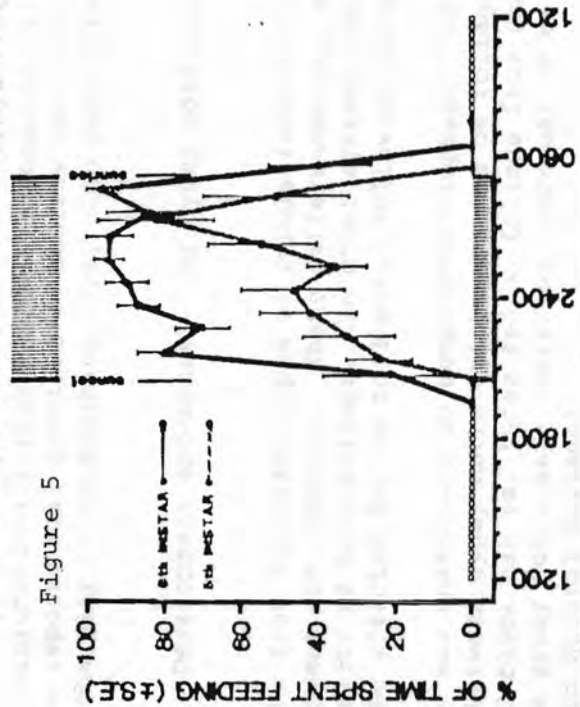


Figure 5

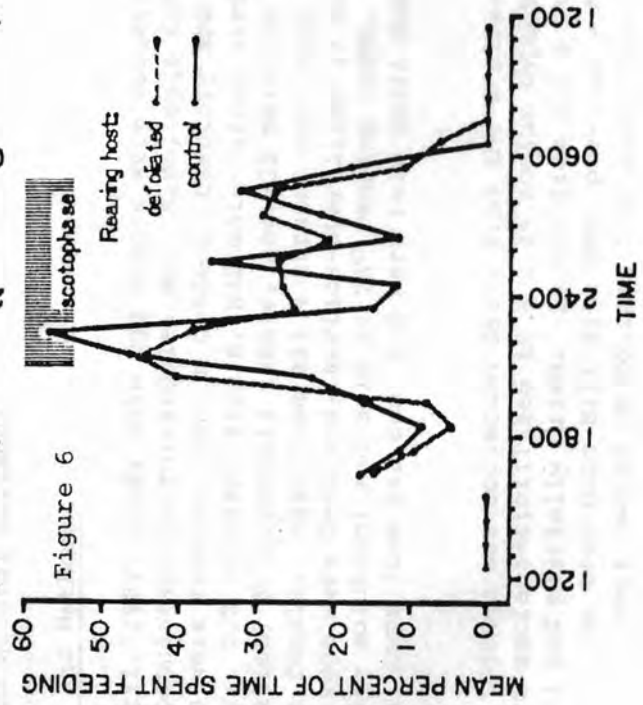


Figure 6

Several workers have varied the density of larvae in laboratory cultures, but the effects on development time have been inconsistent and relatively minor. On the other hand, the effects of microhabitat preference have not been thoroughly investigated. In the laboratory, small decreases in rearing temperature can substantially extend development times. In the field, high density larvae are exposed to sunlight and to the relatively high diurnal temperatures of the upper canopy, while endemic larvae spend the day in cooler microhabitats such as the litter. These differences in microhabitat choice may markedly affect developmental times. Other microclimatic features (e.g., evaporation pressure) also vary with microhabitat, and temperature in the forest will vary with the degree of defoliation. In future reports, we will address the relationships between microhabitat preference and development time.

We still do not fully understand the reasons for density-correlated shifts in larval behavior. Interactions among larval quality, foliage quality, and larval behavior (see also Study #5) indicate that host-tree responses to defoliation may be an important factor. Accompanying behavioral changes (e.g., in microhabitat preference) may secondarily affect population quality. Our future reports will continue to address the role that insect-plant interactions play in determining gypsy moth population quality.

Study #4. 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 between gypsy moths which were fed on foliage from previously defoliated black oak vs. those fed on foliage from undefoliated oaks. Also, F1-sterile gypsy moths were reared on black oak to determine if they developed normally on natural foliage.

Materials and Methods

During April, 1982, gypsy moth egg masses were collected from a healthy building population in Forestdale, MA. Also, eggs from the 3 following treatments were removed from an outdoor insectary and were brought into the laboratory: NJS standard strain, NJS male (irradiated 10 krad at 8-9 days after pupation) X Forestdale female, and NJS male (10 krad at 10-11 days) X Forestdale female. Eggs from all 4 strains were held at 4° until May 8. Then, the eggs were dehaired, surface-sterilized (5 min. in 0.5% sodium hypochlorite solution) and held at 25° pending hatch. Larvae were placed into 180 ml cups (one per cup) and were held under SLRC for the remainder of the test.

Black oak foliage was collected daily from the 4 sites used in Study #2. Leaves were surface-sterilized in a 0.2% sodium hypochlorite solution, and were rinsed and partially dried. Larvae from all 4 strains were fed daily on foliage from the Deer Horn Hill site (not previously defoliated). Forestdale P1 larvae also were reared on foliage from each of the other 3 sites.

The date of each molt was recorded, and pupae were weighed at 6 days of age. The following indices of feeding efficiency were calculated:

$$\text{Approximate digestibility (AD)} = \frac{\text{Food ingested - frass}}{\text{Food ingested}} \times 100$$

$$\text{Efficiency of conversion of ingested food (ECI)} = \frac{\text{wt. gain}}{\text{food ingested}} \times 100$$

$$\text{Efficiency of conversion of digested food (ECD)} = \frac{\text{wt. gain}}{\text{food ingested-frass}} \times 100$$

To develop these indices, 20 newly molted fifth instar larvae were taken from each foliage treatment. The insects were weighed, and their dry weight was estimated by using the percent dry weight of 20 (total) additional larvae which were weighed before and after being dried in an oven at 60°C for 24 hours.

Twenty fifth instars (5 from each foliage treatment) were fed daily on foliage from each site. When the larvae were being fed, each leaf was cut into symmetrical portions; half was given to a larva, and the other half was saved for dry weight determination. Frass and uneaten leaf material were removed from the cups daily and saved.

Feeding was terminated when larvae either became prepupae (males) or were preparing to molt to the 6th instar (females). Then, the larvae, frass, uneaten leaf bits, and original leaf halves were weighed after being oven-dried at 60°C for 24 hours.

Feeding rhythms were analyzed using the methods described in Study #5. Larvae from the Deer Horn Hill (DHH - not defoliated) and Sandwich High School (SHS - previously defoliated) foliage treatments were tested on both DHH and SHS foliage.

Results and Discussion

In other studies (e.g., Wallner and Walton, 1979) foliage quality has been shown to be reduced in trees which were defoliated in the year prior to the study. This decline in foliage quality may result from elevated tannin levels in the foliage of previously defoliated trees (Schultz and Baldwin, 1982), although leaf composition also undergoes other changes following defoliation. Host plant quality has been shown to affect food utilization, development, and behavior. Thus, host defoliation is suspected as a possible driving factor in determining gypsy moth population quality.

In this study, larvae developed similarly on the foliage of either previously defoliated or undefoliated trees (Table 4). However, survival was significantly lower on foliage from the previously defoliated sites. Most mortality (93%) occurred in the 1st instar (since some of the fifth instars were removed for testing, survival only includes instars I through IV). Any defoliation-induced changes in host quality appeared to have little or no effect on larvae once the second instar had been reached.

Table 4. Development and survival of gypsy moth larvae on excised black oak foliage from sites which were and were not defoliated in the year prior to the study.

Foliage Collection Site	No. of larvae	Percent Survival[1]	Female Development[2]		Male Development[2]	
			Mean Larval development (days)	Mean pupal wt (mg. wet)	Mean Larval development (days)	Mean pupal wt (mg. wet)
SHS (defol.1981)	150	66.0 a	40.2 a	1837 a	34.5 ab	535 a
QMHR (defol.1981)	150	76.7 b	40.5 a	1756 a	35.0 b	536 a
CQMA (control)	90	84.4 b	40.3 a	1851 a	34.0 a	560 a
DHH (control)	150	94.0 c	40.3 a	1813 a	34.0 a	566 a

1/ To the fifth instar; most mortality occurred in the 1st instar. Percentages which are not followed by the same letter are significantly different ($p < 0.05$), as determined by contingency table analysis.

2/ Means were separated ($p < 0.05$) using Duncan's new multiple range test.

Table 5. Food utilization efficiency laboratory record for 5th instar gypsy moth larvae gypsy moth on black oak.

Condition of Trees Used In Test	Condition of Trees Used In Rearing Larvae	No. of Larvae	Weight Gain/Larva (Mg. Dry Weight)	Utilization Indices[1/]		
				AD (%)	ECD (%)	ECI (%)
Previously defoliated	Control	16	90.3	16.9	47.4	7.7
Control	Control	18	87.7	19.5	53.8	9.8
Previously defoliated	Prev. Def.	16	81.7	14.9	49.4	7.3
Control	Prev. Def.	17	76.8	19.0	46.4	8.3

1/ AD, ECD and ECI represent approximate digestibility, efficiency of conversion of digested food and efficiency of conversion of ingested food respectively. See text for details on the computation of the indices.

Similarly, conversion efficiencies did not vary greatly among foliage collection sites or larval rearing treatments (Table 5). The higher AD's of the control leaves are suggestive and support insect-plant interaction theory; however, the differences were not statistically significant ($p = 0.1$). Compared to other studies (e.g., Barbosa and Greenblatt, 1979), our AD's were somewhat low, perhaps because the tannin levels were relatively high in leaves from all 4 collection sites (J. Schultz, pers. comm.). Again, site indices (Table 3) indicate that the trees in this study may be stressed regardless of defoliation, and thus our results may not coincide with those obtained with foliage from better sites.

Feeding rhythms also did not vary with rearing treatment (Fig. 6). Larvae from both test treatments (DHH and SHS) showed a sharp peak in feeding immediately following "lights-off", and then fed on and off throughout the remainder of the scotophase. Feeding rhythms similarly did not vary with source of leaves (not shown).

Larvae from high and low density populations show very definite differences in behavior and development (see Study #3 and #5). These differences were not reflected by larvae which were fed on foliage from previously defoliated vs. control trees. Differences in population quality also did not occur either in laboratory-reared larvae which were collected as eggs from various types of populations (P1 generation), or in larvae which were reared at various population densities (e.g., 1 or 10 per 180 ml cup). The factors which contribute to population quality appear to be somewhat complex. Still, defoliation-induced changes in leaf quality can impact on larval development and behavior; these changes appear to be more pronounced in the year of defoliation than in the following year. Hopefully, our future work will clarify the relationships among defoliation, foliage quality and gypsy moth population quality.

F1-sterile larvae showed poor development and survival on black oak foliage (Table 6). Pupal weights of F1-sterile individuals were significantly lower than pupal weights of control insects; this may or may not affect the mating competitiveness of F1-sterile adults. However, compared to controls, survival of F1's was significantly lower and F1 development time was significantly longer. Low survival would have an obvious impact on competitiveness. The longer development times, when compounded with a spread out hatch period (see Study #1), easily could result in a 1 week difference in the timing of adult eclosion between F1-sterile and feral moths. Since adult gypsy moths are short-lived and females often mate soon after eclosion, this degree of asynchrony could drastically reduce the competitiveness of F1-sterile adults.

Table 6. Development and survival of F1-sterile and normal gypsy moths on excised black oak foliage.

Strain/ Treatment	No. of Larvae Set up	Percent Surv. 1/,2/	Percent Males2/	Female Development3/ Larval Development (Days)	Female Development3/ Pupal Weight (mg. wet)	Male Development3/ Larval Development (Days)	Male Development3/ Pupal Weight (mg. wet)
10 krad, 8-9 days, F1	90	72b	72b	44.1c	1339c	39.2b	501bc
10 krad, 10-11 days, F1	90	41c	67b	43.6c	1362c	39.9b	468c
Forestdale P1	150	94a	43a	40.3a	1813a	34.0a	566a
NJSS	90	80b	40a	41.3b	1630b	34.5a	529b

1/ Percent of larvae surviving to the fifth instar.

2/ Proportions were separated using 2 x 2 contingency table analysis. Within a column, percentages which are followed by the same letter are not significantly different ($p < 0.05$).

3/ Means separated by Duncan's new multiple range test ($p < 0.05$).

Study #5 Preliminary 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 sharp peaks of feeding occurring during the crepuscular periods. Larvae in endemic populations feed strictly nocturnally. Field observations (see Study #3) 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 purposes of this study were two-fold: (1) to evaluate a potential technique for assaying larval feeding and activity rhythms on natural foliage, and (2) 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

A feeding activity monitor was constructed using a modification of Kogan's (1973) technique. A leaf was stretched over a plastic membrane and across a horizontal wire; the wire was attached to the probe of a Model DB "Polysonic Detector and Bionic Sensor" (C.W. Dickey Assoc., State College, PA) (Fig. 7) The leaf petiole was held in a water trough to maintain freshness. A late instar (IV-VI) larvae was placed in a 5 cm x 8 cm x 8 cm h box which, in turn, was placed over the leaf. The top and 3 sides of the box were plexiglass; the fourth side was rough-sawn wood which provided the larva with acceptable resting sites. The bottom of the box was left open to provide the larva with access to the foliage.

Masticatory vibrations were transmitted from the leaf to the probe and created signals which were amplified and displayed on a strip chart recorder. The "band pass" filter on the detector's amplifier eliminated signals under 200 Hz, which effectively screened out the low-frequency interference from the laboratory environment. To further ensure isolation from outside noise, the entire leaf-larva-probe assembly (Fig. 7) was placed into 20 x 30 x 40 cm box. Five sides of the box were 1.9 cm plywood covered with anechoic foam; the 6th side (20 x 40 cm) was 0.6 cm plexiglass, which allowed light into the test arena.

In initial tests, NJS diet-reared larvae were offered the foliage of glasshouse-grown red oaks (*Quercus rubra* L., a highly preferred host) and sycamore (*Platanus occidentalis* L., a non-preferred species). Observations on the larvae were made concurrently with recordings of masticatory motions; in this way, it was possible to associate different larval behaviors (e.g., feeding or crawling) with specific patterns on the charts.

In a second series of tests, larvae were collected from a high-density infestation in Cotuit, MA and from an endemic population in Ashumet, MA (for site descriptions, see Study #3). These larvae were placed in the test chamber and were provided with a black oak leaf from one of the two sites. They were allowed to feed for 24 hrs, starting at 1400-1500 hours. These tests were conducted at 25°C, 0500-2100 Pp, with a chart speed of 15 cm/hr. Ashumet larvae, when offered Cotuit foliage, typically showed little or no feeding; when removed from the test chamber, these larvae were held for 24 hrs in a 180 ml plastic cup and were provided with Ashumet foliage. This was done to determine whether the lack of feeding was due to non-preference for the Cotuit 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

Figure 8 a and b shows representative tracings produced by feeding larvae and by crawling larvae, respectively. Feeding produces bouts of very densely packed but relatively short spikes, while crawling activity produces more loosely spaced somewhat taller spikes. Thus, this technique is doubly useful in that it simultaneously monitors for both feeding and locomotor activity.

The results of tests with field-collected larvae are shown in Fig. 9 through 12. The number of tests that were run was quite low for several reasons: (1) we could test only 1 larva per day, (2) there were occasional "bugs" that had to be worked out (usually problems with the strip-chart recorder), and (3) field collected larvae are available for a very short period of time (a few weeks). As a result, only 3-4 larvae were tested for each combination of population type and foliage source.

While these results are very preliminary, they still show consistent trends and differences. For example, when endemic-type larvae (all 5th instars) were offered foliage from the endemic site, they fed almost exclusively nocturnally, with peak feeding activity occurring between 0300 and 0400 hrs (Fig. 9). This agrees closely with both field observations (see Study #3) and with feeding rhythm studies using artificial diet (Lance et al, 1981). On the other hand, when endemic larvae were provided with foliage from the high-density site, they showed little or no feeding. However, they were very active during the scotophase (Fig. 10). Their activity then dropped off in the early morning, but picked up again in the late morning and early afternoon. High-density larvae fed sporadically throughout the day and night, regardless of the type of foliage which was used in the test. Again, this is in agreement with field observations (Study #3). Interestingly, while the high-density larvae fed readily upon either type of foliage, they were much less active when provided with foliage from the endemic site (Fig. 11 and 12).

The feeding rhythms of field-collected larvae, then, continued more or less unaltered when the larvae were brought into the laboratory. Feeding rhythms should provide a useful means of assessing some aspects of gypsy moth population quality. Further, foliage quality (as affected by defoliation pressure) can produce immediate effects on larval locomotor activity and, in the case of endemic larvae, on feeding behavior. Foliage quality appears to play an important if not central role in determining gypsy moth population quality; the nature of this role will be the subject of future investigations.

Fig. 7. Test arena of larval feeding rhythm monitor.
a. leaf
b. sensor (encased in foam rubber)
c. sensor probe (contacts leaf)
d. water trough
e. cage in which larvae are placed

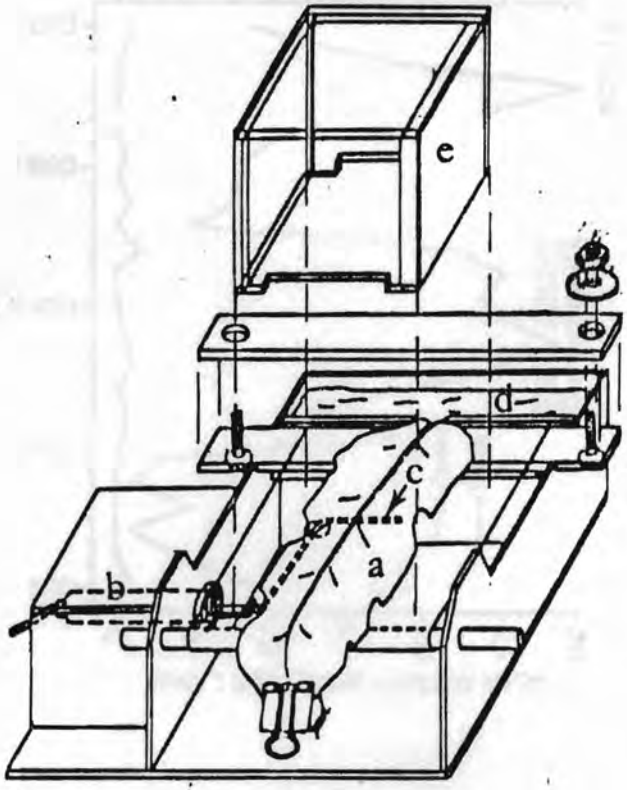
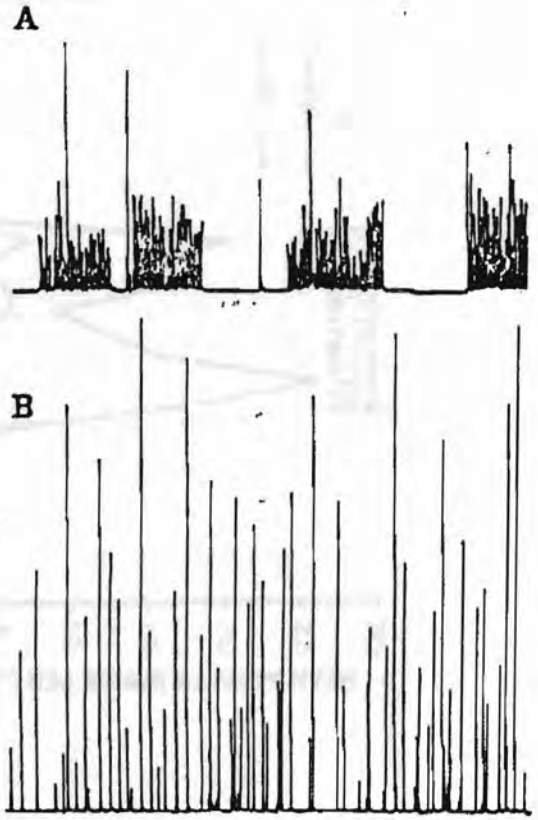
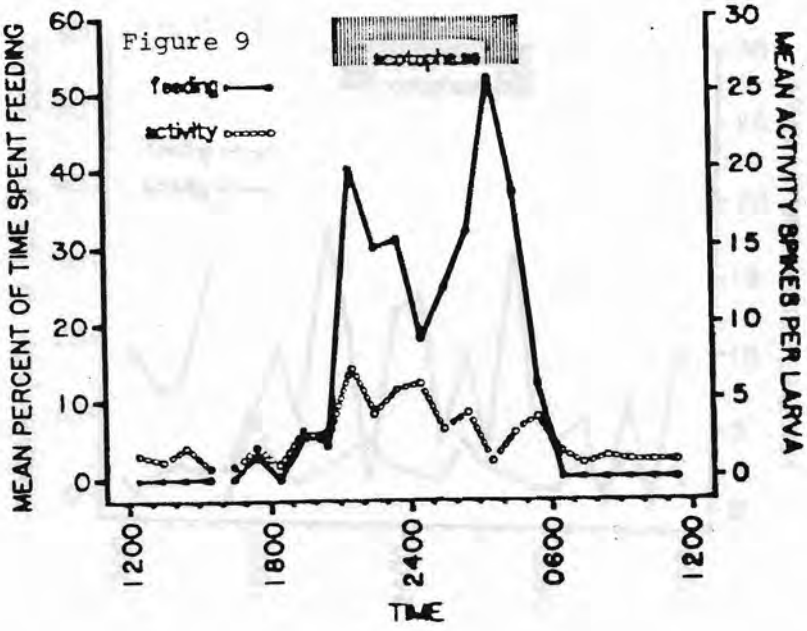
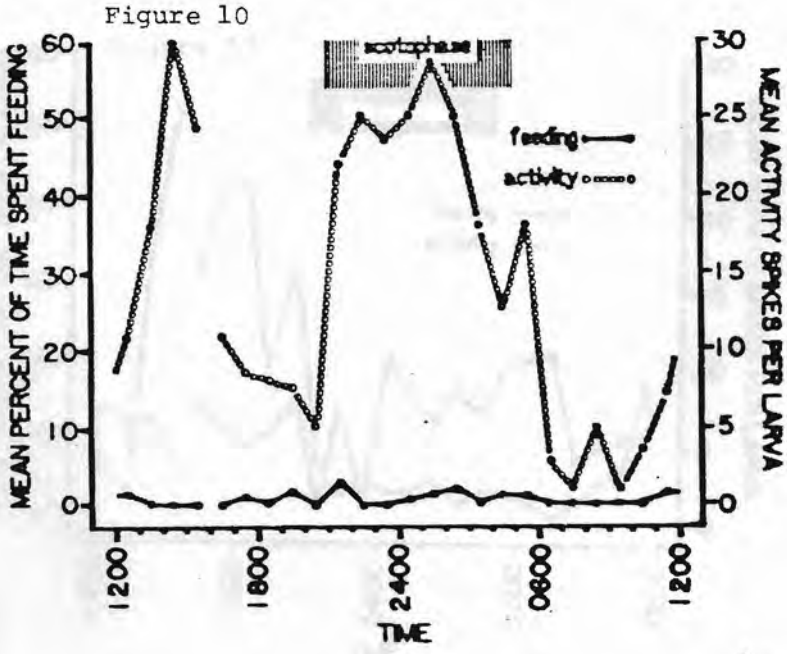


Fig. 8. One hour strip chart recordings produced by feeding rhythm monitor.
A. recording of feeding activity
B. recording of locomotor activity



Figures 9-10. Feeding and activity patterns, in the laboratory, of gypsy moth fifth instars which were collected from an endemic population.
Fig. 9. Test foliage was collected from within an endemic population.
Fig. 10. Foliage was from an outbreak site.

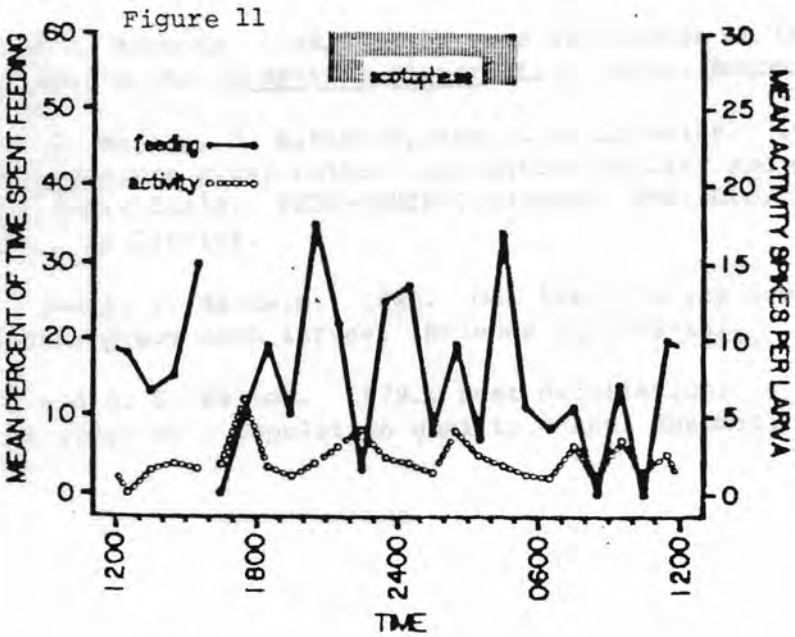


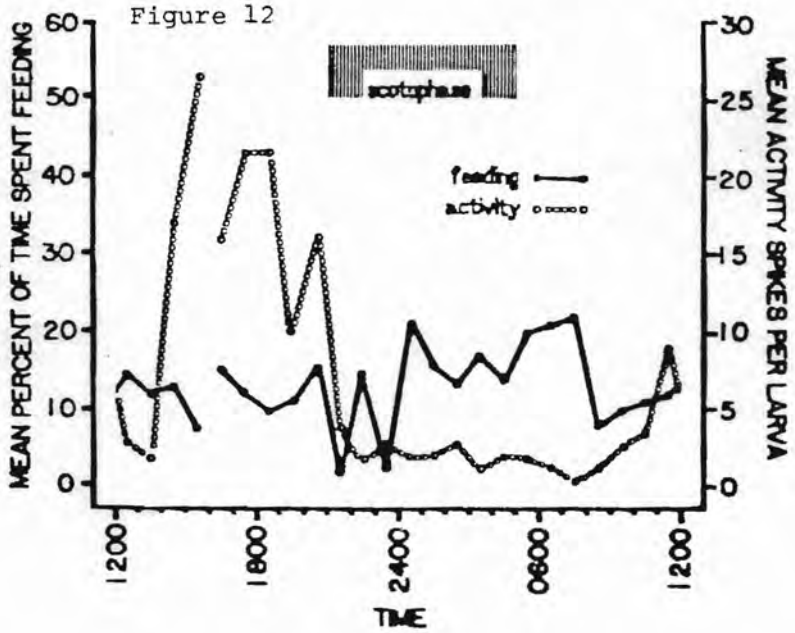


Figures 11-12. Feeding and activity patterns in the laboratory of gypsy moth late instars which were field collected from an outbreak population.

Fig. 11. Test foliage was collected from within an endemic population.

Fig. 12. Foliage was from an outbreak site.





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Project Number: GM 1.2.4
Project Title: Mass Trapping: Effects of Insect and Trap Densities on Mating Success
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: V. C. Mastro, A. P. C. Toles and C. P. Schwalbe

Introduction

Mass trapping of male gypsy moths is an attractive technique for suppression or elimination of isolated infestations. However, successful use of the technique is dependent on a knowledge of how and when to apply it. Insect density, trap density and the ratios of traps to insects, and males to females are factors which likely will influence effectiveness of the technique.

Previous field studies (GM 1.2.3, 1.2.4 and 1.2.5 - 1981) indicate that female mating success is dependent on male density (e.g. sex ratio). The objective of the studies reported here was to describe the relationship between two independent variables (trap and insect densities) and female mating success.

Methods and Materials

Plots (9 ha) were established in mixed pine-oak forest near Otis ANGB, MA. All plots were at least one mile apart. Each plot was assigned one of five trap treatments: no traps (control), 0.75, 2.5, 7.5 and 25 traps per hectare. Standard delta traps baited with hercon dispensers (500 ug) were used throughout the test.

The following is a summary of the trap and insect densities tested.

Traps/ hectare	Pairs/ha 2.5	7.5	25.0
0.75	x		
2.5	x	x	
7.5	x	x	x
25.0	x	x	x
Control	x	x	x

All plots (trap densities) were run simultaneously at a particular insects density.

To facilitate insect release, fifteen parallel lines, 20 m apart, were established on each plot with release points marked every 20 m along the lines. This plot design provided 225 possible points for male and female release. Selection of points for insect release was accomplished by drawing numbers from a random number table. A different randomization scheme was used for male and female placement, however, the same randomization was used for all plots tested at a particular insect density. Females (1 day old virgin) for release were placed in small wooden A-frames; males, which were tagged with fluorescent powder, were simply shaken out of 4 oz. cups at the preassigned points. All insects were released by 11:00 E.S.T. Traps were checked and females and egg masses were recovered two days after release. Recovered females were allowed to complete oviposition in the laboratory, and egg masses were held at 25°C, 50-60% RH for embryonation. After 30 days egg masses were cleaned of hairs and evaluated for embryonation. Captured males were checked under a black light for fluorescent powder to determine if they were released or feral.

Results and Discussion

The proportions of released males recovered at various trap and insect densities are presented in Table 1. Because of the prolonged native flight period in 1982, it was not possible to conduct the tests only during periods with no native flight, and this unusual native flight limited the number of replicates which could be completed. Feral males captured during the period when the first three tests were conducted were migrants; resident insects had not yet begun to eclose, therefore competing feral females did not further confound the results.

As expected, the proportion of males recovered generally increased with increased trap density. Trap density above 7.5 trap/ha, however, does not appear to significantly improve recovery and in several tests the proportions of males recovered were actually lower in plots arrayed with 25 traps/ha. Generally, mating success decreased with increasing trap density (Table 2). The proportion of females mating was the lowest in plots with 25 traps/ha; regardless of insect density. The presumably shorter time interval between release and capture of males at high trap density may explain why higher trap densities reduce mating incidence even though the proportion of males captured at that trap density is less than lower trap densities.

Generally, it appears that more than 7.5 traps/ha are required to reduce mating success to a level which would result in a population reduction the following season. Feral males were present in plots in all but the first series of tests which, in effect, increased insect density. Assuming that the same proportions of feral and laboratory males present were captured in a particular plot, estimates of total male density can be made (Table 2). Using these estimated male densities and trap densities as independent variables to predict percentage mating a regression analysis results in the following equation:

$$\% \text{ mated} = 75.01192 + (-2.144)(t) + (0.574)(m)$$

where t = traps/ha
m = males/ha
$r^2 = 0.603$

Although this equation does not explain all of the variation in mating success, it can be used as a basis for further testing and refinement.

Table 1. Proportions of released males and number of feral males recovered from plots with three simulated insect densities and four trap densities.

Traps/ hectare	Pairs 1/ released per hectare 2.5 Rep. 1 (7/2)		2.5 Rep. 2 (8/25)		7.5 Rep. 1 (7/6)		25.0 Rep. 1 (7/10)	
	Percent male recovery	No. feral males captured	Percent male recovery	No. feral males captured	Percent male recovery	No. feral males captured	Percent male recovery	No. feral males captured
0.75	43.5	0	43.5	10	-	-	-	
2.5	52.2	0	82.6	19	69.1	14	-	-
7.5	91.3	0	91.3	21	82.4	40	73.8	269
25.0	69.6	0	78.3	18	83.8	38	67.2	336

1/ Actual numbers of pairs released per plot were: 23 pairs = 2.5 pairs/ha, 68 pairs = 7.5 pairs/ha, and 256 pairs = 25.0 pairs/ha.

Table 2. Proportions of recovered females that mated successfully which had been placed in plots with three simulated insect densities and four trap densities.

Traps/ hectare	Pairs 1/ released per hectare							
	2.5 Rep. 1 (7/2)		2.5 Rep. 2 (8/25)		7.5 Rep. 1 (7/6)		25.0 Rep. 1 (7/10)	
	Percent females mated	Males per/ha	Percent females mated	Estimated no. males per/ha 1/	Percent females mated	Estimated no. males per/ha	Percent females mated	Estimated no. males per/ha
0.75	76.5	2.5	82.0	5.7	-	-	-	-
2.5	75.0	2.5	86.0	5.7	67.6	9.7	-	-
7.5	61.9	2.5	85.0	9.0	29.4	13.0	94.6	74.6
25.0	5.9	2.5	65.0	15.7	10.3	12.6	75.7	84.0
Control	90.9	2.5	100.0	No estimate	92.6	No estimate	98.9	No estimate

1/ Male density estimates assume the same proportions of feral and released laboratory-reared males were captured.

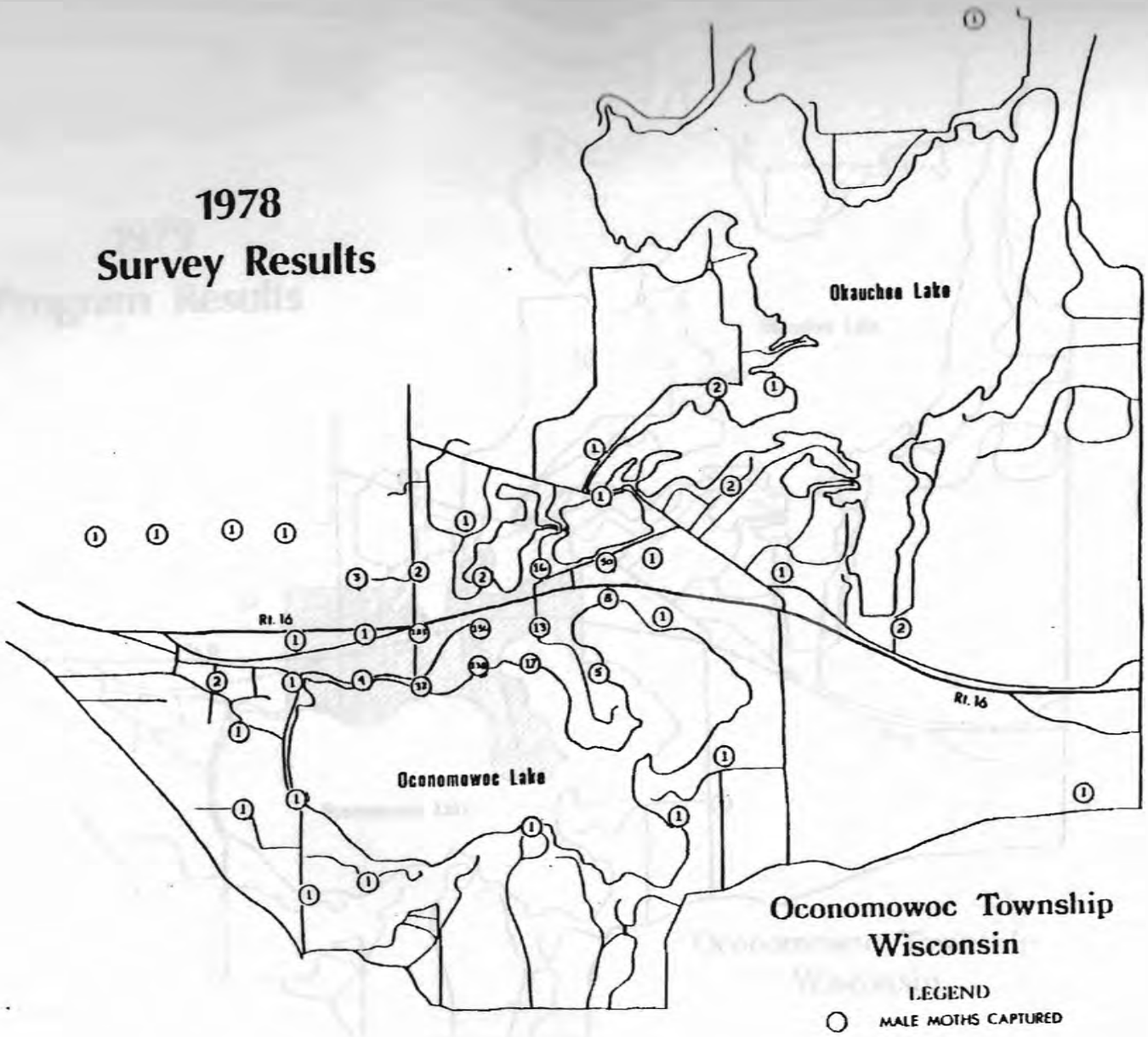
Project Number: GM 1.2.7
Project Title: Gypsy Moth Mating Disruption - Oconomowoc, Wisconsin Demonstration Project
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leader: C. P. Schwalbe

In 1977 a gypsy moth infestation was detected in Oconomowoc, Wisconsin; delimitation survey the following year resulted in the capture of ca. 700 moths from which the infestation was determined to be ca. 425 acres. Since then, an attempt has been made to eradicate that population by nonchemical pesticide means, primarily mating disruption and mass trapping. Results of those efforts were discussed in the October 1, 1980 - September 30, 1981 Laboratory Report. Maps chronicling those tests are also included in this report.

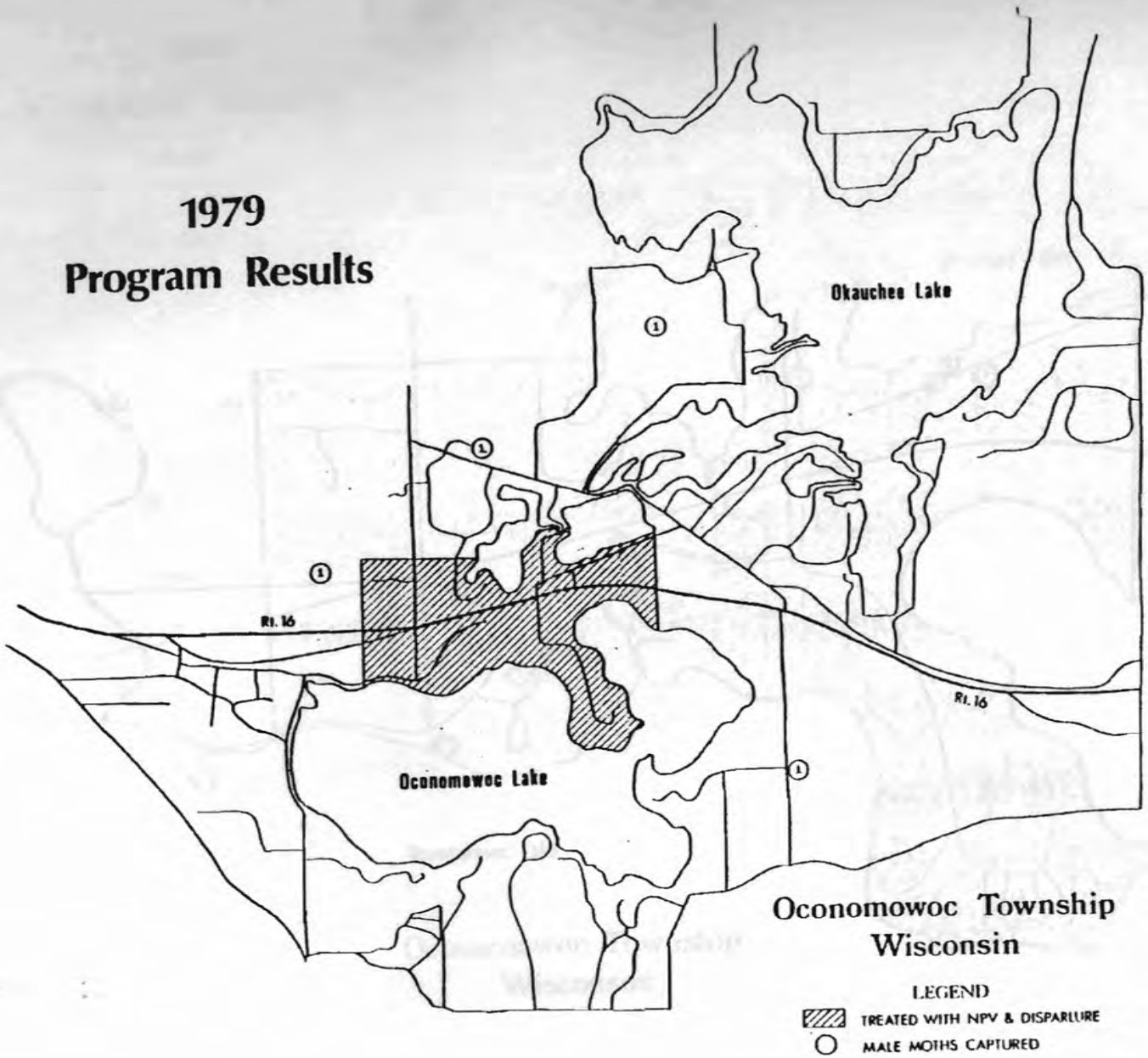
In the fall of 1981, the only area suspected as infested was ca. 30 acres near Okauchee Lake where a large number of males were caught. The entire project area was retrapped at the rate of 3 traps/acre in 1982 and only 19 moths were caught, indicating further population reduction. It is proposed to mass trap the infested area again in 1983 to determine if reproduction occurred in 1982 and, if it did, to exert additional control.

Clearly, this infestation is vastly reduced in size and density from its 1978 condition. But, since Sevin was used (sparingly) in 1981, definitive evidence for eradication with the mating disruption technique will not be possible. If eradication is achieved, the chemical pesticide component will have been used only on small areas, and control over the rest of the area can be ascribed to Gypchek, disparlure sprays, burlap banding, mass trapping and undefined natural mortality factors. While the project was originally planned as a demonstration of the mating disruption method, mass trapping will receive the clearest evaluation, free of the confounding effects of pesticide treatment.

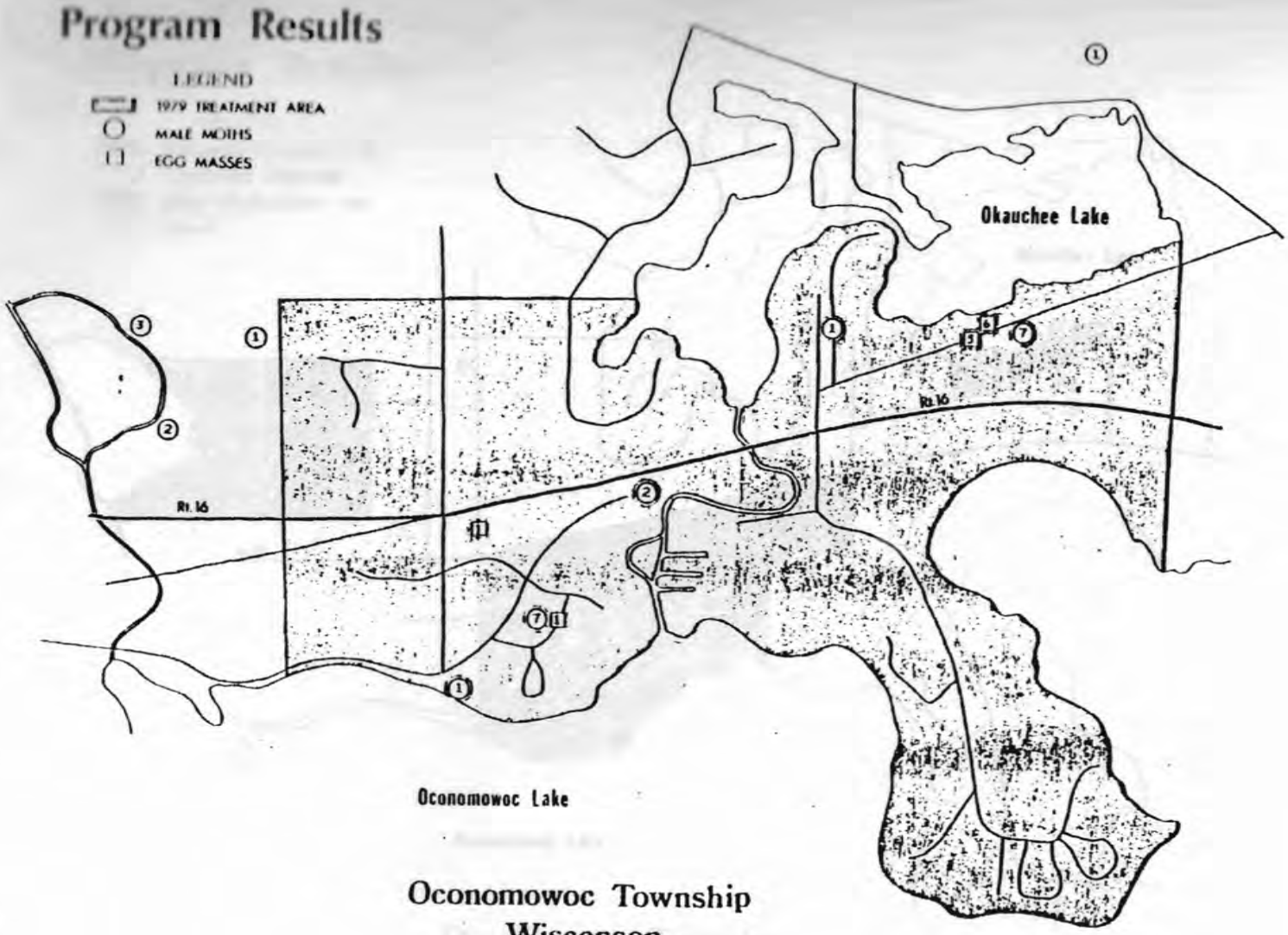
1978
Survey Results



1979
Program Results

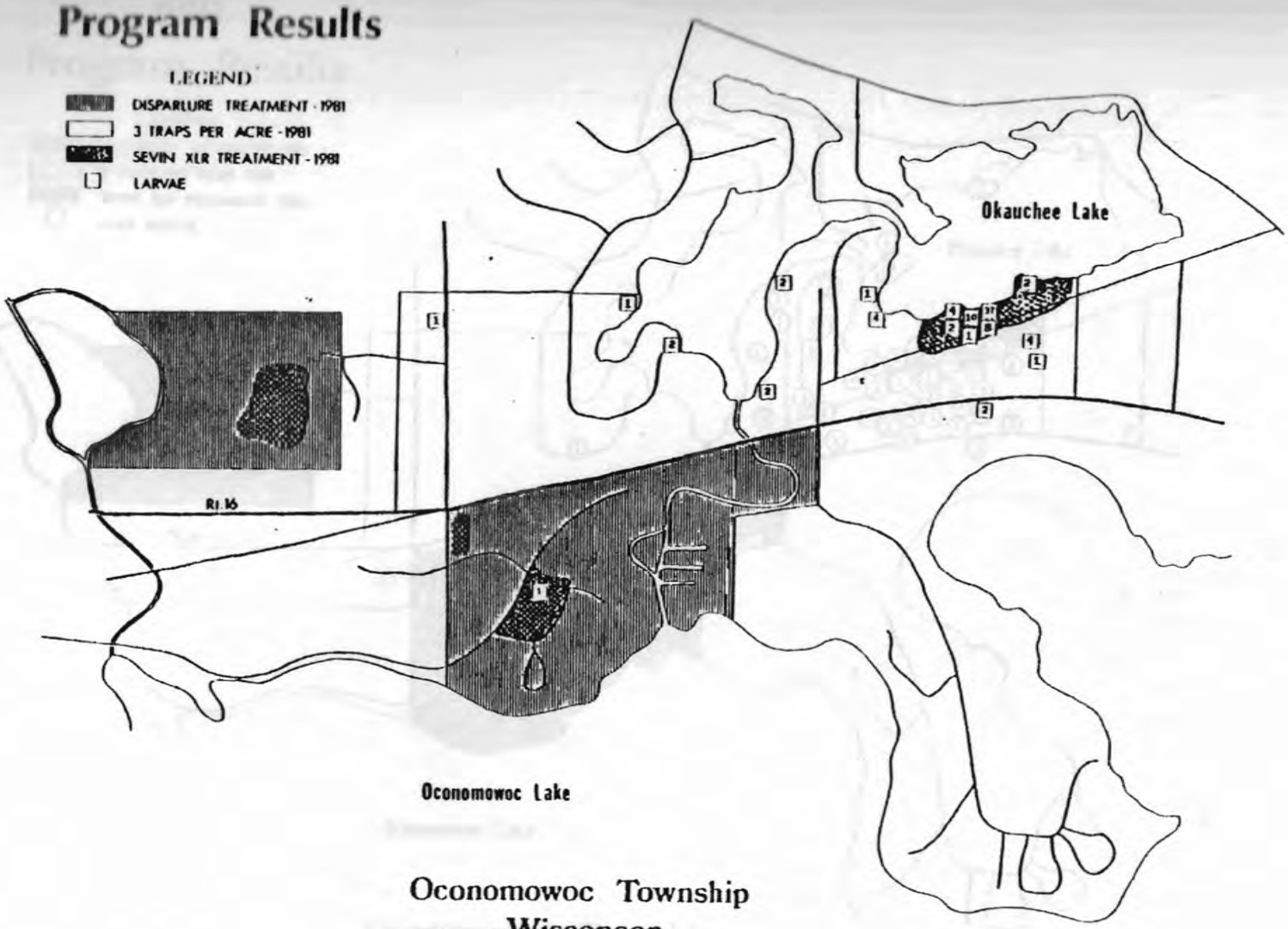


1980
Program Results



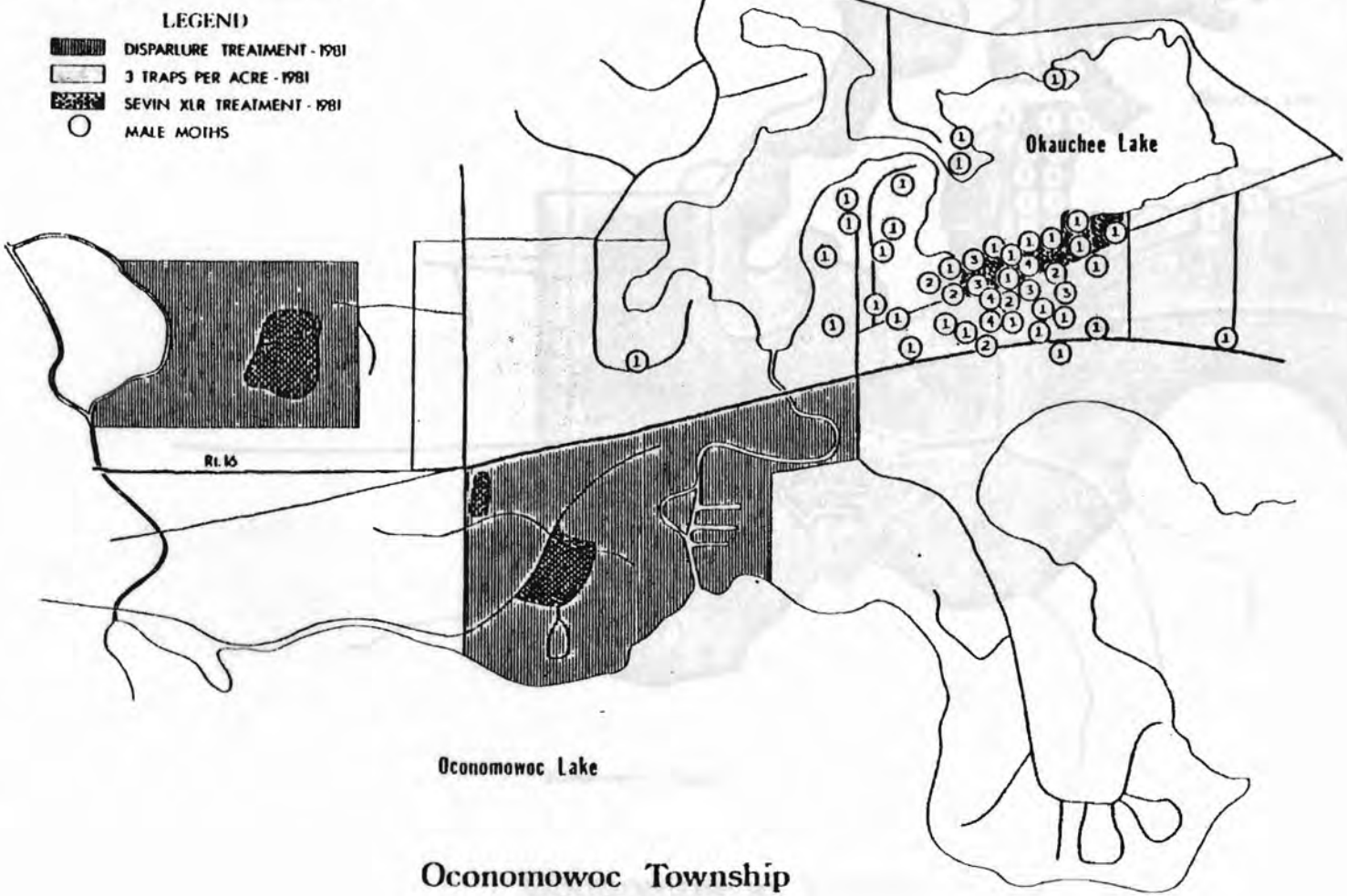
Oconomowoc Township
Wisconson

1981
Program Results



Oconomowoc Township
Wisconson

1981
Program Results

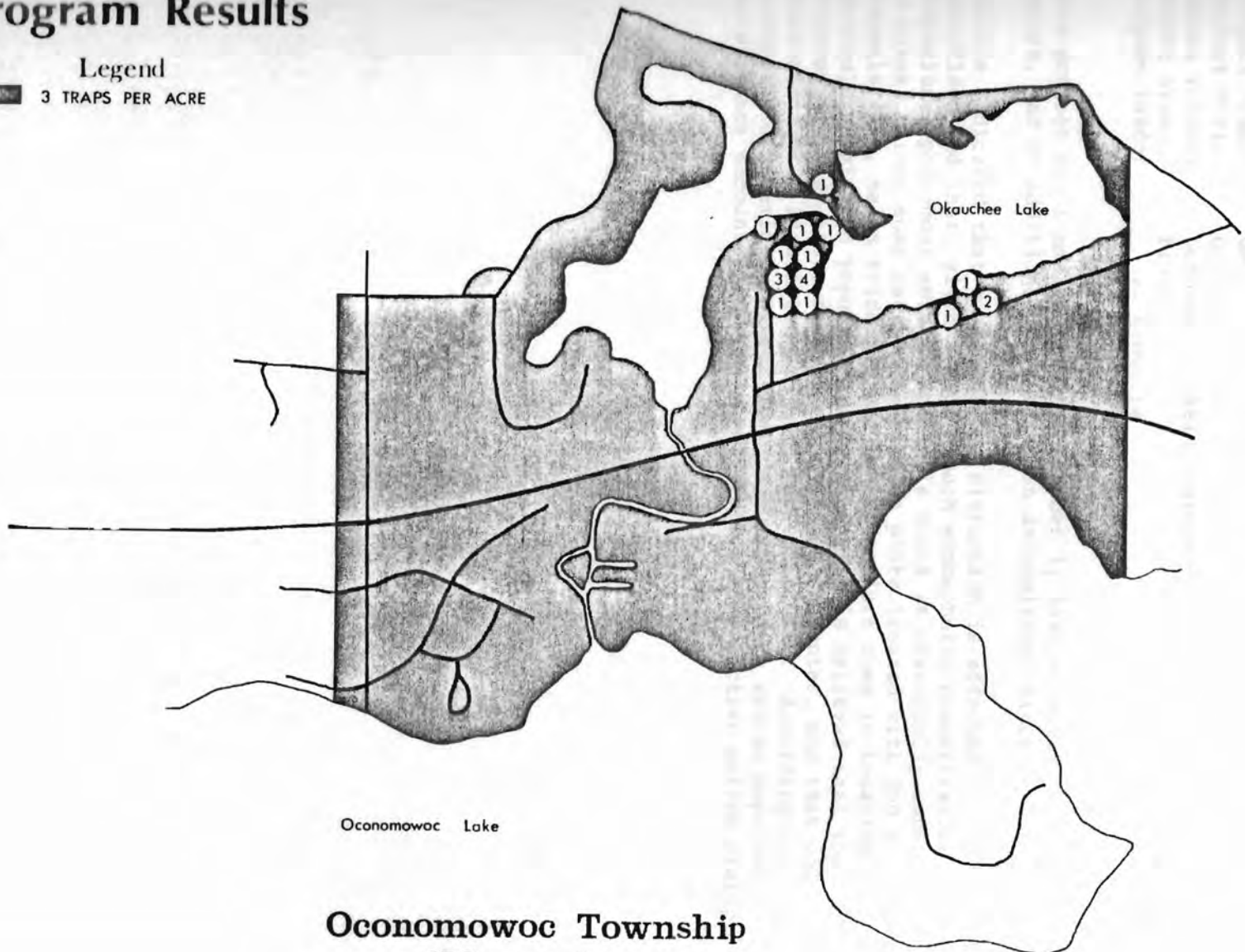


Oconomowoc Township
Wisconson

1982
Program Results

Legend

3 TRAPS PER ACRE



Oconomowoc Township
Wisconsin

Project Number: GM 1.2.8
Project Title: Gypsy Moth Mating Disruption: Behavioral Aspects
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leader: C. P. Schwalbe

This project was summarized in the October 1, 1980 - September 30, 1981 Report, and no additional work has been accomplished since then.

Results indicated that communication disruption is affected by chemical camouflage and that, given a high enough atmospheric concentration of disparlure, pheromone emitted by female moths is essentially imperceptible to males. Since some mating occurred in plots treated with 500 g disparlure/ha, males evidently use non-disparlure cues in locating females, particularly at high population densities. It is believed that the mating observed in these tests resulted from chance encounter, and that only short-range mate identification cues were operating. Accordingly, disruption of chemical (disparlure) communication in sparse populations where chance encounter is rare should result in effective mating disruption.

Project Number: GM 1.2.10
Project Title: Evaluation of Pheromone Dispensers as Trap Baits
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leaders: E. C. Paszek, C. P. Schwalbe, B. A. Leonhardt

In 1981 a comparison study was conducted to determine the activity of 2 types of gypsy moth pheromone dispensers, laminated Hercon strips and Zoecon rubber septa. Those dispensers were loaded with varying amounts of (+) disparlure and aged at several time intervals. The field bioassay indicated that there was no substantial effect of varying the pheromone concentration of the Hercon dispensers. Gas chromatographic analysis indicated that very little lure was lost from the Hercon aged samples. The rubber septa dispensers captured very few moths and the gas chromatographic analysis detected no lure in the aged septa. Every septum however, contained the olefin precursor (2-methyl-cis-7-octadecene) in proportion to the original disparlure content in the unaged dispensers.

This study was repeated in 1982. Rubber septa were loaded with 1, 10, 100 and 1000 ug of (+) disparlure and aged outdoors for 6 weeks. Ten replicates of each of the 4 aged treatments and 10 similar freshly baited unaged treatments were placed on Aug 11 in 1/2 gal milk carton (MC) traps. They were bioassayed in a randomized pattern in a gridded plot with the traps spaced 50 m apart. Ten samples from each of the aged and unaged treatments were packed in amber vials and retained in a freezer for GC analysis. Two treatments, 10 aged and 10 unaged Hercon pheromone dispensers, 500 ug in strength, were used as controls in this study.

Table 1. Comparison of moth capture rates and pheromone release from Zoecon rubber septa and Hercon laminated dispensers.

Dispenser type	Weeks aged	ug disparlure/dispenser nominal	ug disparlure/dispenser found	No. moths captured
Rubber septa-unaged	0	1	ND	3
"	0	10	ND	24
"	0	100	10	41
"	0	1000	370	97
Hercon's laminate-unaged	0	500	360	280
Rubber septa-aged	6	1	ND	3
"	6	10	ND	1
"	6	100	4	13
"	6	1000	360	25
Hercon's laminate-aged	6	500	245	805

Results

This year's tests comparing Zoecon's rubber septa with Hercon's laminated dispensers showed the laminate to be far superior in capturing male gypsy moths. The GC analysis yielded somewhat anomolous results in that the residual disparlure was the same in unaged and 6 week aged 1000 ug samples. Only trace amounts of the olefin precursor were detected. The analyses did not explain why the 1000 ug/rubber septa did not catch as well as the laminates; all had about the same amount of disparlure. Perhaps the septa do not release the last 1/3 of their lure content at a sufficiently high rate for good moth catch. This year's, as well as last year's, data indicate that the rubber septa seem to be a totally unsatisfactory dispensing system for disparlure.

Project Number: GM 1.2.11
Project Title: Bioassay of New and Used Samples of 1980, 1981 and 1982 Hercon Disparlure Dispensers
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leaders: E. C. Paszek and C. P. Schwalbe

Pheromone dispensers are used in traps to survey large areas for gypsy moth. These (+) disparlure dispensers are stored under refrigeration and are annually compared in a field bioassay.

The dispensers were placed in milk carton traps outdoors on July 15. Traps were transferred on July 20 to a light gypsy moth infestation in Yarmouth, MA and placed in 10 x 15 grid with trap spacings 50 m apart. 1980 and 81 used dispensers were from Michigan where they were used in traps from June 2 - September 18, 1981, returned to Otis Methods Development Center, and stored under refrigeration.

The following table lists the number of moths captured in traps baited with new and used 1980, 1981 and 1982 Hercon disparlure dispensers.

Year	Production lot no.	Total no. of moths captured in 10 replicates
1982 (new)	D0332	425
1981 (new)	D0351	173
1981 (used)	D0351	177
1980 (new)	D0430	188
1980 (used)	D0430	157

This bioassay indicates that the 1982 dispensers were more than twice as effective in capturing moths as the 1980 and 1981 dispensers. The 1980 and 1981 used dipensers were still active after a seasons exposure and compared favorably in capturing moths with the unused 1980 and 1981 dispensers.

Project Number: GM 1.2.12
Project Title: Bioassay of 4 Formulations of Hercon (10% DDVP) Insecticidal Strips
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leader: E. C. Paszek

The large capacity milk carton trap used for monitoring gypsy moth populations in infested areas requires a toxicant to immobilize the captured moths. In 1980 a 3.5" piece of Thuron flea collar containing 8.37% DDVP replaced the discontinued 3g (18.6% DDVP) Zodiac flea tag as the toxicant for this trap. Hercon Division of Health Chem Corporation formulated a laminated insect tape containing 10.2% DDVP (44.5 mg/in^2) as a trap toxicant. This tape was tested in 1981 comparing 1 x 4" strips, aged 2, 6 and 10 weeks with similarly aged 3.2" samples of Thuron flea collar. This bioassay indicated that the aged Hercon insect tapes lost their effectiveness to immobilize moths much more readily than the Thuron flea collars. Four new formulations of DDVP toxicant in the insect tape form were prepared by Hercon for testing in 1982.

An aging study of the 4 new 1 x 4" formulations of Hercon (10% DDVP) insecticidal tape was done outdoors in milk carton traps at intervals of 15, 30, 42, 60 and 75 days from May 6, 1982 to July 19, 1982. Thuron Industries did not meet the delivery date for the 1982 flea collar order and the contract was awarded to Reuter Industries. The Reuter flea collars arrived in June and were not aged outdoors. The 20 aged Hercon treatments along with the unaged Hercon and Reuter control were set out in 5 replicates in a natural infestation in Berkley, MA. They were placed in a 20 x 6 grid pattern with the traps spaced 50 m apart. They were checked 4 times July 21, 23, 26 and 29, and moths were counted and removed. Three insecticidal strips from each of the treatments were placed in zip-lock plastic bags and analyzed by Health-Chem Corporation Laboratories.

Table 1 shows the total moth catch for each of the 6 aging periods.

All of the formulations demonstrated good killing action after up to 75 days of aging. Health Chem Corporation has elected for reasons of economy and ease of production to go with the formulation identified as 0092V in Table 1. This will add another killing agent that may be used in the milk carton trap.

Table 1. Number of moths caught in traps using different formulations of Hercon (DDVP) insecticide strip.

	Total Moth Catch for Each Aging Period							
Treatment	0	15	30	42	60	75	Treatment Total	Mean
0082V	369	253	385	273	250	239	1769	295
0092V	408	357	231	304	252	269	1821	304
0102V	359	163	231	169	304	199	1425	238
0112V	318	346	203	242	198	225	1532	255
Block total	1454	1119	1050	988	1004	932	6547	273
Reuter	304							

Statistical analysis was performed[1/] to determine if there were any differences in the total performance of the 4 Hercon formulations for the total catch of the 6 aging periods. Differences among treatments were not significant at the 0.5 level.

1/ By Laura T. Zeoli, Manager, Pesticide Registration Product Development, Hercon Division, Health Chem Corporation.

Project Number: GM 2.2.1
Project Title: Ecological Range of the Gypsy Moth
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: V. C. Mastro, A. P. C. Toles and G. L. McAninch

Introduction

APHIS participates in a survey and detection program for the gypsy moth over a large portion of the uninfested United States. Ecological characteristics (climate, available host) make establishment of the pest in some areas questionable. If, however, it is shown that the gypsy moth can establish in these areas, survey and detection trapping should be maintained and results reviewed from a more critical perspective than, perhaps, has been done to date.

Although we do not know what the geographical limits are in the United States, the known distribution in Europe and Asia suggests that gypsy moth is a very plastic species capable of surviving in a wide variety of climates (Forbush and Fernald 1896, Goldschmidt 1934, Giese and Schneider 1979), and on a wide variety of hosts (Mosher 1915, Doskotch 1977 and Fusco 1980).

The immediate question is: will the gypsy moth establish as far south as Florida? The encompassing question, however, is what is the ultimate range of the gypsy moth in North America, and what types of damage would result from establishment in currently uninfested areas?

Methods and Materials

To determine if climatic conditions in Florida are adequate for egg development and diapause requirements, ten newly laid masses from laboratory reared stock are being shipped every two weeks to a field insectary in Gainesville, Florida. When available, these egg masses were produced from P-1 laboratory reared adults reared from feral egg masses and at other times NJ F-23 stock. Also, to determine if egg masses transported to Florida from the northeast at different times of the year will hatch and thus pose a risk for introduction, 10 egg masses from wild populations near Otis ANGB will be collected from the field every two weeks and shipped to the same insectary. Cages containing egg masses in Florida are checked weekly for hatch. A duplicate control set of egg masses held in an insectary at this facility also is checked weekly.

During March and April, 1982 an effort was begun to screen plants for suitability as hosts for the gypsy moth. All plants were shipped as bare root seedlings. On receipt they were potted and held until new foliage had begun to expand at which time the terminals were caged and 10 neonates were introduced into each cage. Daily data were kept on larval survival and development. When necessary (all foliage consumed) larvae were transferred to a fresh terminal. All larvae were from field collected egg masses.

Seven species of plants were screened as hosts for the gypsy moth: bald cypress, Taxodium distichum, live oak, Quercus virginiana, laurel oak, Quercus laurifolia, sycamore, Platanus occidentalis, loblolly pine, Pinus taeda, sand pine, Pinus clausa and southern red cedar, Juniperus silicola. Tests with live and laurel oaks are not complete, however, both supported larvae until the third instar. High initial mortality was noted on insects infested onto bald cypress; one insect developed to the adult stage in 60 days. Sycamore, southern red cedar and loblolly pine did not support any larvae beyond the first instar. On sand pine, 3 individuals developed to the third instar after which all died.

Project Number: GM 2.2.2
Project Title: Evaluation of Complete Inventory of Disparlure
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leaders: E. C. Paszek and C. P. Schwalbe

Five lots of gypsy moth pheromone, (+) disparlure, that are presently in storage were bioassayed for activity in a natural infestation in Berkley, MA. The lure was impregnated into cotton wicks in 3 concentrations of 1, 10 and 100 ug/100 ul. These wicks were bioassayed August 2-13, 1982 in milk carton traps randomly placed 50 m apart in an 8 x 15 grid. Each treatment was replicated 8 times, and traps were checked 3 times; moths were counted and removed.

The mean number of moths captured in traps baited with the 3 concentrations of lure from all of the 5 lot sources combined are presented in Table 1.

Table 1. Mean number of moths captured in traps baited with 3 concentrations of lure (data from Table 2 pooled).

Lure Concentration	Mean No. of Moths Captured	
	$\bar{x}$	Sd
1 ug	11.1c	6.5
10 ug	26.3b	13.6
100 ug	42.9a1/	15.9

1/ Means in column are significantly different at the 95% level of confidence.

Mean numbers of moths captured by 5 individual lots of the 3 concentrations of pheromone are presented in Table 2.

Analysis of the data indicate that there was a significant difference among the 3 disparlure concentrations. There was no significant difference between the 5 lot sources of disparlure at the 1 ug concentration. However, at the 10 ug concentration there was a significant difference for the 2 lure sources having the highest means MSU 1979, Andrulis 4-18-80 and the lure source having the lowest mean (Andrulis 80-81). At the 100 ug concentration, Hercon L-20-T was significantly more effective than MSU 1979 and Plimmer.

Table 2. Mean number of moths captured in traps baited with 3 concentrations of disparlure from 5 sources.

Lure Concentration	Hercon L-20-T		Andrulis 80-81		Andrulis 4-18-80		MSU 1979		Plimmer	
	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
1 ug	10.3a	5.1	7.9a	3.8	15.9a	8.9	12.5a	3.9	9.3a	7.5
10 ug	25.0bc	6.0	17.1c	6.7	31.6ab	9.2	37.8a	18.7	20.0bc	13.5
100 ug	52.4a	10.1	46.8ab	11.6	49.0ab	18.1	35.9bc	18.0	30.6bc	11.4

1/ Means in rows followed by the same letter are not significantly different at the 95% level of confidence.

Project Number: GM 2.2.3
Project Title: Evaluation of Trap Adhesives for use in the Delta Survey Trap
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leader: E. C. Paszek

In 1982 a series of tests were designed to evaluate trap adhesives that may be used in Delta survey traps with particular emphasis on the storage of these adhesive treated unassembled traps. Tack Trap, the currently used trap adhesive in Delta traps at a 8 ± 1 g application rate has a tendency to run over the allotted 377 cm^2 surface area and smear the stacked traps. When the traps are stored for several months in a hot dry area, the solvent in the tack trap tends to volatize causing the adhesive to shrink and tenaciously adhere to the overlapping panels of the prepared traps. At the time of trap assembly these panels are difficult to pull apart and often tear upon separation.

Five trap adhesives listed in the table below had 6 treatment rates applied onto 377 cm^2 surface areas of Delta traps. They were folded and stored stacked on a shelf at room temperature from 3/24 - 5/4/82.

Table 1. Condition of traps when opened after 6 weeks of storage (wt. in grams applied to 377 cm^2 surface area).

Excelcide		Aeroxon		.4-the-Birds		Tange-Trap		Tack Trap	
6.4	S.Runny	6.7	S.Runny	6.5	S.Runny	6.5	Intact	6.1	Runny
8.6	S.Runny	8.4	S.Runny	8.2	S.Runny	8.6	Intact	8.1	Runny
10.3	Runny	10.4	S.Runny	10.3	S.Runny	10.0	Intact	10.3	Runny
12.2	Runny	12.2	Runny	12.4	S.Runny	12.3	Intact	12.0	Runny
14.1	Runny	14.4	Runny	14.5	S.Runny	14.0	Intact	14.1	Runny
16.1	Runny	16.1	Runny	16.1	S.Runny	16.1	Intact	16.1	Runny

After 6 weeks of storage, the stacked traps were opened and checked for the seepage of adhesive beyond the 377 cm^2 designated surface area of the trap. Excelcide treated traps were slightly runny to runny. Aeroxon treated traps were also slightly runny to runny, and in the 3 highest treatments the folded panels stuck tenaciously together and were difficult to pull apart. 4-the Birds adhesive was slightly runny in all treatments; when compared to the other adhesives, it was less tacky. Tangle-trap did not run in any of the 6 treatments and remained tacky. Tack Trap was runny in all 6 treatments but remained very tacky. Additional storage tests were designed for 3 of the above adhesives, Excelcide, Tangle-Trap, Tack Trap and a new sample of Aeroxon named Bug Glue.

Twenty traps of Excelcide, Tack Trap and Aeroxon Bug Glue were treated with 8 ± 1 g of the adhesive. Tangle-Trap was tested in 2 treatments 8 ± 1 g and 10 ± 1 g. All of these traps were stacked at room temperature for 4 months and checked for weight loss 9/1/82. Five samples from each of the 5 treatments were weighed and the spread (see page) of the adhesive beyond the designated 377 cm^2 area was measured.

Table 2. Weight loss and spread of adhesives after 4 months of storage at room temperature.

Excelcide 8 g ± 1		Aeroxon Bug Glue 8 g ± 1		Tack Trap 8 g ± 1		Tangle-Trap 10 g ± 1			
Spread	Wt +	Spread	Wt -	Spread	Wt -	Spread	Wt +	Spread	Wt +
1.5	+0.4	0	-1.2	0.7	-0.8	0	+0.7	0	+0.5
0.7	+0.5	0.2	-1.0	0.2	-0.8	0	+0.5	0	+0.5
0.7	+0.4	0.2	-1.0	1.0	-0.8	0	+0.5	0	+0.5
1.5	+0.5	0	-1.0	0.5	-0.7	0	+0.5	0	+0.3
1.0	+0.5	0	-0.9	0.2	-0.7	0	+0.6	0	+0.4
Avg. spread 0.9" Runny Avg. Wt gain 0.5g		Avg. spread 0.1" Slightly runny Avg. wt loss 1.0g		Avg. spread 0.5" Runny Avg. wt loss 0.8 g		Not Runny Avg. wt gain 0.6g		Not Runny Avg. wt 0.4	

After 120 days of aging stacked flat on a shelf at room temperature Aeroxon Bug Glue lost an average of 1g/trap and was slightly runny with a spread of 0.1". Tack Trap lost 0.8g/trap and was runny with a spread of 0.5". Excelcide hygroscopically gained an average weight 0.5g and was runny with a spread of 0.9". The 8g and 10g treatments of Tangle-Trap gained 0.6 and 0.4g/trap, and this adhesive remained intact on the designated 377 cm^2 surface area of the traps.

One each of the 5 adhesives that were aged in a stack of 20 traps on a shelf for 120 days 5/3-9/3/82 were hung outdoors 9/3/82 for weathering. Six male moths were released into each of the traps after 12 days and 60 days of outdoor weathering.

Table 3. Six male moths released into Delta traps with the following adhesives at 12 day and 60 day intervals.

Adhesive	Wt in Grams	Moths Captured 24 hrs after release into Traps	
		12 day interval	60 day interval
Excelcide	8 ± 1	6	6
Aeroxon Bug Glue	8 ± 1	6	6
Tack Trap	8 ± 1	6	6
Tangle Trap	8 ± 1	6	6
Tangle Trap	10 ± 1	6	6

All of the above adhesives remained tacky and held moths after 60 days of outdoor weathering. An outdoor bioassay was done in 1976 with the Delta survey trap comparing 5 trap adhesives Tack Trap, Excelcide, Bird Tanglefoot (label changed to Tangle-Trap 1982), Stickem-Special and Tree Tanglefoot. (p.32, Table III, 1976 Gypsy Moth Laboratory Report). This study indicated that after 60 days of field use in a natural infestation, Exelcide and Bird Tanglefoot (Tangle-Trap) could be substituted for the Tack-Trap adhesive.

Based on the evaluation of 5 trap adhesives done in 1982 and the outdoor bioassay of 5 trap adhesives in 1976 I would recommend Tangle-Trap at the 10 lg rate as a substitute for Tack Trap. Tack Trap, Excelcide, 4-the Birds and Aeroxon Bug Glue are runny adhesives and contaminate the traps in storage. 4-the-Birds is not a tacky adhesive. Tangle-Trap is a harnessed adhesive, it stays within the confines of its designated space and captures and holds moths. It may be applied at higher rates than the 8 lg rate without running, and the mechanical application can be facilitated by heating to a liquid state and applied with a brush.

Tack trap, the trap adhesive presently used in the Delta survey traps for gypsy moths is manufactured by Animal Repellents, Inc., 1016 Everee Road, Griffin, GA 30223 (8-404-227-8222). A dealers price quotation dated 11/23/82 for a 35 lb. (5 gal. pail) was $92.09 plus shipping charges.

Tangle-trap, the recommended trap adhesive is manufactured by the Tanglefoot Company, 314 Straight Ave, SW, Grand Rapids, MI 49504 (8-616-459-4130). A dealers price quotation dated 11/23/82 for a 25 lb. pail was $54.57 plus shipping charges. The dealers price of Tangle-Trap is $2.18/lb., $0.45 less than Tack Trap which is $2.63/lb.

Project Number: GM 2.2.4
Project Title: Induced Inherited Sterility Trial - Horry County, SC
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: V. C. Mastro and G. L. McAninch

Introduction

Irradiation studies have determined that males treated at the proper pupal age with 10 krads instead of fully sterilizing dose (15 krads), and mated with untreated females produce progeny which are completely sterile (Project No. 8.2.2). Release of these partially sterile males in a native population offers many advantages over a totally sterile male release. The year after release potentially approximately 200 sterile males and 100 sterile females are produced from each sub-sterile male mating so that the overall population suppression effect is much greater. Sterile progeny are reared in the field, which eliminates rearing cost and perhaps synchronizes the periodicity of adult activity better. Field release costs are also decreased if a second season of release is not required.

Evaluation of several areas of F-1 sterility have been undertaken. F-1 eggs successfully overwinter under field conditions and hatch begins (in the spring) at the same time as normal egg masses. However, hatch from F-1 egg masses is less synchronous than normal egg masses and they require an average of 2-6 days longer to complete hatch. In laboratory tests, survival is similar for F-1 and control larvae. Larval behavior and survival in the field remains to be studied and compared, but competitiveness comparisons in laboratory and field trials have demonstrated F-1 males were fully competitive with wild type males.

Methods and Materials

To evaluate the induced inherited sterility principle under field conditions a sparse isolated infestation was chosen for release of sub-sterile males. The objective was to determine if F-1 progeny of released males survive and interact with the native population. Intensive trapping in 1981 (traps not placed on a uniform grid) captured 47 males in a small area. An egg mass survey during the fall of 1981 succeeded in locating 2 egg masses and two pupal cases. In April of 1982 a systematic grid (47 m^2) of larval sampling points with three trees banded at each point was established over an area of ca. 170 ha. Burlap bands were checked weekly and the numbers of larvae and/or pupae recorded.

Male releases began on May 26 and ended on June 28. Approximately 9,000 - 10,000 males which had been irradiated (10 krads) between 8 and 11 days after pupation were released daily. A weighted release system, similar to the system used in the Benton Harbor, MI sterile male pilot test, was used to distribute male pupae so that the greatest number were placed where 1981 trap catches were the greatest and where larvae were found under burlap bands in 1982. Release cage design and placement was the same as that used in the Benton Harbor pilot study.

To monitor sterile overflooding ratios and determine the isolation and limits of the infestation, a grid of traps (32/sq mi) was established over approximately a 9 sq mi area. Traps in the center square mile (where the infestation was confirmed) were checked daily and the rest were examined weekly. All captured males were identified: released moths were internally marked red and wild ones were, naturally, unmarked. These counts were used to estimate population density and achieved overflooding ratios.

All traps used in the survey were standard 2 quart milk carton traps. To monitor mating success of sterile and wild males, 20 unmated one-day old laboratory reared females were placed each morning in the infested area and recovered each evening. Females were placed in standard A-frame mating arenas. Egg masses from these females were returned to the Otis laboratory where determinations will be made on mating type. At the end of the flight season the area was extensively searched for feral egg masses and (to minimize the impact of sampling) only one-half of each mass, when possible, was removed and returned to the laboratory for evaluation.

Results and Discussion

The burlap band survey succeeded in locating 16 larvae. All larvae were found within the area where 1981 trap catches had been highest. A total of 13 feral males were captured all in monitor traps within the "core" area. Sterile male recovery in traps for the entire flight period was 8,099 or ca. 2.5% of the total number of male pupae placed. Overflooding ratios, computed daily during the period of feral male flight, using data only from the three traps where feral males were captured, averaged 77S:1F. Samples from a total of 21 feral egg masses were collected at the end of the field season. These samples along with egg masses recovered from monitor females, are being held under chill (6°C) for 180 days. When diapause requirements are met, hatching larvae will be reared in the laboratory. Comparison of the fertility of F-1 adult progeny with laboratory control matings will permit separation between mating types in the parental generation.

Even though the proportion of sterile males recovered in Horry Co. is similar to proportions recovered from Benton Harbor (1980 - 3.5% and 1981 - 2.9%) several differences were noted. First, the proportion of pupae eclosing successfully in South Carolina was lower. High relative humidities which caused higher moisture levels in release cages appear to have retarded eclosion some, however, mortality caused by delays in shipment probably contributed to pupal mortality to a greater degree. Also, casual observations suggest that avian predation was not as great a problem in South Carolina as observed in Michigan.

Higher temperatures in South Carolina resulted in eclosion from each shipment of pupae over a shorter period of time compared to that in Benton Harbor. The same proportion of eclosion (80%-90%) which occurred over 7 or 8 days in Michigan required only 3 or 4 days in South Carolina. This rapid eclosion is not detrimental unless consecutive male shipments are missed or delayed. If many shipments are missed or delayed the number of sterile males in the field declines rapidly, resulting in windows where overflooding ratios are lower than desirable.

Using male capture data from the three positive traps (i.e. traps which captured feral males during the period of feral flight), estimates can be made of the effects of the release and the size of the residual feral population in 1983. Several assumptions were included in the calculation of these estimates: 1) 3% of the adult feral male population was captured in traps, 2) the feral population had a 1:1 sex ratio, 3) the intrinsic rate of natural increase (Rm) for the feral population is 3 x. (This is the rate of increase found in the Benton Harbor pilot study site, however, at other sites it has been found to vary greatly), and 4) survival and development of F-1 progeny in the field is similar to that of laboratory studies. These estimates of the results of the 1982 release do not consider the effects of F-1 females in 1983 because their competitiveness has not been investigated. If they are not selectively competitive for either F-1 males or feral males, their net effect should not substantially change the estimated results.

Summary of Horry County, SC Inherited Sterility Pilot Test

	F-1		Estimated No. of Sterile	Estimated No. of Feral		Mean S:F Male Ratio	F♀ x F♂	F♀ x S♂
	♂	♀	♂	♂	♀			
1982[1/]	0	0	33,800	433	433	77:1	5.6	427.4
1983	855[2/]	427[2/]	None Released	16.8[3/]	16.8[3/]	52:1	0.3	16.5

1/ Estimates of sterile and feral insects based on a 3 percent trapping rate

2/ Number of feral females mating with released males in 1982
(427.4) x 2 (Rm for F-1♂) = 855
x 1 (Rm for F-1 ♀ = 427

3/ Number of feral females mating with released males in 1982
(5.6) x 3 (Rm for feral ♂ +♀) = 16.8

Project Nunber: GM 2.2.5
Project Title: Mass Trapping Pilot Study - Monona, WI
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leader: V. C. Mastro

Introduction

Delimitation survey in 1981 clearly defined a small infestation of gypsy moths adjacent to the eastern shore of Lake Monona, WI. The highly residential nature of the area was well suited for treatment with one of the non-pesticide eradication techniques now under development. Mass trapping was selected for testing in the area.

Methods and Materials

The area outlined on Figure 1 was, from the results of 1981 delimitation trapping, considered infested. This area (approximately 125 ha) was trapped at the rate of 7.4 traps per ha using a uniform grid for trap placement. Where possible, all traps were placed on host trees at ca. 1.5 m in height. Where host trees were not available, man made objects were used.

To provide an estimate of larval distribution and density within this core area, trees were banded with burlap strips at the rate of 7.4/ha. Trees were selected by the following order of preference:

1) Oak - white
2) Oak - red
3) Oak - all others
4) Willow - all species
5) Birch - all species
6) Aspen
7) Ash
8) Larch
9) Apple

Burlap bands were checked weekly June 9 to July 15. All larvae and pupae were collected and reared in the laboratory for determination of parasitism.

In a buffer zone 400 m surrounding the core area, traps were also placed at the same rate of 7.4 per ha. A buffer area was established to compensate for any possible spread from the core area or from undetected populations near the infestation. To determine the degree of isolation and detect any outlying infestation, the area around the mass trapping area was trapped at a rate of 32 traps per square mile.

Results and Discussion

Twenty-nine larvae and two pupae were found under burlap on banded trees, and no evidence of parasitism was detected. All collections were within the central core area where 1981 trapping results indicated the population was located. A total of 114 adult males were captured, also all within the core area, with no additional captures in the buffer zone. However, delimitation trapping succeeded in locating a small infestation approximately 7 miles northwest of the core area.

Results of burlap banding and trapping in 1983 will be used to evaluate the impact of the 1982 trapping program. After the 1983 program is complete a final report will be submitted. Trapping results in 1982 indicate that there was a significant reduction in the population from the previous year without any intervention, with the exception of delimitation trapping. Although the precise reason for this reduction is unknown, the severe 1981-1982 winter weather probably contributed to mortality in the egg stage. Unless the 1982-1983 winter is severe, results of the test should not be compromised.

Project Number: GM 2.2.6
Project Title: Design of Traps for Capturing Live Male Gypsy Moth
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leaders: V. C. Mastro, G. L. McAninch, A. P. C. Toles, J. D. Peterson, D. B. Hawley

The objective of tests described in this report was to develop an efficient pheromone baited trap that would capture and retain male gypsy moths alive. Trap comparisons were conducted in both low and high native populations and in simulated male populations. Several different techniques were used to assess the performance of various trap designs, including direct observations and counts of total males captured. At the initiation of the test series, past trap design information and male behavior experience were considered and fourteen new or modified trap designs were constructed for testing. As the series of tests progressed, poor designs were either modified or dropped and, as new insights into design efficiency were obtained, new trap designs were added. Because the final number of trap designs was large (n = 30), all will not be described in detail. Traps that were highly efficient or design characteristics which enhanced efficiency will, however, be described. To provide a base line for comparison the standard two quart milk carton trap was included in the test (trap type 1). Also, the standard delta trap was used in some tests for comparison (trap type 19). Several modifications of both traps were also tested.

In an area where larval gypsy moth population densities were low and where it was anticipated that adult flight would be light, five parallel lines (60 m spacing) were established. Trap positions were established at 20 m intervals on all lines, and a trap type was randomly assigned a position on each line. All traps were hung ca. 1.5 m from the ground on branches except delta-type traps which were stapled at the same height to the boles of trees. Traps were checked daily, the number of males captured were recorded, and the trap positions randomized. Data were analyzed using a randomized complete block design, and trap differences were separated using Duncan's multiple range test. To remove possible edge effects (tendency for traps on the edge of a block to capture more males), a standard milk carton trap was placed on the end of all lines. Data from these "buffer traps" were not included in the analysis.

To evaluate trap performance in a dense native population, a circular plot design was used. Trap positions were established at 20 meter intervals along the circumference of a circle. Each trap type was represented twice (once on each half of the circle). Traps were checked and rerandomized daily. In the first test, traps were hung from the limbs of trees, (1.5 m ht). However, in the following tests, to minimize possible position effects, traps were hung from one-half inch steel L-shaped rods driven in the ground at least one meter from the nearest tree.

Additional tests were conducted after native flight using the circular plot design and releasing laboratory reared males (as pupae) in the plot center.

Observational data were also gathered on some trap designs. Depending on the number of observers available 4 or 5 trap types were placed on 2" diameter pine posts at 1.5 m in height. For 20 minute observation periods all males flying within a meter of the trap were observed and the following behaviors recorded: 1) orienting to the trap, 2) touching the trap and 3) entering the trap. At the end of 20 minutes, observers and trap position were randomized. All observational test data were collected during native male gypsy moth flight.

Trap Comparison - low native insect density

Table 1 presents the results of the first series of tests in which 14 trap types were compared (7/28 - 8/3). Trap type 1 (standard 2-quart milk carton), which was the only design tested in this series which killed the captured males, served as a control. With the DDVP strip removed from the standard milk carton (trap type 2), the mean number of males captured was less than the standard milk carton in Tests 1-3, although it was only statistically different in the first test. One trap design (type 9) consistently captured more males (not significant) than the standard milk carton. Several designs (trap types 4, 5, 9, 10 and 13) demonstrated the ability to capture and retain males. However, no common design features can be attributed to the performance of these traps. Some traps provided better airflow through the (5, 9, 13) while others had entry ports designed to better retain captured males (4 and 5).

In the second test (8/5 - 8/10 - Table 1) several traps (types 3, 7 and 11) with poor performance were eliminated, and four new traps added (type 15, 17, 18 and 19). Trap type 15 was a modification of trap type 3 which attempted to correct entry port problems. Trap type 19 was the standard delta trap, type 17 the delta trap with a rectangular entry port cut the length of the trap bottom, and type 18 the delta trap with an additional triangular entry port cut in the bottom. These traps were included to determine if the design of the delta trap could be improved. In trials where delta-type traps were tested, traps were checked and if necessary, replaced at least twice daily to prevent overloading of the tacky surface. Again, in this test, the trap design which resulted in the greatest mean number of males captured was trap type 9 followed by the standard milk carton trap and the delta-type traps.

In the third test conducted in the sparse native density plot, four unsatisfactory designs were dropped (trap types 4, 6, 12 and 15) and four new designs were added (trap types 20, 21, 22 and 23). These trap designs attempted to incorporate features from the traps which had better performance in earlier trials. Again, in this third test trap type 9 captured the greatest number of males. This design consistently captured almost twice as many males as any other live male trap type tested. Comparison of the standard delta trap with the standard milk carton trap in Tests 2 and 3 indicates that at least under these conditions the traps performed similarly.

Table 1. Mean male captures for various trap types in a sparse native population.

Trap Code	Test 1 (7/28 - 8/3)	Test 2 (8/5 - 8/10)	Test 3 (8/11 - 8/19)
1	29.4 a	52.0 abc	32.6 abc
2	5.6 cd	28.8 abcde	31.6 abc
3	0.2 f	-	-
4	10.6 cd	19.4 bcde	-
5	11.6 bc	18.2 abcd	15.4 cde
6	9.0 cd	36.4 bcde	-
7	0.4 f	-	-
8	1.2 ef	35.0 bcde	21.8 bcd
9	32.6 ab	74.2 a	61.0 a
10	7.0 cd	25.0 abcd	8.0 ef
11	0.0 f	-	-
12	5.8 cd	13.4 de	-
13	16.8 cd	22.4 ef	7.0 f
14	4.0 de	25.6 bcde	29.4 bc
15	-	3.6 f	-
17	-	41.4 abc	39.4 ab
18	-	68.0 ab	33.8 abc
19	-	52.8 abc	29.4 abc
20	-	-	19.0 bcd
21	-	-	6.2 ef
22	-	-	19.6 bcd
23	-	-	8.2 def

Trap Comparison - high native insect density

Three tests using a circular plot design were run in a high density native population. The first test was initiated on July 30 using the same trap designs tested during this period in the low native insect densities except that trap types 7 and 11 were not included. Traps in the first test were suspended from the limbs of trees approximately 1.5 h. Results of this first test (Table 2) indicate that in dense populations several trap types (2, 4, 5. 14) appear to perform better than the standard milk carton without DDVP (trap type 2). Trap type 9 did not perform as well in the first test as in the low native density plots. Even though it captured only the fifth largest number of males, this was not significantly different from the four trap types capturing more males.

Because of possible positional effects which may have biased the results of the first test, traps in the second two tests were hung from 1/2" dia. steel L-shaped rods driven into the ground at least 1 meter from the nearest tree. During the period of the first test native females were eclosing in large numbers, presenting numerous potential pheromone sources on trees from which traps were hung. With this change, results of the second and third tests were similar to results of tests in low insect density test. Trap type 9 again had the highest mean number of males captured, although not significantly more than the standard milk carton trap.

Table 2. Mean number of male moths captured in traps of various designs in a high native insect density.

Trap Code	Test 1 (7/30 - 8/3)	Test 2 (8/4 - 8/10)	Test 3 (8/11 - 8/16)
1	62.5 ab	64.0 ab	24.5 a
2	25.5 bcd	21.5 c	6.0 abc
3	7.0 c	-	7.5 abc
4	50.5 abc	43.0 abc	4.0 bc
5	76.5 a	50.5 abc	11.5 ab
6	40.0 abcd	31.5 abc	-
7	-	-	-
8	7.0 e	64.0 abc	9.5 abc
9	47.0 abc	142.5 a	27.0 a
10	28.5 bcd	19.0 bc	2.5 c
11	-	-	-
12	18.5 d	23.0 bc	7.5 abc
13	23.0 cd	16.0 c	3.0 bc
14	77.0 ab	80.0 ab	13.5 a
15	-	4.0 d	2.0 c
22	-	-	3.0 bc

Trap comparison - simulated male population

A series of trap comparisons were conducted after native flight using the circular plot design with laboratory reared males released from the plot center. Several new traps were included in this test and were designed to include characteristics which were thought to improve trap efficiency such as: better ventilation, entry ports designed to take advantage of male walking-orientation behavior and a larger vertical area to take advantage of searching males orienting to vertical silhouettes.

Trap types 20, 29 and 30 performed as well as the standard milk carton trap (type 1). Trap type 9 in this test surprisingly caught significantly fewer males than the standard. Characteristics of this trap (a long vertical silhouette and good airflow) had been incorporated into two other designs, traps 20 and 30. Traps 20 and 30 had different types of entry ports and captured a large number of males; trap type 30 captured a far greater number of males than any other trap. Modifications were made on the standard milk carton in an attempt to improve air movement through it; type 29 had 180 3/16" holes punched in the bottom half of the trap, type 25 had 180 holes punched in the top half of the trap (around the entry ports) and type 27 had 360 holes punched in both the top and bottom halves. All three modifications contained a DDVP strip. Under these test conditions modifying airflow characteristics of a milk carton trap did not appear to affect its performance. However, this may not be true under all populations and environmental conditions.

Table 3. Mean number of released laboratory-reared males captured.

Trap Code	Test 1 (8/17 - 8/31)
1	168.0 ab
2	79.0 bcd
9	104.0 bcd
14	53.5 de
20	169.0 ab
24	124.0 bc
25	105.5 bcd
26	65.0 cd
27	102.0 bcd
28	26.5 e
29	164.0 ab
30	320.5 a

Considering the results of the three series of tests, trap type 9 should be an efficient live male trap which can be used in evaluating F-1 sterile male releases. This trap was at least as efficient as the standard milk carton trap in all but the last tests. The last test series indicates that trap efficiency may be still further improved through incorporation of design features leading to improved sensitivity of survey and detection programs.

Direct observation of traps to compare trap efficiency was done throughout the native flight season, whenever time and personnel permitted. Each observation period was 20 minutes and the data reported in the following tables summarizes 27 of these observation periods or 9 hours of observation. The 4 traps chosen for most observations were designs used in the preceeding tests; trap codes remain the same. Table 4 presents data on the relative attractancy of traps i.e. the ability to attract males that are near it or "orientation efficiency". Of the four traps, the standard two quart milk carton attracted the least number of males. With this particular trap, orientation efficiency appeared to vary with the wind conditions during the test period. On very calm days the trap orientation efficiency decreased while on windy days it increased. These data indicate that, overall, the milk carton trap appears to be deficient in attracting males.

Table 4. Orientation efficiency - the percentage of males flying within one meter of the trap that were observed orienting to it.

Trap Type	Trap Code	No. of males Observed Within 1 m of Trap	Percent Orientating	
Milk Carton, Standard	1	289	28.7	a1/
Delta, V-bottom	17	387	34.9	ab
Delta, Standard	19	338	36.4	b
Delta, Slit-bottom	18	320	39.0	b

1/ Percentages followed by the same letter are not significantly different at the .05 level according to Chi square analysis.

When trap efficiency (no. of males captured divided by no. of males orienting to a trap) was used to compare the various traps, no significant differences between traps were found (Table 5). Therefore, once males orient to any of these four trap types, they are equally likely to be captured.

Table 5. Trap efficiency - the percentage of males that oriented to a trap that were captured.

Trap Type	Trap Code	No. of Males Observed Within 1 m of Trap	Percent Captured	
Milk Carton, Standard	1	83	41.0	a1/
Delta, V-bottom	17	135	43.0	a
Delta, Standard	19	123	38.2	a
Delta, Slit-bottom	18	125	43.2	a

1/ Percentages followed by the same letter are not significantly different at the 0.5 level according to Chi square analysis.

Orientation efficiency and trap efficiency data for traps that were observed for shorter periods of time are presented in Table 6. Although the numbers of observations were not enough for statistical analysis, results are generally consistent with the results of the previously described trap comparisons. Traps that captured large numbers of males generally had either a high orientation efficiency or trap efficiency characteristic or both. Scrutiny of trap characteristics to determine how they influence trap performance may allow design of new traps in a more informed manner.

Table 6. Orientation efficiency (OE) and trap efficiency (TE) for various trap types.

Trap Code	No. of Male obs within 1 m	Orientation Efficiency	Trap Efficiency	No of Observation Periods
4	88	49.1	37.0	4
5	99	34.3	44.1	4
6	61	42.6	34.6	4
8	59	25.4	26.7	4
9	148	35.1	76.9	4
10	68	16.2	45.5	4
12	63	46.0	62.1	4
13	50	44.0	45.5	4
14	101	28.7	48.3	4
15	60	23.3	50.0	4
20	70	25.7	72.2	6
30	182	45.6	73.5	10

Project No. GM 2.2.7
Project Title: Influence of Trap Position on Trees in Capturing Moths in Delta Survey and Milk Carton (MC) Monitoring Traps
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leader: E. C. Paszek

Objective

This test was designed to compare the influence of trap position on a tree trunk as opposed to free hanging traps placed on the ends of branches in attracting moths.

Method

In this study the delta survey traps and the MC monitoring traps were stapled 3' high above the forest litter on trees with 12" diameter trunks. The free hanging traps were hung 10' away from large sized trees 3' above the forest litter on the end of a branch. They were set out 8/11/82 in a 10 x 4 grid with trap spacings 50 m apart. Traps were checked 3 times and removed on 8/13/82.

Results

Table 1. Number of moths captured in traps placed on tree trunks and at the end of branches.

Trap Type	Placement	Total No. Moths Captured	Average No./Trap	Average No./Reading
MC	trunk	369	37	12
Delta	trunk	345	35	12
MC	free hanging	204	20	7
Delta	free hanging	240	27	9

Both delta and MC traps stapled to tree trunks captured more moths than the free hanging traps. In the free hanging position the delta trap appeared to be slightly more effective in capturing moths than the MC trap.

Project Number: GM 7.3.4
Project Title: Insect Production and Distribution
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: J. J. Baker, J. A. Tanner, and L. F. Kennedy[1/]

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 1982 production and distribution of such material.

A. Production

I. Number of larvae started by manually placing neonates on diet.

	Neonates
Laboratory	605,760
Colony	422,090
Insecticide	250,982
Virus	304,160
Total	1,582,992

II. Number of eggs dispensed with the egg infest method. This semiautomated infesting technique was introduced into the facility on April 5, 1982 and is now exclusively used.

	Eggs
Laboratory	767,190
Colony	15,920
Insecticide	632,880
Virus	178,620
Pheromone	214,752
Michigan Sterile Male	3,782,002
Otis Sterile Male Red	383,400
Otis Sterile Male Plain	533,440
Total	6,508,204

III. Egg Masses Refrigerated: 102,956 masses

1/ Retired

B. Distribution of reared insects, 1982.

Project	Egg Masses	L-1	L-2	L-3 L-4	L-4	L-5 L-6	Male Prepupae	Male Pupae	Female Prepupae	Female Pupae
W. McLane (APHIS)		36	218,340	15,670	8,550	1,846				
M. Shapiro (ARS)		22,560	242,680	12,880	3,580	2,500				
Ring Carde' (U. Mass)								95,400		13,800
Pedro Barbosa (U. Maryland)	123							1,500	27,500	
J. Kennedy (England)								1,900		1,900
Ralph Webb (ARS)								11,500	2,000	8,000
J. Elkinton (U. Mass)								1,500		
O. Cranshaw (Michigan State)								3,000		3,000
Mike Ma (U. Maryland)						25,000				
C. Yin (U. Mass)				14,000						
T. ODell (USFS)	1,350									
R. Chianese (NJDA)	2,347									
R. Fusco (PA FPM)	366									
W. Yendol (Penn State)	230									
V. Mastro (APHIS)							466,717			
Total	4,416	22,596	461,020	42,550	12,130	29,346	466,717	114,800	29,500	26,700

C. Summary of sterile male production, FY 1982.

Project	Rearing Period (days)	Male Prepupae	Male Pupae	Female Pupae
Michigan-Red	102	106,299	908,536	
Michigan-Red	85			64,856
Otis-Red	69	8,720	87,889	
Otis-Red	2			1,000
Otis-Plain	70	34,626	115,943	
Otis-Plain	56			54,638
Total	384	149,645	1,112,368	120,494

Project Number: GM 9.3.6
Project Title: Substituting Supro-610 for Casein in the Gypsy Moth Diet (B-4) Under Mass Rearing Conditions
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leaders: John Allen Tanner and Bernadine P. Weeks

Introduction

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

Objectives

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

Methods and Materials

P_1 Generation:

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

The insects were allowed to develop to the pupal stage at 25.5°C, 50% RH, and a 15L:9D photoperiod. The pupae were held at 25.0°C, 50% RH, and 15L:9D photoperiod until adult emergence occurred. Both growth chambers were known to have a 2-3° C temperature difference between the upper and lower level during the summer rearing program, and larval and pupal development rates varied significantly between them. To minimize this effect, each rack was divided into three levels (upper, middle and lower). Each level contained 8 trays (240 cups). Complete developmental and reproductive data were taken from insects at each level. Approximately 25 egg masses were obtained from each level/treatment replication, and embryonated 28 days. The egg masses were chilled 180 days at 6-8 C.

F_1 Generation:

Developmental data were taken on F_1 progeny of insects reared on diet containing Supro-610 or casein. One half of the F_1 neonates from each treatment were reared on diet containing Supro-610 as the protein source. The remaining F_1 neonates were reared on diet containing casein. This resulted in the following four protein source combinations:

Protein Source P_1 Generation	Protein Source F_1 Generation
Supro-610	Supro-610
Supro-610	Casein
Casein	Supro-610
Casein	Casein (Control)

The F_1 neonates were also separated by the rearing cart levels the parent population was reared on. The F_1 neonates from each P_1 rearing cart level were likewise divided and reared on the top, middle or bottom portion of the rearing cart. This resulted in the following rearing rack combinations for each protein source combination:

Rearing Rack Level P_1 Generation	Rearing Rack Level F_1 Generation
Top	Top
Top	Middle
Top	Bottom
Middle	Top
Middle	Middle
Middle	Bottom
Bottom	Top
Bottom	Middle
Bottom	Bottom

The above rearing rack level combinations allowed us to partition any temperature gradient effects out of the statistical analysis.

The development of 40 neonates (4 cups with 10 neonates/cup) were followed for each protein source - rearing rack location combination. A total of 360 neonates were used for each protein source combination/replication. Three replications were conducted, each commencing at weekly intervals. All three replications were conducted in the growth chamber which originally held the P_1 parent pupae (25.0° C, 50% RH, 15L:9D).

The developmental and reproductive data taken for the parental generation and the F_1 generation consisted of the following measurements. Each measurement is preceded by the letter P, F or P-F which indicates if the measurement was made in the parental (P), the F_1 (F), or in both generations (P-F).

	Generation measurement taken	Measurement
1)	P-F	Percent establishment of neonates on diet.
2)	P-F	The mean larval stage on the 7, 14 and 21 post infest date (PID).
3)	P-F	The mean male and female pupal DT_{50}.
4)	F	The mean male and female pupation curve.
5)	P-F	The mean male and female pupal weights on the 35th PID.
6)	P-F	The percent male and female pupal deformity.
7)	P-F	Percent survival of neonate to pupae.
8)	P-F	The mean male and female adult DT_{50}.
9)	P-F	The percent male and female adult deformity.
10)	P-F	The percent male and female adult recovery.
11)	P-F	The sex ratio.
12)	P	The percentage of mating pairs.
13)	P	The mean number of eggs laid/female.
14)	P	The percent hatch of eggs chilled 120, 150 and 180 days.

The developmental and reproductive data for P_1 generation gypsy moth reared on diet containing either casein or Supro-610 as the protein source are presented in Table 1. The developmental data of their offspring (F_1) also reared on diet containing either casein or Supro-610 are presented in table 2.

In general, development and reproduction of insects reared on Supro-610 diet in the P_1 generation was comparable to those reared on casein diet, but in the F_1 generation development on Supro-610 was inferior to that on casein diet.

The mean larval stages of P_1 larvae reared on Supro-610 diet were slightly lower than P_1 larvae reared on casein diet. However, the differences in the mean larval stages were only 0.1 stage at each post infest date (PID) that the measurement was taken. This indicates that neonates reared on Supro-610 diet were slower to establish on the diet and begin growth, however, once growth commenced they developed at the same rate as neonates reared on casein diet.

Significantly fewer established neonates survived to the pupal stage when reared on Supro-610 diet compared to neonates reared on casein diet (Table 1). However, the reduction in survival was only 4.2%.

The P_1 reproductive data and the subsequent F_1 hatch data were similar for insects reared on each diet.

The mean larval stage of F_1 larvae reared on Supro-610 diet was considerably lower than F_1 larvae reared on casein diet (Table 2). This was true regardless of the diet the P_1 larvae were reared on. The differences in the mean larval stages ranged from .4 to .7 stage and was fairly consistent at each PID the measurement was taken. This indicates that this time the F_1 neonates reared on Supro-610 diet required a considerably longer time to establish on the diet and begin growth than F_1 neonates reared on casein. However, as in the P_1 generation, once the neonates became established and growth began, the F_1 larvae on Supro-610 diet developed at a rate similar to the F_1 larvae on casein diet.

Significantly fewer established F_1 neonates survived to the pupal stage when reared on Supro-610 diet than F_1 neonates reared on casein diet. Reduction in survival was 10-11 percent.

The F_1 female pupae tended to weigh more when reared on the Supro-610 diet. They also had more deformity and gave rise to more deformed adult females. This was not the case in the P_1 generation (Table 1).

The poor results with Supro-610 in the F_1 generation may be due to the source of Supro-610 used. In the P_1 generation a small test sample was purchased from the company. In the F_1 generation the Supro-610 test samples were taken from a larger quantity purchased by this facility for intended use in the sterile male program.

The differences in the results observed in the F_1 generation compared to the P_1 generation indicate that further testing is needed on the Supro-610 before it can be considered for use as a substitute for casein in the B-4 diet. One question that seems to need answering is how much does the quality of the Supro-610 vary between lot numbers?

Conclusion

The substitution of Supro-610 for casein in the B-4 diet can not be recommended at the present time.

References Cited

Bell, R.A., C. Owens, M. Shapiro, and J.G.R. Tardif. 1981 Development of Mass Rearing Technology, Chapter 6.5 in C.C. Doane and M.L. McManus, Ed. The Gypsy Moth: Research Towards Integrated Pest Management, U.S. Dept. of Agri. Tech. Bull. 1584: 599-655.

Table 1. Development and reproduction of P_1 generation gypsy moth under mass rearing conditions as affected by the substitution of Casein with Supro-610 in the B-4 diet[1/].

Parameter	Casein $\bar{x}$	Casein Sd	Supro-610 $\bar{x}$	Supro-610 Sd
% Establishment	96.2	3.8a	98.9	1.3a
x Larval stage				
7 (PID)	2.0	0.1a	1.9	0.1a
14 (PID)	3.9	0.1a	3.8	0.2a
21 (PID)	5.4	0.2a	5.3	0.2a
DT_{50} Pupae (days)				
Male	27.8	1.1a	28.5	1.3b
Female	29.9	1.2a	30.6	1.6b
Pupal Wts (gms)				
Male	0.69	0.02a	0.70	0.03a
Female	2.28	0.11a	2.31	0.13a
% Pupal deformity				
Male	11.3	3.5a	11.3	4.6a
Female	82.7	5.8a	81.5	6.9a
% Survival neonate to pupae	93.8	2.5a	89.6	1.0b
DT_{50} Adults (days)				
Male	41.5	1.1a	42.1	0.7b
Female	41.0	0.5a	41.3	0.8b
% Adult deformity				
Male	0.7	1.0a	0.0	0.0a
Female	15.4	6.8a	18.6	7.9a
% Adult emergence				
Male	99.6	0.9	98.9	1.4a
Female	92.4	5.0	93.2	5.5a
Sex ratio				
Male:Female	50:50	2.3	52:48	2.5b
% Mating pairs	96.5	4.2a	97.3	2.8a
# Eggs/mass	1061.9	75.0a	1102.9	83.7a
Diet contamination:				
% of cups with:				
Virus	0.0		0.0	
Bacteria	0.0		0.0	
Mold	0.1	0.2	0.4	0.5a
% Hatch of eggs chilled:				
120 days	91.9	7.6a	90.7	10.2a
150 days	92.0	9.4a	89.4	12.6a
180 days	91.3	8.6a	89.0	11.2a
HT_{50} of eggs chilled:				
120 days	10.1	2.0a	10.5	1.5a
150 days	4.5	1.4a	4.7	1.2a
180 days	1.7	0.6a	1.7	0.8a

1/ Means within a row not followed by the same letter are significantly different at the .05 level.

Table 2. Development of F_1 generation gypsy moth as affected by the substitution of casein with Supro-610 in the B-4 diet of the P_1 and F_1 generation[1/2/].

Parameter	S-S		S-C		C-S		C-C	
	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
% Establishment	96.5	4.4a	97.6	4.0a	95.5	6.3c	97.5	4.6a
x Larval stage								
7 (PID)	1.4	0.5b	1.9	0.4a	1.5	0.5b	1.9	0.3a
14 (PID)	3.1	0.8b	3.7	0.5a	3.1	0.8b	3.8	0.5a
21 (PID)	4.7	0.9b	5.3	0.6a	4.8	0.9b	5.3	0.6a
DT_{50} Pupae								
Male	30.0	1.1a	27.6	0.6c	30.1	0.9a	28.3	0.7b
Female	32.2	0.8a	30.3	0.8b	32.1	0.9a	30.6	1.0b
Pupal Wts (gms)								
Male	0.72	0.04ab	0.73	0.03ab	0.71	.04b	0.74	0.06a
Female	2.45	0.22a	2.15	0.18c	2.41	.22a	2.26	0.22b
% Pupal deformity								
Male	13.8	21.4a	12.9	17.8a	14.6	22.6a	16.0	20.1a
Female	95.9	11.1a	84.0	18.8b	93.7	16.6a	86.3	17.9b
% Survival neonate to pupae	83.8	6.6b	94.8	4.0a	84.2	9.2b	94.2	5.3a
DT_{50} Adults								
Male	44.3	1.5a	41.5	0.7c	44.5	1.5a	42.2	0.7b
Female	44.4	1.0a	42.2	1.5b	44.3	1.0a	42.5	1.0b
% Adult deformity								
Male	1.5	2.8a	1.8	3.3a	0.8	2.2a	1.3	2.3a
Female	19.4	11.0a	12.8	10.4b	21.6	14.6a	10.7	10.0b
% Adult emergence								
Male	99.2	2.2a	97.2	5.6b	96.6	6.4b	97.9	4.7ab
Female	98.1	3.3a	95.8	4.8bc	94.4	6.2c	96.4	4.8ab
Sex Ratio								
Male:Female	49:51	9.1a	48:52	8.5a	51:49	7.6a	52:48	10.7a

1/ S = Supro-610
C = Casein

2/ Mean within a row not followed by the same letter are not significantly different at the .05 level.

Project Number: GM 1.3.1
Project Title: Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Rearing Facility
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: John Allen Tanner and Bernadine P. Weeks

The purpose of this project is to measure the quality of insects produced 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 variable on the production insects and to make changes in the rearing techniques when possible.

Insects reared solely for colony maintenance (colony insects) are currently serving as the internal standard. Colony insects are placed on diet, once a week, at weekly intervals. Their development is compared 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.

Colony insects are reared on diet made from dietary ingredients that are preweighed at the beginning of each rearing year (September 1) to ensure a complete years supply. The ingredients are stored at -15°C until needed. This ensures that each dietary ingredient is selected from one lot number. This reduces the variation in colony insect development that may be associated with differences in the quality of dietary ingredients obtained from different lot numbers.

Production insects are reared on diet made from dietary ingredients that are weighed out on a day to day basis. During the course of a rearing year, each dietary ingredient may come from several different lot numbers. This may result in increased variation in the development of the production insects.

Colony insects are reared at 8 neonates/diet cup with 30 diet cups on each rearing tray. Each rearing tray is placed in a single layer in each rearing truck section. Production insects are started 10 or 12 neonates/diet cup with 30 diet cups being placed on each rearing tray. Three rearing trays are stacked in each rearing truck section.

Developmental data and, in the case of the colony insects, reproductive data, are collected with the modified Tanner - Houle procedure (Tanner and Buck 1979a and 1979b, Tanner and Weeks 1980). Data are collected on the colony insects (8 per cup), the production insects (12/cup), and a special group of production insects reared at 8/cup. Comparison of the last two groups enables us to observe both the effects of density and diet on the development of the gypsy moth.

Table 1 compares the developmental data of colony insects started 8 neonates/cup and production insects started 8 or 12 neonates/cup for the 1982 rearing year (October 1981 to September 1982).

Table 1. Developmental data of colony insects reared at 8 neonates/cup and production insects reared at 8 or 12 neonates/cup.

Parameter	Number of Neonates/cup					
	Colony		Production			
	8		8		12	
	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
Mean larval stage						
- 7 (P.L.I.D.)1/	2.02	.14 a2/	2.02	.14 a	2.02	.09 a
- 14	3.86	.13 a	3.82	.13 a	3.82	.13 a
- 21	5.39	.10 a	5.41	.12 a	5.35	.13 a
Pupal weights (gms)						
Males	0.74	.04 a	0.74	.05 a	0.66	.04 b
Females	2.35	.13 a	2.39	.14 a	2.01	.17 b
Pupal DT50 (P.L.I.D.)						
Males	28.5	4.3 a	29.3	0.8 a	29.4	0.8 a
Females	30.3	4.5 a	31.0	0.8 a	31.1	0.9 a
% Pupal Deformity						
Males	10.5	7.6 a	9.7	6.5 a	8.6	6.5 a
Females	82.4	8.8 a	79.2	9.7 a	66.9	10.7 b
% Survival (Neonates to Pupae)	88.7	5.6 b	91.7	6.2 a	84.2	7.8 c
Adult DT50 (P.L.I.D.)						
Males	43.6	0.9 a	43.4	0.8 a	43.2	0.8 a
Females	42.6	0.9 a	42.5	0.8 a	42.2	0.9 a
% Adult Emergence						
Males	98.9	1.7 a	98.8	2.2 a	99.1	1.2 a
Females	88.9	15.0 b	86.9	13.3 b	94.9	7.8 a
% Adult Deformity						
Males	1.2	1.9 a	1.2	1.8 a	1.1	1.4 a
Females	18.0	13.2 b	20.0	12.7 b	10.2	8.1 a
Sex Ratio						
% M:% F	54:46	5.8 a	54:46	7.1 a	52:48	5.5 a
% Contamination						
Bacteria	0.00 a		0.00 a		0.02	0.15 a
Fungus	0.85	0.28 a	0.02	0.15 a	0.11	0.37 a
Virus	0.08	0.28 a	0.06	0.25 a	0.21	0.51 a

1/ P.L.I.D. = Post Larval Infest Days

2/ Means within a row not followed by the same letter were significantly different at the .05 level.

Several significant developmental differences were observed between the New Jersey colony (8 neonates/cup) and the New Jersey production insects reared at 8 or 12 neonates/cup. Colony insects had:

1) Higher male and female pupal weights than production insects reared 12/cup but similar weights to production insects reared at 8/cup.

2) A higher percentage of deformed female pupae and adults than production insects reared 12/cup but a similar percentage of deformed female pupae and adults as production insects reared 8/cup.

3) A lower percent survival to the pupal stage than production insects reared 8/cup but a higher percent survival than production insects reared 12/cup.

4) A lower percent adult female emergence than production insects reared 12/cup but a similar percent adult female emergence as production insects reared 8/cup.

With the exception of percent survival to the pupal stage, colony and production insects, both started at the 8 neonate/cup density had similar growth and developmental data. These data show that dietary ingredients can be held in a freezer for up to one year without any noticeable deterioration.

The 8 neonates/cup density produced heavier male and female pupae compared to the 12 neonates/cup density. The 8 neonates/cup density also produced a higher percentage of deformed female pupae and adults and a lower percent emergence of female adults.

Table 2 compares the 1981 and 1982 developmental data from colony insects. The developmental rate of the colony insects in 1982 was considerably slower than than in 1981, as evidenced by the lower mean larval stage at 7, 14 and 21 post larval infest day (P.L.I.D.) observations. The 1982 colony insects spent almost 2 days longer in the larval stage as indicated by the higher number of P.L.I.D. needed to reach the pupal DT_{50}. This longer larval stage may be the reason the pupae were heavier in 1982 than in 1981.

The sex ratio was more heavily skewed towards male in 1982 than 1981. Most other measurements were similar for 1982 and 1981.

Table 2. Comparison of the developmental data of colony insects infested in 1982 to the developmental data of colony insects infested in 1981.

Parameter	Colony Insects Infested 1982 $\bar{x}$	1982 Sd	1981 $\bar{x}$	1981 Sd
- 7 (P.L.I.D) 1/	2.02	.14 a2/	2.13	.17 b
-14	3.86	.13 a	3.93	.12 b
-21	5.39	.10 a	5.45	.20 b
Pupal weights (gms)				
Males	0.74	.04 a	0.73	.04 b
Females	2.35	.13 a	2.22	.15 b
Pupal DT^{50} (P.L.I.D.)				
Males	28.5	4.3 a	26.6	1.0 b
Females	30.3	4.5 a	28.6	1.1 b
% Pupal Deformity				
Males	10.5	7.6 a	16.9	7.3 b
Females	82.4	8.8 a	84.1	10.3 a
% Survival (Neonates to Pupae)	88.7	5.6 a	87.7	5.1 a
Adult DT_{50} (P.L.I.D)				
Males	43.6	0.9 a	40.7	1.4 b
Females	42.6	0.9 a	40.0	0.9 b
% Adult Emergence				
Males	98.9	1.7 a	99.1	1.5 a
Females	88.9	15.0 a	89.7	9.0 a
% Adult Deformity				
Males	1.2	1.9 a	1.2	1.5 a
Females	18.0	13.2 a	20.0	10.4 a
Sex Ratio				
% M:% F	54:46	5.8 a	51:49	4.1 b

1/ P.L.I.D. = Post larval infest day

2/ Means within a row not followed by the same letter are significantly different at the .05 level.

The sole function of the colony insects is to produce enough egg masses to provide for future rearing needs. These are the only insects that are mated in our rearing facility, thus, reproductive and eclosion data are only collected on the colony insects. To determine if there has been a change in the availability of neonates in the following generation, the reproductive and eclosion data collected in 1982 were compared with similar data collected in 1981 (Table 3). The results of the reproductive and eclosion data collected in 1982 were similar to the results of the data collected in 1981 with the sole exception that in 1982 a significantly lower percentage of females deposited egg masses.

Table 3. Reproductive and egg eclosion data of colony insects reared in 1981 and 1982.

	1982		1981	
	$\bar{x}$	Sd	$\bar{x}$	Sd
% of females depositing egg masses	83.8	13.9 b 1/	90.4	8.6 a
# Eggs deposited/female	1017	89.4 a	988	105.3 a
% Hatch				
120 day chilled eggs	91.4	6.4 a	93.0	6.7 a
150 day chilled eggs	87.6	20.8 a	93.5	4.6 a
170 day chilled eggs	92.0	5.5 a	91.4	6.5 a
Time to 50% hatch (P.E.I.D.) 2/				
120 day chilled eggs	8.8	0.8 a	9.1	1.1 a
150 day chilled eggs	4.0	0.6 a	4.0	0.8 a
170 day chilled eggs 3/	2.3	.05	1.7	0.6

1/ Means within a row not followed by the same letter are significantly different at the .05 level.

2/ P.E.I.D. = Post egg incubation days

3/ Eggs were chilled 170 days in 1982 but 180 days in 1981.

References Cited

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Tanner, J. A. and B. P. Weeks 1980. Establishing Standards. APHIS Laboratory Report October 1, 1979 - September 30, 1980: 167-175.

Project Number: GM 1.3.2
Project Title: Development of Mechanical Egg Infestation Procedures
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: John Allen Tanner, J. G. R. Tardif, J. J. Baker and Bernadine P. Weeks

This project concerns reducing the labor cost of infesting larvae onto diet, one of the major costs in gypsy moth rearing operations.

Gypsy moths are presently infested onto diet as neonate larvae. Neonates are carefully removed from hatch dishes (100 x 15 mm petri dishes) with a camel hair brush and gently knocked off the brush onto diet. Since care must be taken not to damage the neonates, this operation is tedious and time consuming.

Tests have shown that eggs can be incubated directly on the B-4 diet without sacrificing developmental synchrony or insect quality (Tanner and Weeks 1981). A mechanical egg dispensing apparatus was developed in late 1981. The unit operates on a simple shuttle principle and consists of a slide bed (.500" x 2" x 26.25"), a feed head (.750" x 2" x 21.75") and a shuttle (.125" x 1" x 24.5"). (Fig. 1). A 1.010" x .130" groove was milled down the center of the slide bed, and six .250" diameter holes were drilled in the center of the groove on 3.750" centers. The slide bed was then fitted with .250" I.D. x 3" L stainless steel tubing pressed into the .250" dia. holes which were counterbored .375" Dia. x .250" deep to allow an interference fit with the tubing O.D. These act as delivery tubes. The feeder head was drilled and machined to accept six 1.0" Dia. x 3.0" L stainless steel tubing reservoirs interference fitted to a depth of .500", and six .250" Dia. x 1.50" L stainless steel air delivery tubes. The holes were precisely indexed to match the 1-1/2" travel of the shuttle from reservoir to discharge.

A small .250" L x .250 W x .025" D channel was milled into the bottom of the feed head to allow a small flat spring (.250" x .005" with upturned end) to deflect a small distance (0 - .020"). This channel was then extended .400 L x .250 W x .100 D to allow spring mounting clearance (Fig. 2). This prevented the shearing of eggs. The shuttle was drilled with six properly spaced holes which were sized for the proper number of eggs required. Many shuttles were fabricated to allow quick changeovers. The hole sizes varied from .029" to .032" which deposited 9 to 18 eggs, respectively. The feed head was then hinged to the slide bed which was also hinged to the cart mounting plate. Grooves were cut into this mounting plate to allow the hinged machine to revolve 180° for emptying and cleaning. The machine was then fitted with a double acting air cylinder that actuates the shuttle, a millipore filter which keeps air biologically clean, and miscellaneous air line regulators, filters, gauges and valves. The whole assembly was then mounted on a special cart designed to allow trays of diet-filled containers to be passed under the machine's dispensing tubes (Fig. 3).

During operation the feed head is latched to the slide bed which in turn is latched to the cart mounting plate. The reservoirs are then filled with a quantity of clean dry eggs, and covered with a plastic cap. An operator then slides a tray of diet-filled containers under the delivery tubes, presses a foot valve which allows the shuttle to ram forward carrying the required number of eggs from the reservoirs to the delivery tubes. At the end of its travel the shuttle actuates an air valve which delivers a puff of clean air down through the delivery tubes. This prevents eggs from sticking in the shuttle cavities. The speed of the ram and the air puff are controlled by filtered and regulated air pressure. To further prevent eggs from shearing at the shuttle and reservoir interface, a small flat spring allows eggs which are slightly above the shear line to pass without damage by deflecting upward from 0 to .015". This allows up to 38% of an egg diameter to pass while restricting normal eggs with average diameters of .039". Each time the shuttle returns it is filled with eggs for the next cycle as the operator advances the trays. The unit can fill 6 containers per second (21,600 containers per hour). The machine's principle is adaptable to any type configuration and will lend itself well to future fill, infest and cap sequences requiring total automation.

Eggs to be used in the dispensing machine must be surface disinfected in a 10% Formalin solution to suppress nucleopolyhedrosis virus (Shapiro and Bell 1977). The setae which enclose the eggs must also be removed (dehaired) to allow free dispensing of the eggs by the shuttles. The setae are removed by gently rubbing the egg masses over a wire screen connected to a vacuum to draw off the setae. Removing the setae after the Formalin treatment may increase the risk of recontaminating the eggs. An alternate method would be to treat the dehaired eggs in the Formalin solution. This method may reduce the risk of recontaminating the eggs but direct exposure of the eggs to the Formalin solution may also be detrimental to the eggs.

The following test was conducted to evaluate treating dehaired eggs with the Formalin solution.

Methods and Materials

Five egg masses (New Jersey F_{21}) were randomly selected from colony egg masses chilled 180 days (4.9 - 8.2°C). Each mass was partitioned into three sections. One section was treated with a 10% Formalin solution (one hour) then rinsed (one hour) in running water (25°C). The eggs were then dried and dehaired in a wire funnel (Tardif and Secrest 1970). A second section was first dehaired, then treated, rinsed and dried. The remaining section was only dehaired.

Egg samples were placed by hand into rearing cups (XE-6 Sweetheart plastic cups) containing 85 ml of B-4 diet. The cups were held in an inverted position (diet on top) within the darkroom of an environmental chamber (25°C, 50-60% RH) for the first three days of incubation, after which the cups were placed in an upright position within the lighted section (16L:8D) of the above environmental chamber.

A sample consisted of 10 embryonated eggs/cup. A total of 10 cups were used for each section/mass. A total of four replications were conducted over a period of time. The results were analyzed by Chi-square contingency tables.

Treating dehaired eggs in the Formalin solution did not reduce the percent hatch of the eggs (Table 1) nor did it reduce the percent establishment (Table 2) or percent survival of the neonates (Table 3) compared to treating non-dehaired eggs. The Formalin treatment significantly reduced the hatch of eggs whether dehaired or non-dehaired compared to untreated controls (Table 1). The cumulative male pupation curve was the same for all treatments (Fig. 4).

Formalin treating the dehaired eggs resulted in the formation of egg clumps which had to be broken up before the eggs could be properly dispensed by the machine. This added handling increases the risk of recontaminating the eggs, thus defeating the original purpose for treating dehaired eggs. We also observed that dehaired eggs were more difficult to prepare for drying and that more eggs were lost during the drying process.

Currently whole egg masses are treated with Formalin. The masses are then rinsed, dried and dehaired in a sterile, wire funnel.

Table 1. The percent hatch of gypsy moth eggs as affected by exposing non-dehaired and dehaired eggs to a 10% Formalin solution.

	Dehaired-Formalin	Non-dehaired-Formalin	Dehaired-No Formalin
No. of eggs	1971	1878	1997
Percent hatch1/	86.7 ± 2.9 a2/	85.0 ± 3.9 a	88.3 ± 2.9 b

1/ Mean within a row not followed by the same letter is significantly different at the .05 level.

2/ ± S.D.

Table 2. The percent establishment of gypsy moth neonates as affected by exposing non-dehaired and dehaired eggs to 10% Formalin solution.

	Dehaired-Formalin	Non-dehaired-Formalin	Dehaired-No Formalin
No. of larvae	1709	1602	1763
Percent establishment1/	96.2 ± 2.1 a2/	95.3 ± 2.6 a	96.5 ± 2.7 a

1/ Means were not significantly different at the .05 level.

2/ + S.D.

Table 3. The percent of established gypsy moth neonates surviving to pupation as affected by exposing non-dehaired and dehaired eggs to 10% Formalin solution.

	Egg Treatment		
	Dehaired-Formulin	Non-dehaired-Formulin	Dehaired-No Formulin
No. of neonates	1644	1525	1701
Percent pupation1/	94.3 ± 2.8 a2/	92.7 ± 2.1 a	92.6 ± 3.6 a

1/ Means were not significantly different at the .05 level.

2/ ± S. D.

Advance knowledge of the percent hatch of eggs to be dispensed is necessary to select the proper dispensing shuttle which results in a predetermined number of larvae per cup. Accurately predicting the percent hatch of gypsy moth eggs has been difficult, especially when the final percentage was below 80% (Tanner and Buck 1979).

Most hatch tests consist of either dehaired or non-dehaired egg samples extracted from the core of several masses and incubated in petri dishes. A more precise method may be to select the hatch samples from the whole population of eggs to be dispensed. This sampling would occur after the masses have been treated and dehaired. The samples could then be incubated on diet and under conditions similar to what the whole population will eventually be exposed to.

Eggs chilled 180 days require one week of incubation to determine total hatch. During this time the remaining treated dehaired eggs must remain in the refrigerator. This may be detrimental to the eggs because removal of the setae (hairs) exposes the eggs directly to the cold temperatures and high humidity found in the refrigerator.

The following test was conducted to determine if treated-dehaired eggs can be stored in the refrigerator for up to seven days.

Methods and Materials

Five egg masses (NJF_{23}) were selected from laboratory egg masses chilled 173 days (8-9°C). The masses were treated in a 10% Formalin solution (1 hr), rinsed in 25°C water (1 hr) and air dried in a clean air hood before the setae were removed (dehaired) by gently rubbing the masses over a wire screen connected to a vacuum cleaner.

The newly dehaired eggs were divided into three equal groups. Two groups were placed into individual 8 oz. paper containers and returned to the refrigerator. The remaining group was hand dispensed into 6 oz. plastic cups (Sweetheart XE-6) containing 85 ml of B-4 diet. The cups were capped (untreated paper) and the eggs incubated within an environmental room (25°C, 55-60% RH, 14L:10D).

A second group of eggs was removed from the refrigerator after three days of additional refrigeration. The eggs were allowed to room dry for a few minutes before being passed through a sieve to remove any hatched neonates. The eggs were then dispensed and incubated in the above environmental room. The last group of eggs was removed from the refrigerator after seven days of additional refrigeration and handled as above.

All cups within an individual treatment were examined seven days after the eggs were dispensed. The number of unhatched embryonated eggs and unembryonated eggs, plus the number of established and nonestablished neonates were determined for each cup. The locations within the cup of the nonestablished neonates were also recorded.

Commencing on the 21st post dispensing day (PDD) each cup was checked daily and the number of male and female pupae tallied.

A total of 30 diet filled cups were used for each treatment/replication. A total of five replications were conducted over a period of time. The results were analyzed by Chi-square tables.

Results

Returning treated gypsy moth eggs to the refrigerator for 3 or 7 additional days, did not effect the hatch (Table 4) but did increase slightly the percentage of neonates which established on the diet (Table 5) compared to treated eggs given no additional refrigeration. Treated eggs given 7 additional days of refrigeration had a slightly higher percentage of neonates completing their development to the pupal stage compared to neonates from the other treatments (Table 6).

The rate (slope) of male pupation was similar for all three treatments but male larvae from eggs given 3 or 7 additional days of refrigeration pupated sooner than male larvae from eggs given no additional refrigeration (Fig. ~~6~~ 5). The maximum percentage of male larvae pupating within four days of each other was similar for all three treatments (Table 7). This four day period is critical when irradiating the male pupae for the sterile male program (Mastro 1980).

We did have one problem associated with this test. Treated-deharied eggs given 7 days of additional refrigeration tend to commence hatching while still in the refrigerator. The percentage that hatched was low (visual observation) and most of these were removed by passing the eggs through a sieve. However, more would hatch during the dispensing operation and these often "gummed up" the dispensing bar causing the bar to dispense fewer eggs than normal. This requires the bar to be cleaned often during the dispensing operations.

Table 4. The percent hatch of gypsy moth eggs (chilled 173 days) that were given 3 or 7 days of additional refrigeration after being treated (10% Formalin solution) and dehaired.

	Days of Additional Refrigeration		
	0 (Control)	3	7
Total embryonated eggs	1963	1911	1820
Percent hatch[1/]	93.2 ± 2.6 a[2/]	92.8 ± 2.5 a	91.4 ± 4.5 a

1/ Means within the row are not significantly different at the .05 level.

2/ ± S. D.

Table 5. The percent establishment of gypsy moth neonates that eclosed from eggs (chilled 173 days) that were given 3 or 7 days of additional refrigeration after being treated (10% Formalin solution) and dehaired.

	Days of Additional Refrigeration		
	0 (Control)	3	7
Total hatch eggs	1827	1773	1664
Percent establishment[1/]	95.3 ± 3.0 b[2/]	97.4 ± 1.9 a	96.4 ± 2.9 ab

1/ Means within the row not followed by the same letter were significantly different at the .05 level.

2/ ± S. D.

Table 6. The percent pupation of established gypsy moth neonates that eclosed from eggs (chilled 173 days) given 3 or 7 days of additional refrigeration after being treated (10% Formalin solution and dehaired.

	Days of Additional Refrigeration		
	0 (Control)	3	7
Total established neonates	1743	1725	1603
Percent pupation[1/]	86.5 ± 4.3 b[2/]	84.6 ± 5.0 b	90.2 ± 2.4 a

1/ Means within the row not followed by the same letter were significantly different at the .05 level.

2/ ± S. D.

Table 7. The maximum percentage of male larvae that pupated within four days of each other as affected by the 3 or 7 days of additional refrigeration the eggs (chilled 173 days) were given after being treated (10% Formalin solution) and dehaired.

	Days of Additional Refrigeration		
	0 (Control)	3	7
Total male pupae	802	763	730
Maximum percent pupation 1/	67.8 ± 6.8 a 2/	72.0 ± 7.2 a	70.2 ± 4.6 a

1/ Means within a row were not significantly different at the .05 level.

2/ ± S. D.

The following test was conducted to ascertain the accuracy of the above sampling procedure for predicting the ultimate percent hatch of eggs incubated 7 days later. This test was conducted shortly after the automated egg dispensing machine was evaluated for use in the summer sterile male program. The eggs used in this test were also used in the early portion of the sterile male program.

One hundred and ten egg masses (New Jersey F_{23}) were randomly selected and treated in a 10% Formalin solution (1 hour). The masses were rinsed (1 hour, 25°C H_2O) then dried (app. 30 minutes), and dehaired. A large sample of eggs was selected and immediately mechanically dispensed into 120 diet filled cups. The cups were then capped and the eggs incubated in the environmental room (25.5°C, 55-60% RH, 16L:8D) which would eventually house the remaining eggs. The remaining dehaired eggs were placed into a 16 oz. dixie cup and returned to the refrigerator for seven days.

Six days after the 120 sample cups were placed into the environmental room, all the cups were examined. The number of unhatched embryonated and unembryonated eggs were recorded for each cup. Also recorded for each cup was the number of established and nonestablished neonates. A mean percent hatch was determined from the 120 cup sample. Based on this percentage, a dispensing bar was selected to dispense the remaining eggs stored in the refrigerator. A bar was selected which would result in approximately 12 neonates/cup. The eggs were dispensed into 2520 diet filled cups and incubated in the above environmental room. One week after the eggs were incubated, 126 cups (5% of 2520) were randomly selected, and the number of unhatched and nonestablished neonates were determined for each cup.

The hatch results observed with the 120 sample cups were compared to the hatch results observed for the 126 cup sample by Chi-square contingency tables. The data were also compared for linear correlation. A total of 12 replications was conducted over a period of time.

Results

The 120 cup hatch sample gave a good general estimate of the percent hatch that occurred when all the eggs were incubated (Table 8). The percent hatch of the 120 cup hatch sample varied from the percent hatch of the 126 sample cups by only an average of 4.2%. The difference ranged from 0.1 to 10.1 percent. However, there was a lack of correlation (r = .52) between the paired percentages of the two sample groups.

Table 8. The accuracy of the 120 cup hatch sampling in predicting the percent hatch of gypsy moth eggs incubated seven days later.

Replication	Percent Hatch: Hatch Sample		Population Sample		Difference	
1	80.5		77.0		3.5	
2	71.5		71.6		0.1	
3	74.2		76.9		2.7	
4	83.4		75.1		8.3	
5	76.6		76.4		0.2	
6	82.2		78.4		4.2	
7	81.1		74.8		6.3	
8	84.9		78.6		6.3	
9	81.6		78.6		3.0	
10	73.4		76.2		2.8	
11	84.6		74.5		10.1	
12	73.7		71.1		2.6	
Mean 1/	79.0	4.8	75.8	2.5	4.2	3.0

r = .52 nonsignificant correlation

1/ ± S. D.

The automated egg dispensing machine was evaluated under large scale dispensing operation for use in the summer sterile male program.

Methods

One hundred egg masses (New Jersey F_{23}, chilled 170 days) were randomly divided into two-50 mass groups. One group was returned to the automated refrigerator (8 to 10°C). The second group was treated (10% Formalin solution), rinsed (25°C H_2O) and dried before being dehaired. Egg samples (12 eggs/sample) were placed into 20 cups containing 85 ml of diet and incubated in an environmental room (25.5°C 1.5°C, 55-60% %H, 16L:8D). The remaining eggs were returned to the automated refrigerator.

The hatch samples were examined six days after their incubation, and a mean percent hatch was calculated based on the total number of embryonated and unembryonated eggs. This percentage was used to select a dispensing bar which would dispense a sufficient number of eggs/cup to provide an average of 12 neonates/cup.

The dehaired eggs and non-dehaired egg masses were then removed from the refrigerator. A portion of the dehaired eggs were machine dispensed into 840 rearing cups with the bar selected based on the hatch sample results. A portion of the remaining dehaired eggs were manually placed into 840 additional rearing cups at the density calculated that would provide 12 neonates/cup. All the cups were then capped and placed into the above environmental room.

The non-dehaired egg masses were treated (10% Formalin) rinsed, dried, and then placed into a high humidity chamber within a darkened portion of the above environmental room. Three days later the newly hatched neonates were placed into 840 cups (12 neonates/cup) and returned to the dark room for one day before being placed with the other two treatments.

Seven days after the neonates were placed on diet (10 days after egg incubation), thirty cups were randomly selected from each treatment and examined. The number of unhatched embryonated and unembryonated eggs were recorded for each cup containing eggs, plus the number of established and nonestablished larvae were recorded for all the selected cups from each treatment.

Twenty-one days after the neonates were placed on diet (24 days after egg incubation), thirty cups were randomly selected from each treatment. All cups were examined daily. The number of male pupae within each cup was recorded until all male larvae had either pupated or died.

A total of four replications were conducted over a period of time.

Results

The percent hatch of the mechanically dispensed eggs was similar to the percent hatch of hand dispensed eggs (Table 9). The percent establishment was significantly but only slightly lower when neonates emerged from eggs mechanically dispensed onto diet compared to neonates that emerged from eggs hand dispensed onto diet (Table 10). However, the difference was only 1.5% which would be considered not significant in a mass rearing program. The percent establishment of neonates emerged from eggs mechanically dispensed was similar to the percent establishment of neonates started by the neonate infest method (Table 10).

The maximum percentage of male larvae pupating within four days of each other was about 9 to 10% higher when neonates were placed on diet than when eggs were placed on the diet (Table 11). This was similar to the results observed by Tanner and Weeks (1981) when the egg infest method was originally compared to the neonate infest method. Mechanically infesting the eggs onto diet had no effect on the maximum percentage of male larvae pupating within four days of each other compared to hand infesting eggs onto the diet (Table 11).

From these results it can be concluded that the egg dispensing machine has no effect on the eggs being dispensed and could be used successfully in the sterile male program.

The mechanical egg dispenser was used during the 1982 sterile male program. Compared to a similar program conducted in 1981, 4 fewer employees were needed to produce a similar number of male pupae.

Table 9. The percent hatch of gypsy moth eggs as affected by the dispensing method.

	Dispensing Method	
	Hand	Machine
No. of embryonated eggs	1596	1519
Percent hatch[1/]	84.5 ± 2.9 a[2/]	85.2 ± 4.1 a

1/ Means within the row were not significantly different at the .05 level.

2/ ± S. D.

Table 10. The percent establishment of neonates as affected by the egg dispensing method.

	Dispensing Method		
	Hand	Machine	Control[1/]
No. of neonates	1348	1295	1444
Percent Establishment[2/]	97.6 ± 1.0 a[3/]	95.8 ± 6.0 b	96.7 ± 1.5 ab

1/ Neonates started by the neonate infest method

2/ Means within row not followed by the same letter were significantly different at the .05 level.

3/ ± S. D.

Table 11. The percentage of male pupae which completed pupation within four days after 10% male pupation had occurred as affected by the egg dispensing method.

	Dispensing Method		
	Hand	Machine	Control[1/]
No. male pupae	635	582	664
Percentage[2/] [3/]	69.5 ± 3.1 b[4/]	68.5 ± 3.3 b	78.2 ± 9.7 a

1/ Neonates started by the neonate infest method.

2/ The percentage of male pupae which completed pupation within four days after 10% male pupation had occurred.

3/ Means within the row not followed by the same letter were significantly different at the .05 level.

4/ ± S. D.

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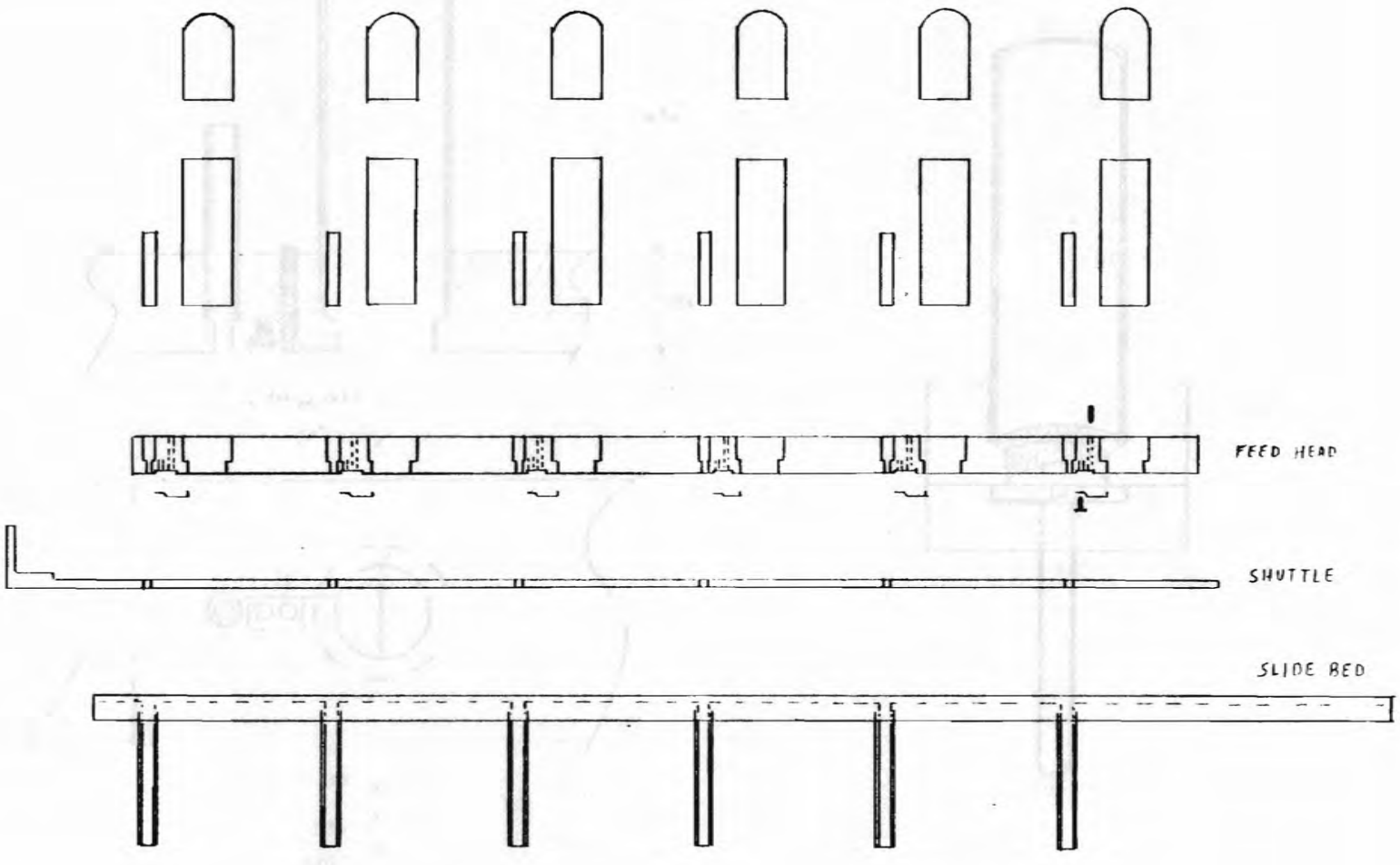


Figure 1. Egg dispenser blown away view.

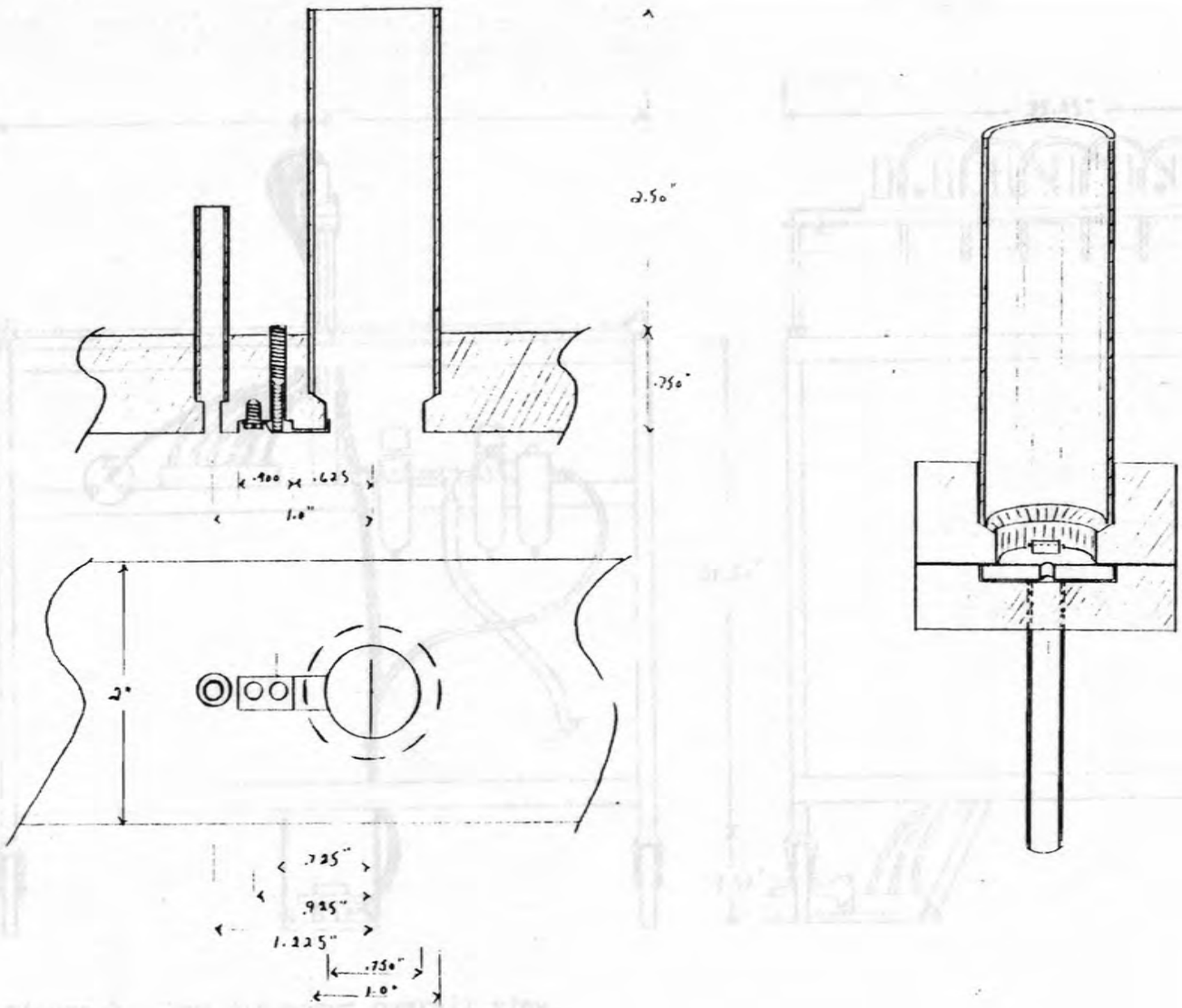


Figure 2. Feed head side and cross-sectional views.

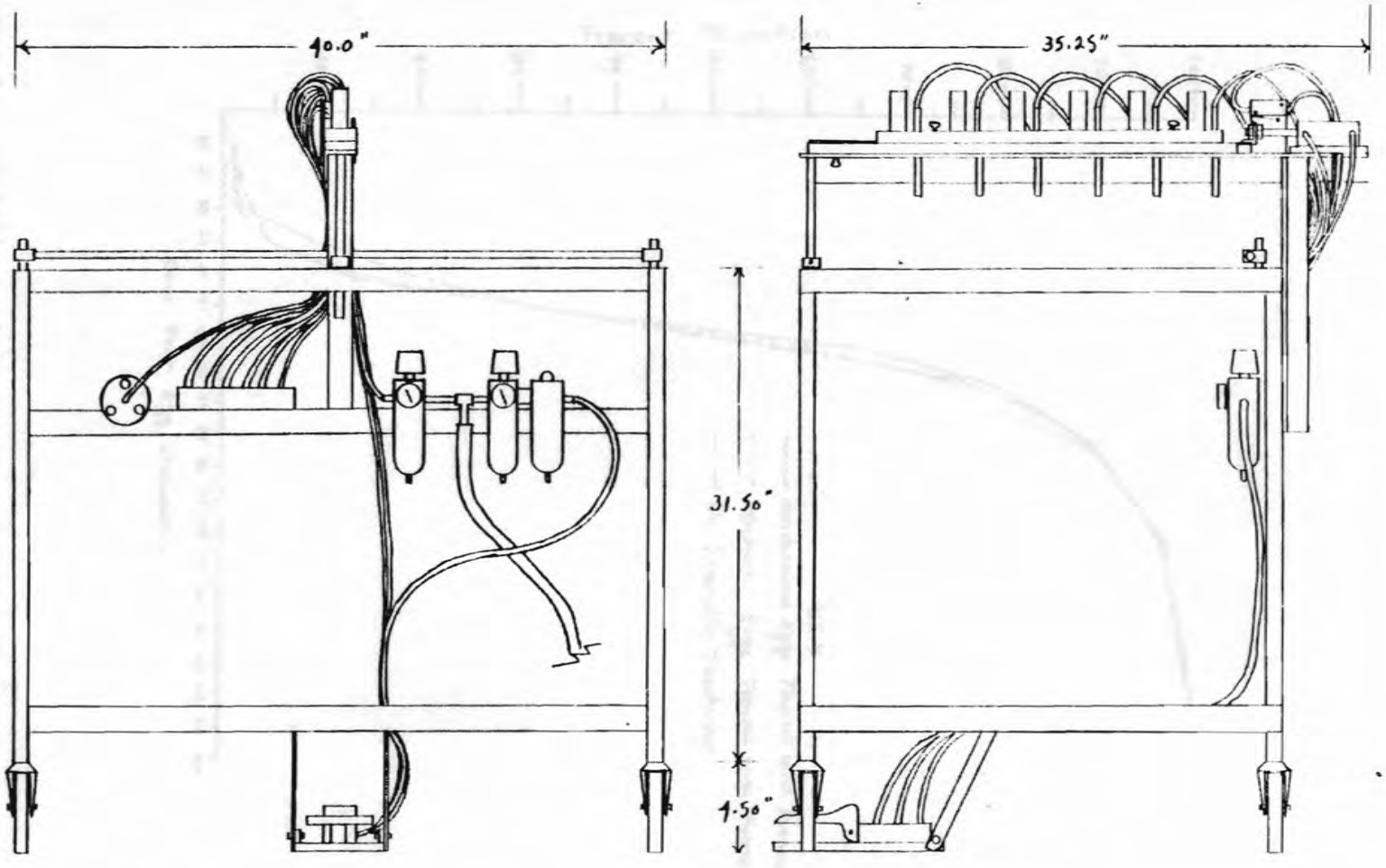


Figure 3. Egg dispenser overall view.

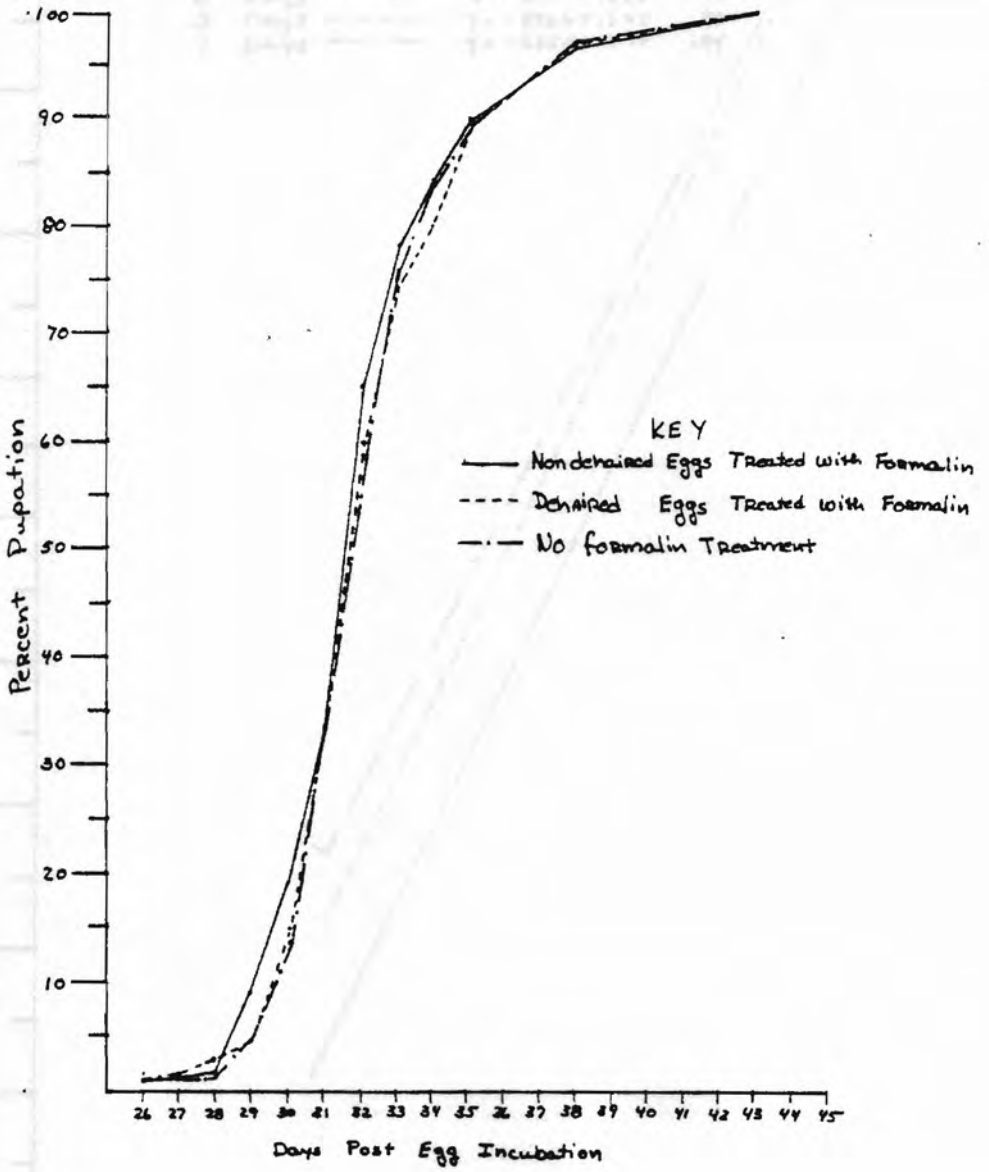


Figure 4. Cumulative male pupation as affected by treating dehaired eggs with a 10% Formalin solution.

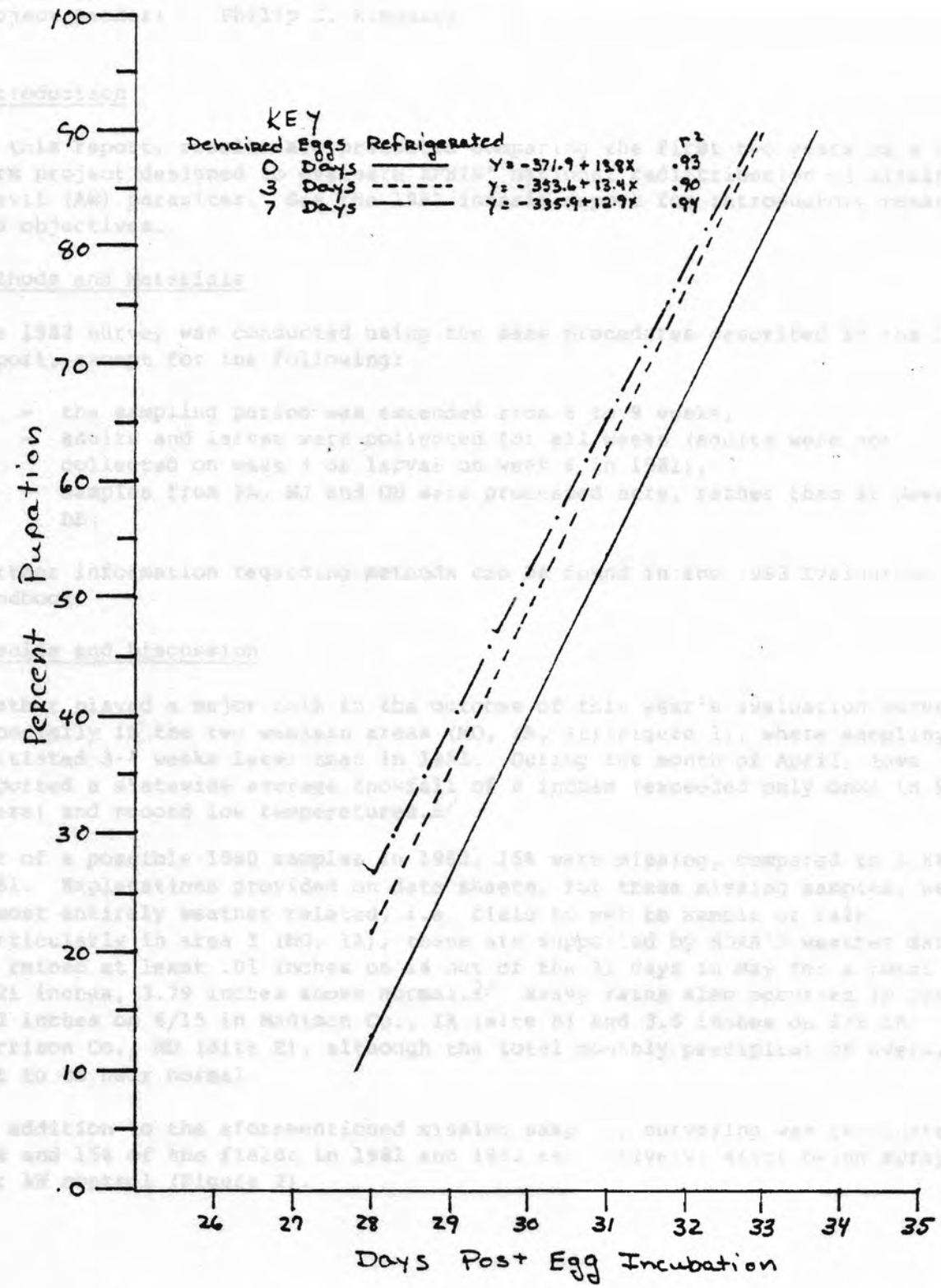


Figure 5. Rate of male pupation as affected by the length of refrigeration given to Formalin treated dehaired eggs.

Project Number: AW 1.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasite Redistribution Program
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leader: Philip C. Kingsley

Introduction

In this report, results are presented comparing the first two years of a long term project designed to evaluate APHIS' national redistribution of alfalfa weevil (AW) parasites. See the 1981 interim report for introductory remarks and objectives.

Methods and Materials

The 1982 survey was conducted using the same procedures described in the 1981 report, except for the following:

- the sampling period was extended from 8 to 9 weeks,
- adults and larvae were collected for all weeks (adults were not collected on week 4 or larvae on week 8 in 1981),
- samples from PA, NJ and OH were processed here, rather than at Newark, DE.

Further information regarding methods can be found in the 1982 Evaluation Handbook.

Results and Discussion

Weather played a major role in the outcome of this year's evaluation survey, especially in the two western areas (MO, IA, IL)(Figure 1), where sampling was initiated 3-4 weeks later than in 1981. During the month of April, Iowa reported a statewide average snowfall of 8 inches (exceeded only once in 92 years) and record low temperatures.1/

Out of a possible 1080 samples in 1982, 15% were missing, compared to 5.8% in 1981. Explanations provided on data sheets, for these missing samples, were almost entirely weather related, i.e. field to wet to sample or rain. Particularly in area I (MO, IA), these are supported by NOAA'S weather data. It rained at least .01 inches on 14 out of the 31 days in May for a total of 8.21 inches, 3.79 inches above normal.2/ Heavy rains also occurred in June, 3.2 inches on 6/15 in Madison Co., IA (site B) and 3.5 inches on 6/8 in Harrison Co., MO (site E), although the total monthly precipitation averaged out to be near normal.

In addition to the aforementioned missing samples, surveying was terminated in 19% and 15% of the fields in 1981 and 1982 respectively, after being sprayed for AW control (Figure 2).

1/ Climatological Data. National Oceanic and Atmospheric Administration, National Climatic Center, Asheville, NC.

2/ NOAA's weather data were averaged from 6 weather stations, one near each site.

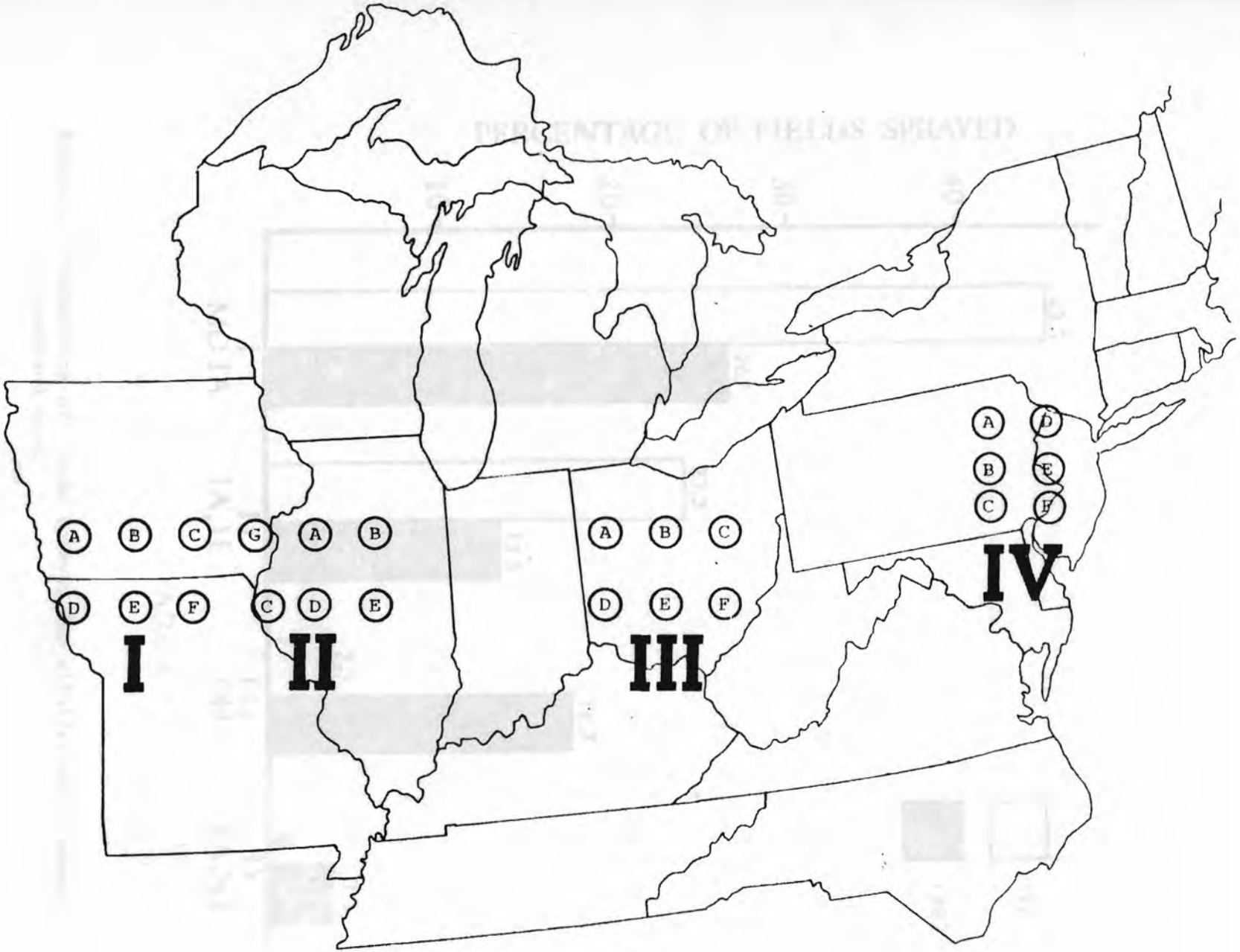


Figure 1. Alfalfa Weevil Evaluation survey areas and sites (5 fields/site).

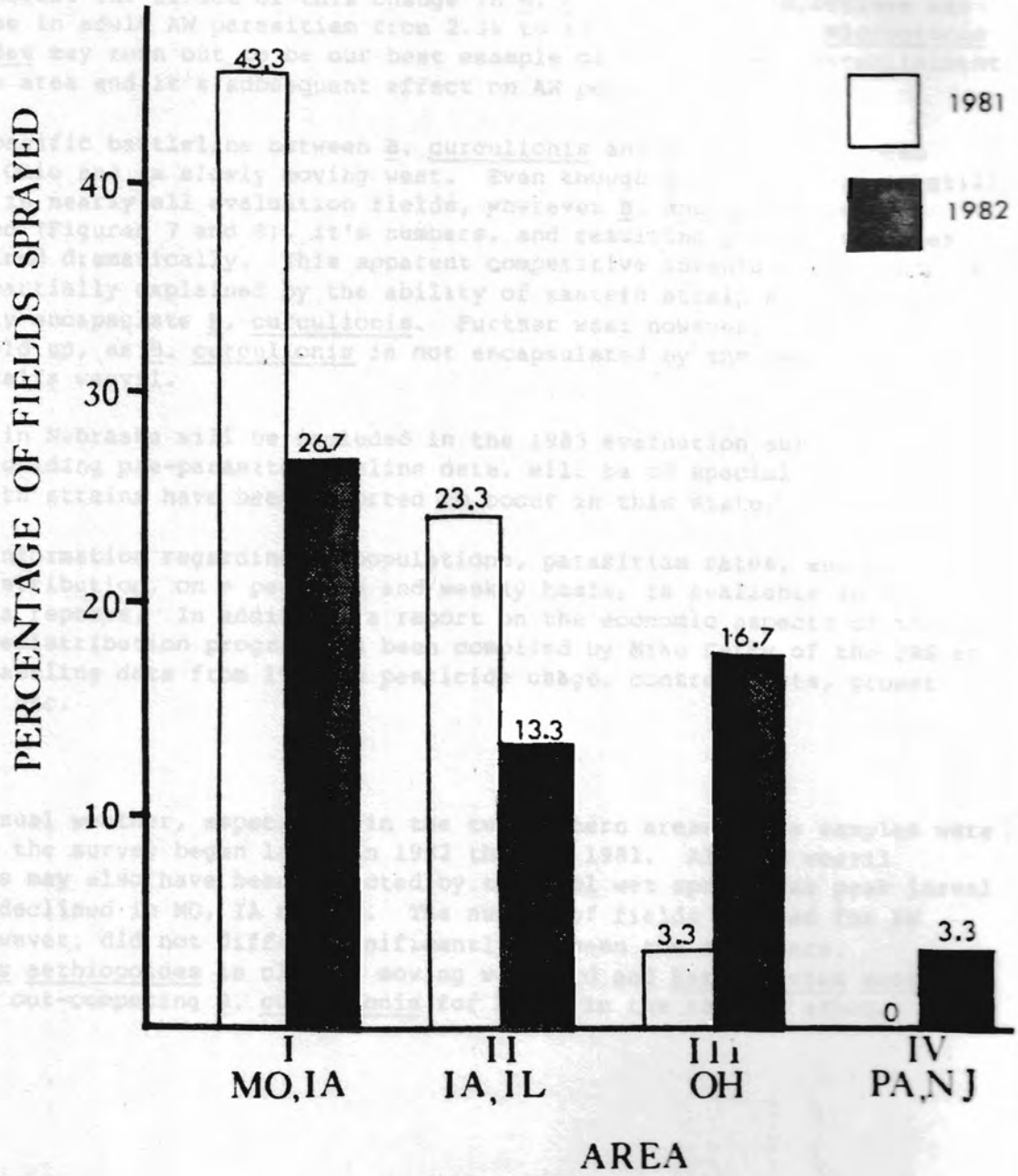


Figure 2. Percentage of fields sprayed for alfalfa weevil control by year and area.

Comparing last year's peak larval densities (Table 1, Figures 3 & 4), AW populations crashed in areas I and II (MO, IA, IL). These declines were presumably the combined results of heavy rains, especially in MO and IA, affecting the incidence of disease, and a general increase in parasitism rates. In area II (IA, IL), parasitism by Bathyplectes curculionis increased from 8% in 1981 to 36% in 1982 (Figure 7). As indicated by a rise in parasite species diversity in MO and IA (Table 1), the percentage of area I fields where the adult parasite Microctonus aethiopoides was collected, increased from 35.3 to 65.0 over the two years (Table 2), accounting for three new county records. The effect of this change in M. aethiopoides' importance was an increase in adult AW parasitism from 2.3% to 13.2% (Table 1). Microctonus aethiopoides may turn out to be our best example of a parasite's establishment into a new area and it's subsequent effect on AW populations.

An interspecific battleline between B. curculionis and B. anurus has been formed in Ohio and is slowly moving west. Even though B. curculionis is still collected in nearly all evaluation fields, wherever B. anurus has become established (Figures 7 and 8), it's numbers, and resulting parasitism rates have declined dramatically. This apparent competitive advantage by B. anurus might be partially explained by the ability of eastern strain AW to effectively encapsulate B. curculionis. Further west however, this advantage may not hold up, as B. curculionis is not encapsulated by the western strain of the alfalfa weevil.

Six sites in Nebraska will be included in the 1983 evaluation survey and, besides providing pre-parasite baseline data, will be of special interest because both strains have been reported to occur in this state.

Detailed information regarding AW populations, parasitism rates, and parasite species distribution, on a per site and weekly basis, is available in two annual data reports. In addition, a report on the economic aspects of the parasite redistribution program has been compiled by Mike Duffy of the ERS and includes baseline data from 1981 on pesticide usage, control costs, grower attitudes, etc.

Summary

Due to unusual weather, especially in the two western areas, more samples were missed and the survey began later in 1982 than in 1981. Alfalfa weevil populations may also have been affected by the cool wet spring, as peak larval densities declined in MO, IA and IL. The number of fields sprayed for AW control however, did not differ significantly between the two years. Microctonus aethiopoides is clearly moving westward and Bathyplectes anurus is apparently out-competing B. curculionis for hosts in the eastern areas.

Table 1. Two year area summary for peak larval densities of the alfalfa weevil, percent parasitism[1], and parasite species diversity.

Area	Mean (SE) Peak Larval Density (#/100 Sweeps)		Adult Parasitism (%)		Larval Parasitism (%)		Mean (SE) Number of Parasite Species/Site	
	1981	1982	1981	1982	1981	1982	1981	1982
I	619.5b[2]	193.3ab	2.3a	13.2a	14.4c	18.1a	1.7a	2.3a
MO, IA	(105.0)	(36.2)					(0.21)	(0.21)
II	846.3b	251.5ab	39.1b	35.9c	8.1a	40.0c	2.2a	2.5a
IA, IL	(199.3)	(59.0)					(0.17)	(0.33)
III	92.1a	97.5a	32.0b	26.6b	11.6b	26.0b	3.5b	3.8b
OH	(22.3)	(22.9)					(0.22)	(0.31)
IV	218.1a	381.7b	26.2b	32.6bc	30.9d	26.3b	4.5c	4.0b
NJ, PA	(39.9)	(87.9)					(0.29)	(0.26)

1 A total of 20,149 and 33,076 alfalfa weevils were dissected in 1981 and 1982 respectively.

2 Values in columns followed by the same letter are not significantly different at the 95% level of confidence. Peak larval densities and the number of parasite species were tested with a Newman-Keuls multiple comparison test, while parasitism frequencies were tested using a Chi-square analysis.

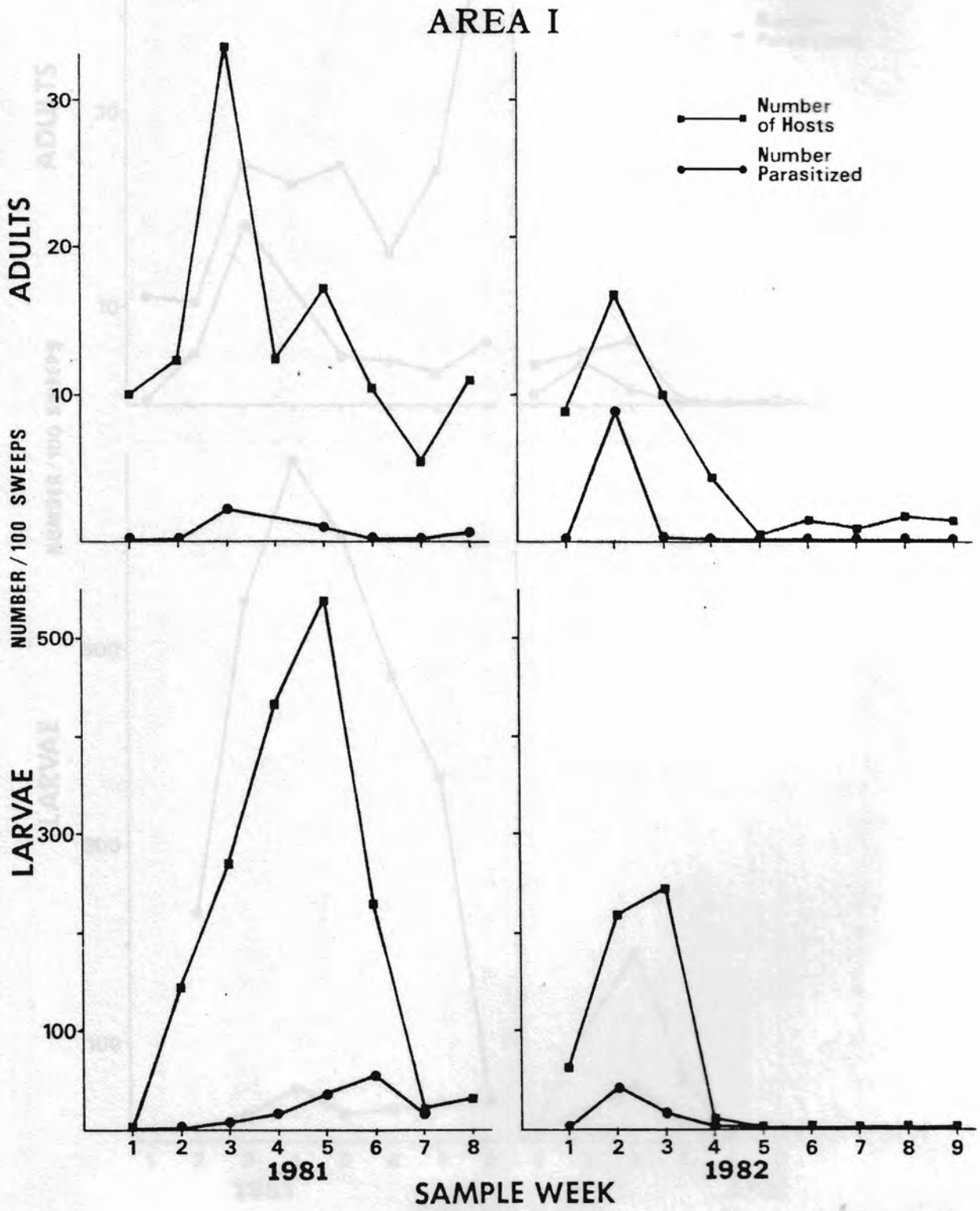


Figure 3. Alfalfa weevil adult and larval densities and the number parasitized in Area I (MO, IA) during the first two years of the Alfalfa Weevil Parasite Redistribution Evaluation Project.

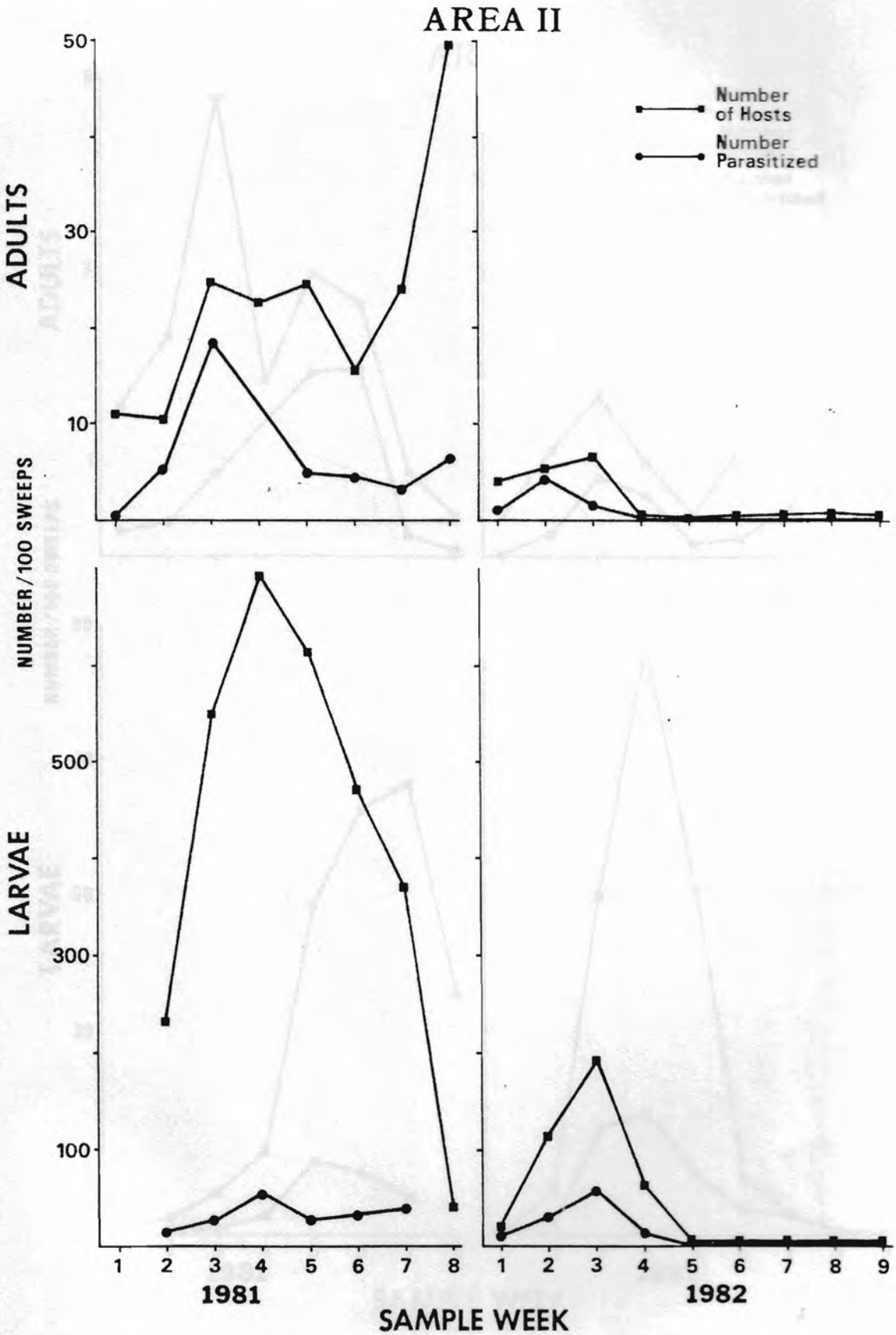


Figure 4. Alfalfa weevil adult and larval densities and the number parasitized in Area II (IL, IA) during the first two years of the Alfalfa Weevil Parasite Redistribution Evaluation Project.

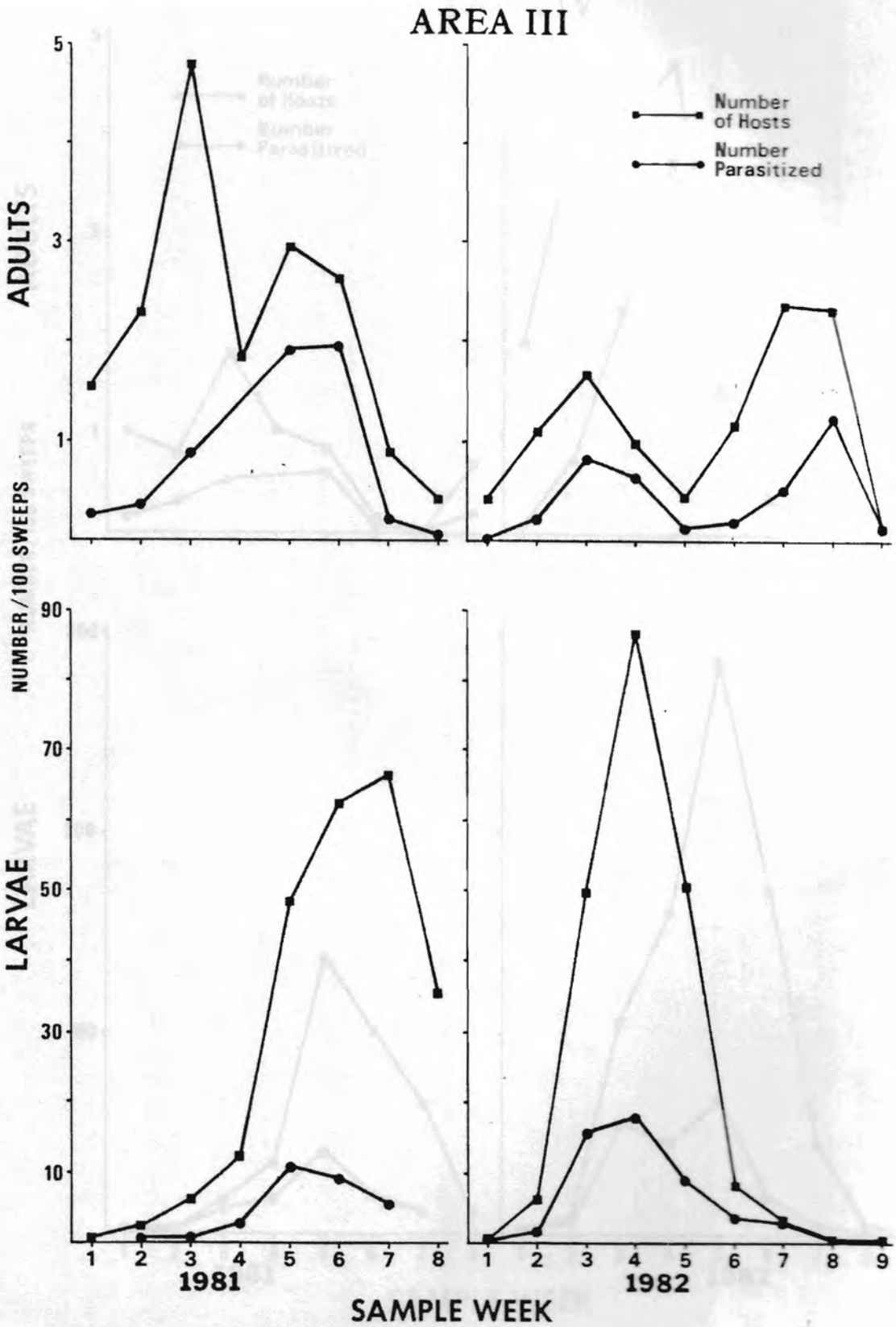


Figure 5. Alfalfa weevil adult and larval densities and the number parasitized in Area III (OH) during the first two years of the Alfalfa Weevil Parasite Redistribution Evaluation Project.

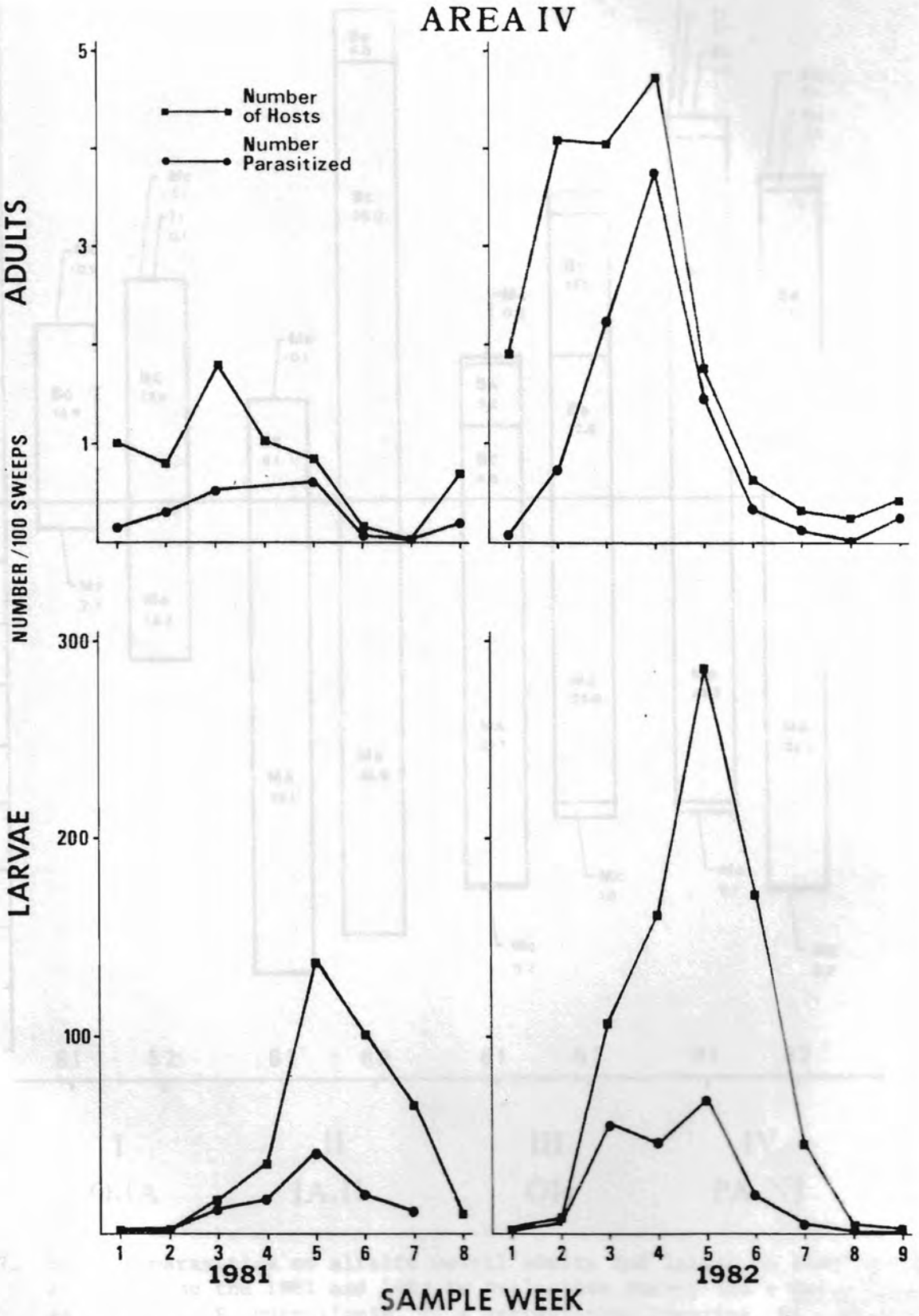


Figure 6. Alfalfa weevil adult and larval densities and the number parasitized in Area IV (PA, NJ) during the first two years of the Alfalfa Weevil Parasite Redistribution Evaluation Project.

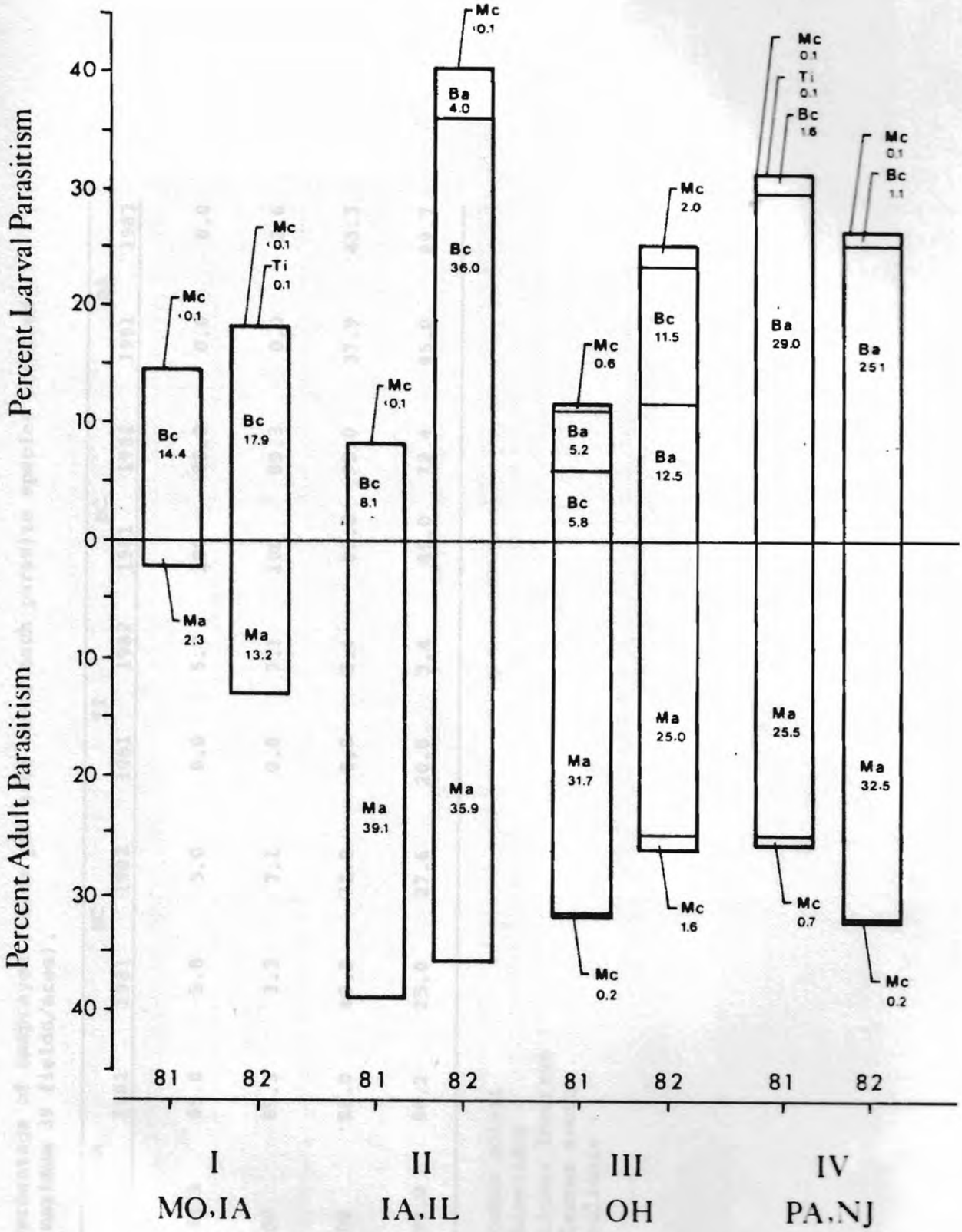


Figure 7. Percent parasitism of alfalfa weevil adults and larvae in each of four areas during the 1981 and 1982 AW Evaluation Survey (Ba = Bathyplectes anurus, Bc = B. curculionis, Ti = Tetrastichus incertus, Ma - Microctonus aethiopoides, Mc = M. colesi).

Table 2. Percentage of unsprayed fields per area where each parasite species occurred (maximum 30 fields/area).

Area	MA		MC		TI		BC		BA	
	1981	1982	1981	1982	1981	1982	1981	1982	1981	1982
I MO, IA	35.3	65.0	5.8	5.0	0.0	5.0	100	85.0	0.0	0.0
II IA, IL	100	89.3	3.3	7.1	0.0	7.1	100	89.3	0.0	3.6
III OH	100	88.0	48.3	72.0	0.0	0.4	82.8	92.0	37.9	43.3
IV PA, NJ	95.0	86.2	25.0	27.6	20.0	3.4	85.0	72.4	95.0	89.7

MC = Microctonus colesi
MA = M. aethiopoides
TI = Tetrastichus incertus
BA = Bathyplectes anurus
BC = B. curculionis

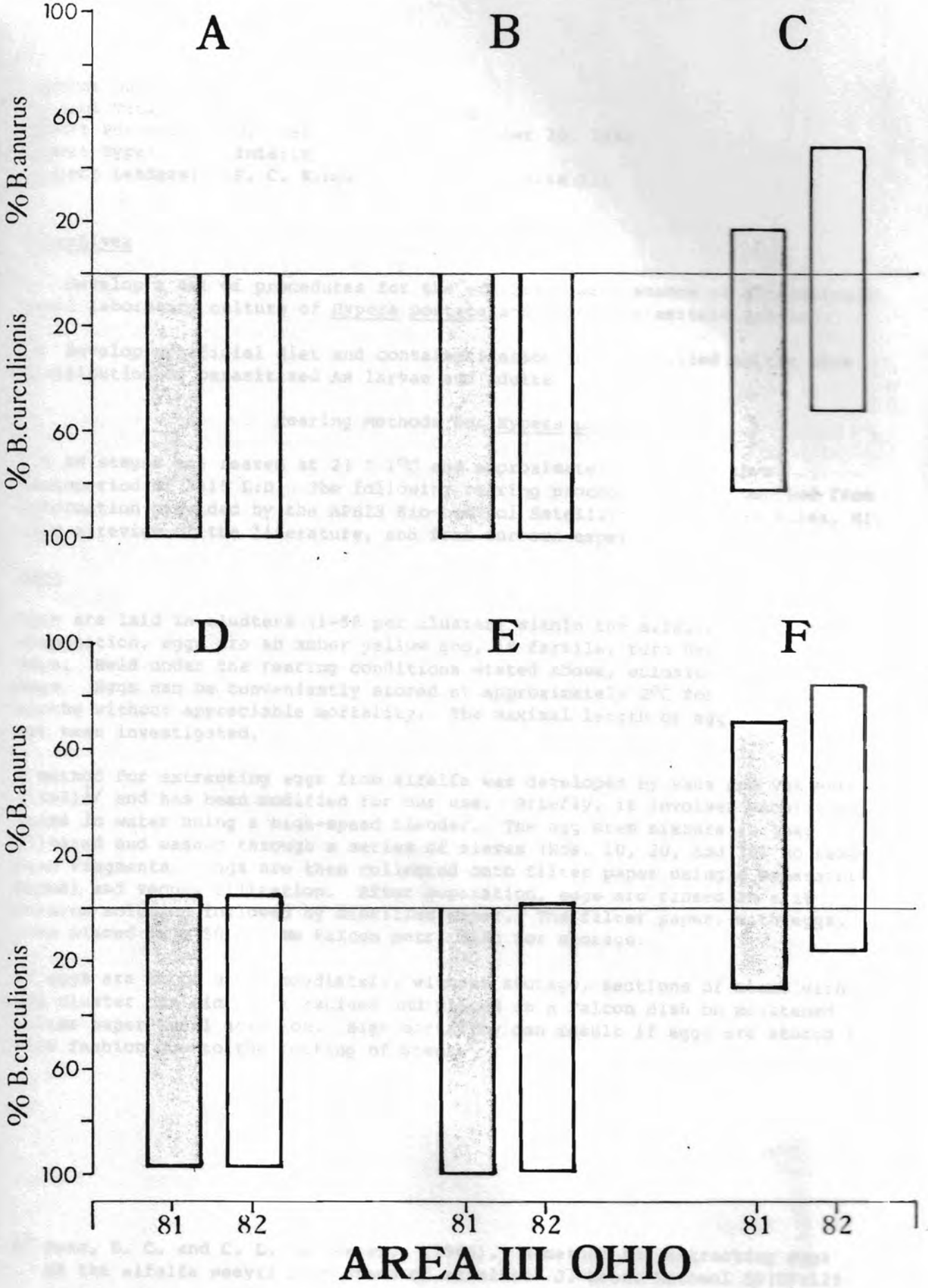


Figure 3. Relative frequencies of Bathyplectes curculionis and B. anurus by site and year in Ohio.

Project Number: AW 2.1.1
Project Title: Alfalfa Weevil Rearing
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: P. C. Kingsley and A. C. Felix III

Objectives

1) Develop a set of procedures for the efficient maintenance of a relatively small laboratory culture of *Hypera postica* and select parasitoid species.

2) Develop artificial diet and containerization to be utilized in the mass distribution of parasitized AW larvae and adults.

Rearing Methods for *Hypera postica*

All AW stages are reared at 23 ± 1°C and approximately 33% RH with a photoperiod of 9:15 L:D. The following rearing procedures were compiled from information provided by the APHIS Bio-control Satellite Facility at Niles, MI, from a review of the literature, and from our own experimentation.

Eggs

Eggs are laid in clusters (1-50 per cluster) within the alfalfa stem. At oviposition, eggs are an amber yellow and, if fertile, turn brown within three days. Held under the rearing conditions stated above, eclosion occurs in 7-8 days. Eggs can be conveniently stored at approximately 2°C for at least two months without appreciable mortality. The maximal length of egg storage has not been investigated.

A method for extracting eggs from alfalfa was developed by Pass and Van Meter (1966)[1/] and has been modified for our use. Briefly, it involves macerating stems in water using a high-speed blender. The egg stem mixture is then filtered and washed through a series of sieves (Nos. 10, 20, and 30) to remove stem fragments. Eggs are then collected onto filter paper using a separatory funnel and vacuum filtration. After separation, eggs are rinsed in a 1% chlorox solution followed by distilled water. The filter paper, with eggs, is then placed in a 50 x 9 mm Falcon petri dish for storage.

If eggs are to be used immediately, without storage, sections of stems with an egg cluster can simply be excised and placed in a Falcon dish on moistened filter paper until eclosion. High mortality can result if eggs are stored in this fashion due to the rotting of stems.

1/ Pass, B. C. and C. L. Van Meter. (1966). A method for extracting eggs of the alfalfa weevil from stems of alfalfa. J. Econ. Entomol 59(5):1294.

Larvae

Two methods for rearing larvae are used; one for our lab culture and another for diet tests. For culturing en masse, approximately 100 eggs on filter paper are placed among a bouquet of alfalfa stems in water filled aqua-pics. Bouquets are then placed in cylindrical one quart cardboard containers with a Sweetheart translucent six oz. plastic cup as a lid. Containers are laid on their sides with the alfalfa touching the lid to ensure that the phototrophic first instar larvae remain in contact with the alfalfa leaves. Additional bouquets are added as needed. Although this method is relatively simple and can yield adequate numbers of adults, mortality is fairly high ($\sim$40%). In addition, after seven days, larvae show a large variation in development, and therefore are not suitable for use in diet tests.

In order to produce more consistently sized seven day old larvae, we place five newly hatched larvae, using a camel hair brush, in a 1.5 oz. Thunderbird plastic container with a sprig of alfalfa. The alfalfa stem extends outside the cup through a "V" shaped slit in the edge of the paper lid. A small cotton plug is fit around the stem and lid to prevent escape. Inverted cups are then placed over a tray of water with stem ends immersed. In seven days larvae have consumed most of the alfalfa and are ready for transfer to diet.

Development time from egg hatch to adult eclosion is approximately 21.5 days with the pupal period lasting around 8 days.

To aid in the identification of larval instars, in terms of size and age, we sampled a laboratory culture on a daily basis and measured head capsule widths. Results are as follows:

Head Capsule Widths in millimeters

Instar	$\bar{x} \pm$ SE	Range
1st	0.204 ± .003	0.198 - 0.220
2nd	0.281 ± .003	0.264 - 0.308
3rd	0.421 ± .004	0.374 - 0.440
4th	0.599 ± .003	0.550 - 0.660

These results correspond closely to a similar study done by Bartell and Roberts (1974).[2/] We also calculated the mean instar at days 1 to 10.

days -	1	2	3	4	5	6	7	8	9	10
$\bar{x}$ instar -	1.0	1.0	1.2	1.9	2.2	3.0	3.0	3.2	3.6	4.0

Adults

Oviposition cages consist of a two quart cylindrical cardboard container with a screened lid. Approximately 20 adults are placed in each cage with a bouquet of two to three alfalfa stems in an aqua-pic. Bouquets are changed every other day and eggs extracted as previously described.

2/ Bartell, D. P. and S. J. Roberts. (1974). A head capsule caliper: new tool for determining instars of the alfalfa weevil. J. Econ. Entomol. 67(6):801.3.

Egg production was monitored for 30 females over a 70 day period. Survival and fecundity information are shown in figure 1. The mean preoviposition period was 30.7 ± 1.35 days and peak egg production (27 eggs/day) occurred at an age of approximately 45 days.

Rearing Methods for *Bathyplectes anurus* and *E. curculionis*

See Appendix I for a review of the literature regarding rearing methods for these parasitoids.

Diet Testing

Throughout our diet experiments we have used the standard high wheat germ gypsy moth diet (Table 1) varying the amounts of water and acetone extracted alfalfa powder (added as a feeding stimulant).

First instar *H. postica* larvae feed on such a modified gypsy moth diet reluctantly. In early experiments we found that container size and diet consistency were important factors in survival and development. By reducing container size from 1.5 oz. Thunderbirds to 1 dram shell vials, survival in one experiment increased from 65% to 92% over a ten day period. Drowning was also a significant mortality factor. In these early tests, where first instars were placed on diet, adult weights were significantly lower for those reared on diet vs alfalfa (5.41 ± 0.163 mg and 7.56 ± 0.085 mg respectively). Developmental times were also significantly longer on diet, 35.4 ± 0.50 days vs 21.5 ± 0.06 days, from egg eclosion to adult emergence.

Table 1. Composition of the high wheat germ diet for the alfalfa weevil.

			% 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

The first of two experiments to be described in detail was begun on May 14th. Larvae used in this test were field collected as part of the Alfalfa Weevil Parasite Redistribution Evaluation Project described in AW 1.1.1. Samples from seven fields in three states were divided among two diets, artificial and alfalfa, for rearing. Survival and parasitism were monitored, and results are presented in Table 2.

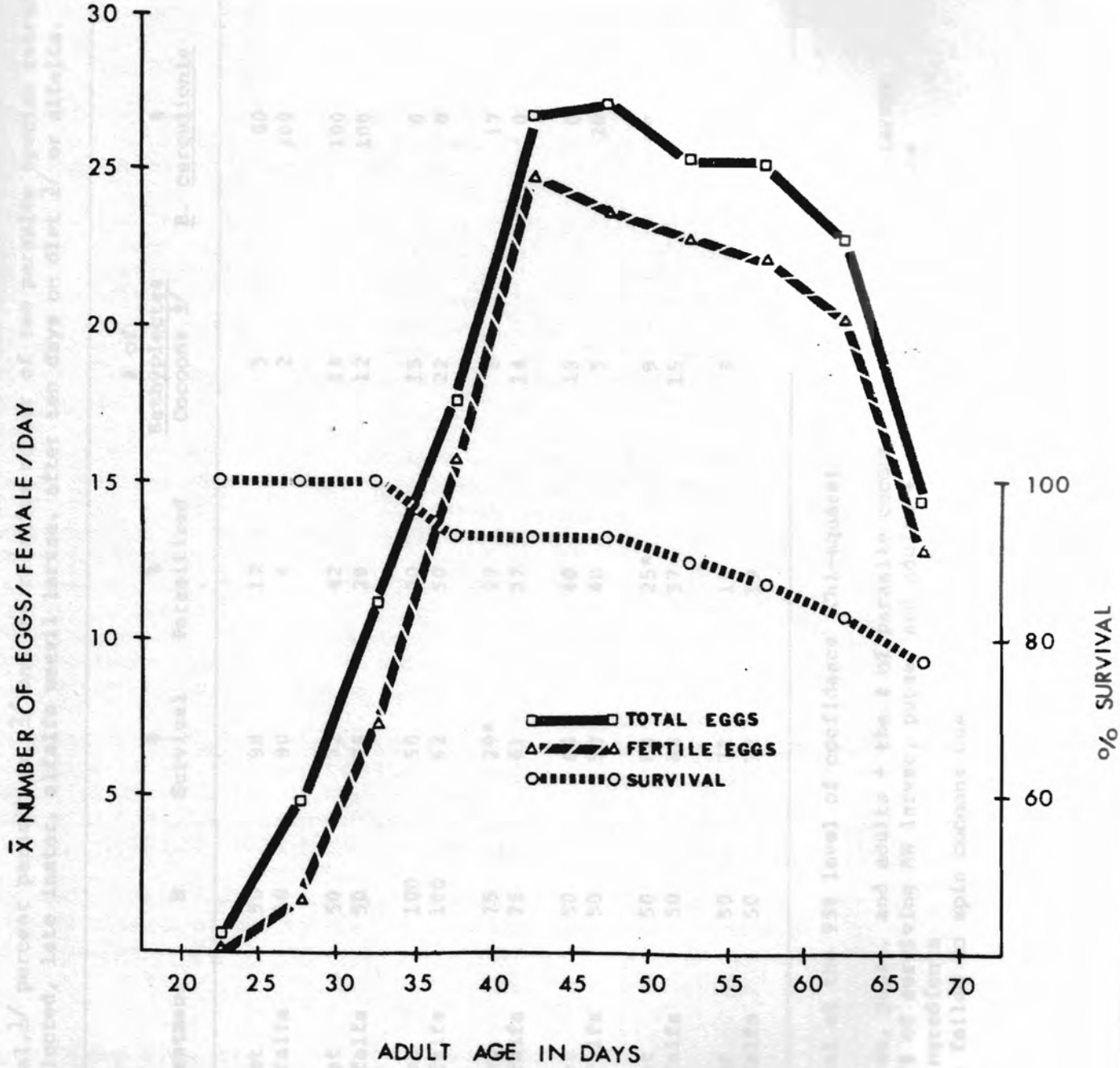


Figure 1. Fecundity and survival of Hypera postica females.

Table 2. Percent survival,[1] percent parasitism,[2] and the relative number of two parasite species reared from field collected, late instar, alfalfa weevil larvae, after ten days on diet[3] or alfalfa.

Field Location State	County	Treatment	N	% Survival	% Parasitized	# of Bathyplectes Cocoons[4]	% B. curculionis	% B. anurus
OH	Stark	Diet	50	98	12	5	80	20
		Alfalfa	50	90	4	2	100	0
OH	Miami	Diet	50	72	42	14	100	0
		Alfalfa	50	76	29	12	100	0
OH	Morgan	Diet	100	50	30	15	0	100
		Alfalfa	100	62	50	22	0	100
PA	Luzerne	Diet	75	29*	27	6	17	83
		Alfalfa	75	61	37	14	0	100
PA	Luzerne	Diet	50	64	40	13	0	100
		Alfalfa	50	50	40	5	20	80
NJ	Sussex	Diet	50	88	25*	9	0	100
		Alfalfa	50	88	57	15	0	100
NJ	Hunterdon	Diet	50	72	17	3	0	100
		Alfalfa	50	72	19	2	0	100

* Significantly different at the 95% level of confidence (Chi-square)

1/ # of surviving AW larvae, pupae, and adults + the # of parasite cocoons/the initial # of AW larvae
2/ # of parasite cocoons/# of surviving AW larvae, pupae, and adults + the # of parasite cocoons
3/ See table 1 for diet ingredients
4/ 33 Bathyplectes larvae failed to spin cocoons due to a poor substrate

Out of 21 comparisons, only two significant differences were found between the two rearing methods. Larvae from one of the two fields in Luzerne Co., PA showed a significantly higher mortality on artificial diet than on alfalfa. A probable explanation for this difference, and the reason for our next series of experiments, was an apparent bacterial contamination on the diet surface. The difference in the percent parasitism of larvae from Sussex Co., NJ remains unexplained.

Overall survival on diet vs alfalfa was 63.3 and 69.6%, respectively, whereas the percent parasitism for all fields was 26.8% for those reared on diet and 34.8% for those on alfalfa.

It is clear from this experiment that rearing field collected, late instar larvae on diet has no appreciable effects on survival, percent parasitism, or the relative number of each parasite species reared. However, the problem of mortality due to mass contamination became apparent. Ten cups out of 26 were badly contaminated, requiring larvae to be transferred to fresh diet. If diet is to be used in a system for the mass redistribution of parasitized larvae, the potential for diet contamination must be minimized.

Our initial effort to deal with this problem was to change diet consistency by varying the relative amounts of water and agar, reasoning that a drier diet would be less susceptible to contamination. These efforts were mostly unsuccessful; as a consequence, we began testing various antibiotics against the major bacterial contaminant using Sensi discs. These antibiotic soaked discs were placed on diet in six oz. Sweetheart cups. A suspension of inoculum, made from a bacterial culture and distilled water, was atomized over the diet and discs. A contamination free ring of diet surrounding a disc indicated control. From this screening, two antibiotics were selected for further tests: Chlorotetracycline and Tetracycline.

In order to find the minimal concentration needed to control common contaminants, we incorporated various amounts of each antibiotic into diet. Cups with diet were inoculated as described above, using an atomizer, and monitored for contamination. Results indicated that less than .002% of either antibiotic would eliminate contamination by the bacteria tested.

The second experiment to be described in detail was designed to test any adverse effects of the two antibiotics on AW survival, development time and adult weights.

Antibiotic Test

Methods and Materials

Alfalfa weevil larvae used in this experiment were from a colony maintained at our laboratory under the following environmental conditions: 23.3°C, RH 50-80% and 9-hr photoperiod. Newly hatched larvae were reared on indoor grown alfalfa for five days. They were then transferred to cartons containing bouquets of outdoor grown alfalfa, to allow exposure to any microbes that may contaminate the diet. Seven day old larvae (3rd instars) were then removed from the alfalfa and placed in six oz. Sweetheart cups (5 larvae/cup) containing a modified high wheat germ gypsy moth diet (Table 1).

Diet was prepared in a one gallon blender. Agar was blended in 2400 ml of boiling water for 30 seconds. The remaining constituents were then added and the entire mixture was blended at the highest setting for 30 seconds. This was followed by a repeated sequence of hand stirring and 30 second blending. The hot mixture was poured into six oz. plastic Sweetheart cups which were covered with paper towels and refrigerated. Weevil larvae were placed on diet one day following its preparation. Four Kimwipe pieces (6x6 cm) were placed in each diet cup, as a substrate for cocoon spinning, before sealing with a cardboard lid.

Eleven cups (replicates) were set up for each of seven diet treatments; three concentrations (.00125, .0050, .0250% of the total diet mixture) of two antibiotics (chlorotetracycline and tetracycline) and a control (no antibiotic). Diet cups were monitored daily for adult emergence. Fourteen days following the placement of larvae on diet, cups were checked for contamination, AW survival, and development. As adults emerged, they were sexed, weighed and emergence time (# of days from egg hatch to adult emergence) was recorded. The experiment was terminated after 20 days with any remaining larvae considered dead.

Results

Highest alfalfa weevil survivorship was achieved using diet with a .00125% concentration of tetracycline (Table 3). Diet contamination for this treatment was minimal, and age and weight at emergence compared favorably with the control. The same concentration of chlorotetracycline resulted in significantly lower survival; attributable to a relatively high level of contamination. Although contamination was completely eliminated by using higher concentrations of both antibiotics, alfalfa weevil development time was extended and weight at emergence was reduced. For comparative purposes, development time was 21.5 ± .06 days, adult weight 7.56 ± .085 mg, and survival ranged from 60-80% for those reared exclusively on alfalfa.

Experiments are presently underway to study parasite survival, development, fecundity, and sex ratio, when reared on AW larvae fed diet with the antibiotic tetracycline incorporated.

Table 3. Adult *Hypera postica* age, weight, and survivorship at emergence after rearing on a modified gypsy moth diet with three concentrations of chlorotetracycline and tetracycline. [a]

Treatment		Adult Emergence [c] Age (Days) [d] ($\bar{x}$ ± SE)	Weight (mg) [d] ($\bar{x}$ ± SE)	Survivorship [e] (%)	Diet Cups Contaminated (%)
Chloro-tetracycline	.00125[b]	23.3 ± .35 a	5.5 ± .28 ab	29.1 b 16/55	36.4 4/11
	.005	24.0 ± .38 a	6.1 ± .17 b	36.4 b 20/55	0.0 0/11
	.025	26.6 ± .54 b	4.9 ± .23 a	30.9 b 17/55	0.0 0/11
Tetracycline	.00125	24.0 ± .59 a	6.3 ± .21 b	74.0 a 37/50	10.0 1/10
	.005	24.9 ± .51 ab	5.6 ± .26 ab	30.9 b 17/55	0.0 0/11
	.025	25.0 ± .53 ab	5.6 ± .25 ab	41.8 b 23/55	0.0 0/11
Control		23.2 ± .82 a	6.2 ± .22 b	30.9 b 17/55	63.6 7/11

a) Larvae were reared on alfalfa bouquets for seven days before transfer to 8 oz. diet cups (5 larvae/cup).
b) Percentage of total diet mixture.
c) Emergence from cocoon.
d) Student-Newman-Keuls multiple range test was used to compare treatment means at the 0.05 level. Column means with the same letter are not significantly different.
e) Chi-square test was used to compare survivorship frequencies at the 0.05 level. Column means with the same letter are not significantly different.

Project Number: MBB 1.1.1
Project Title: Determining the Optimum Temperature for Storing Larval *Pediobius foveolatus*
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leaders: Patricia J. Coughlin, Othel T. Forrester

Introduction:

Previous storage tests using mummies containing pupated *Pediobius foveolatus* (Pf) stored 14 days indicated a decrease in fecundity of the emerging adults, evidenced by a lower percent eclosion and a lower ratio of females:males. The objective of this test was to determine the effect of various storage temperatures and duration of storage on larval stages of Pf in parasitized Mexican bean beetle larvae (MBB).

Methods and Materials

The storage tests were conducted in Percival I3BLL reach-in environmental chambers. Groups of 30 late 4th instar MBB larvae were exposed to 15 Pf and held at 25°, RH 50% for 24, 48 and 72 hours before storage. Groups of parasitized larvae from the above treatments were stored for 14, 17, 21 and 28 days. Storage regimes tested were a) 10°C 22 hours - 25°C 2 hours, b) 12°C 22 hours - 25°C 2 hours, c) 12°C 24 hours and d) 25°C - 24 hours.

Samples of parasitized MBB larvae were removed and dissected at 4 day intervals to determine Pf pupation.

After storage, samples of mummies from each treatment were individually eclosed in No. 000 Lily gelatine capsules to determine yield and sex ratio.

Fecundity of the adults from the stored mummies was determined by exposing groups of 30 late 4th instar larvae to 15 two day old adults from each of the treatments.

Results and Discussion

The results of the fecundity tests are given in Table 1. The results of the yield tests are in Table 2. Larval Pf can be stored after 24, 48 or 72 hours development for 14 to 17 days without impairing fecundity at 12-25°C, 10-25°C or 12°C. The greatest decrease in development rate occured at 12°C, with 10-25°C being second and 12-25°C the fastest to eclose. However, the average number of females was slightly higher for mummies stung with Pf held at 12-25°C. The Pf held at 12-25°C from 72 hours development begin to pupate at 21 days, while the ones at 10-25°C were still larvae. Those held at 12°C failed to survive in sufficient numbers to test beyond 17 days storage. Pf stored 28 days at 12-25°C after 72 hours development produced progeny with a decrease in the number of females and fewer eclosing mummies. This change may be caused by continued storage extending into pupation. Pf stored at 12-25°C after 24 hours development did not pupate as rapidly as those stored after 72 hours development at 12-25°C, and these parasites demonstrated fecundity within the acceptable range of 3:1 (or higher) female/male ratio, and an average of 20 or better Pf per mummy produced.

12-25°C appears to be the optimum temperature for storing larval Pf to lengthen the time to eclosion; 72 hours post-parasitization was the best time to place them in storage up to 21 days. For 28 days storage, placing them in storage at 24 hours results in best retainment of fecundity.

The yield of stored mummies up to 21 days at 12-25°C appears well within the acceptable range.

Table 1. Fecundity of adult Pf from stored mummies.

# of Mummies	Age (Hours)	Time Stored (Days)	Storage Temp. (°C)	% Eclosion	Days To Eclosion	Mean Females	Mean Males	Mean Total	Sex Ratio
25	72	14	25	96	14	16.8	6.4	23.2	2.6:1
30	72	14	12 - 25	93	26	16.8	3.0	19.8	5.6:1
30	72	14	10 - 25	100	28	17.3	3.6	20.9	4.8:1
30	72	14	12	93	29	18.9	6.1	25.0	3.1:1
29	72	21	25	93	14	14.8	3.6	18.4	4.1:1
30	72	21	12 - 25	96	33	18.7	3.9	22.6	4.8:1
30	72	21	10 - 25	56	34	16.8	4.1	20.9	4.1:1
30	72	21	12	0	0	0	0	0	0
28	72	28	25	83	14	19.3	7.1	26.4	2.7:1
30	72	28	12 - 25	70	38	3.6	14.0	17.7	0.3:1
22	72	28	10 - 25	86	41	8.3	7.7	16.0	1.1:1
30	72	28	12	0	0	0	0	0	0
30	48	17	25	97	14	14.9	6.7	21.6	2.2:1
30	48	17	12 - 25	97	29	18.8	5.8	23.9	3.2:1
30	48	17	10 - 25	97	31	18.4	6.2	24.6	3.0:1
30	48	17	12	97	33	15.7	7.3	23.0	2.2:1
27	48	21	25	77	14	7.3	3.4	10.7	1.3:1
26	48	21	12 - 25	96	33	16.6	6.0	22.6	2.8:1
30	48	21	10 - 25	93	33	14.0	4.6	18.6	3.0:1
30	48	21	12	0	0	0	0	0	0
30	24	21	25	97	14	16.4	5.1	21.6	3.2:1
30	24	21	12 - 25	93	31	17.7	7.1	25.8	2.5:1
30	24	21	10 - 25	0	0	0	0	0	0
30	24	21	12	0	0	0	0	0	0
24	24	28	25	54	14	7.8	7.0	14.8	1.1:1
30	24	28	12 - 25	97	43	18.5	5.1	23.6	3.6:1
30	24	28	10 - 25	0	0	0	0	0	0
30	24	28	12	0	0	0	0	0	0

Table 2. Yield of Pf from mummies stored at various temperatures for varying periods.

# of Mummies	Age (Hours)	Time Stored (Days)	Storage Temp. (°C)	% Eclosion	Mean Females	Mean Males	Mean Total	Mean Sex Ratio
14	72	14	12 - 25	86	14.0	4.8	18.8	3.1:1
13	72	14	10 - 25	87	11.8	5.0	16.8	2.4:1
14	72	14	12	93	12.4	5.0	17.4	2.5:1
*out of 15 MBB stung								
30	54		25	83	16.4	3.0	19.4	5.5:1
30	54	14	12 - 25	96	16.4	2.3	18.7	7.1:1
30	54	17	12 - 25	96	18.9	3.3	22.2	5.7:1
30*	54	21	12 - 25	93	15.8	3.6	19.4	4.4:1
*out of 30 MBB stung								

Project Number: MBB 1.1.3
Project Title: Determination of the Most Effective Release Formula for *Pediobius foveolatus* in Nurse Plots
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leader: O. T. Forrester

This project report has been submitted to the Annals of the Entomological Society of America for publication. See Appendix II.

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, 1981 - September 30, 1982
Report Type: Interim
Project Leader: O. T. Forrester

Introduction

The use of nurse plots planted with green beans to congregate overwintered adult Mexican bean beetles is a successful method of supplying host material for field production of Pediobius foveolatus (Pf). A single 100' x 50' nurse plot may provide enough to service 200 acres of soybeans.

An alternative concept is that of releasing Pf directly into soybean fields. A direct release will not have the advantage of concentrated numbers of hosts, and accurate host population assessment for release will be more difficult. Therefore, early identification of fields and release of Pf will be important to successful establishment.

The objective of this study is to determine the feasibility of controlling Mexican bean beetle by releasing Pf directly into soybean fields.

Methods and Materials

The Pf used in this test were produced at the Niles, Michigan satellite facility, the Gast Rearing Laboratory, Starkville, Mississippi and Contract No. 54-6395-2-65.

A 100 acre soybean field in Southern Pike County, Ohio was selected for this test. The field was bounded on the north and south by 100 acre soybean fields and on the west by the Scioto River.

On July 27, two samples consisting of two 3 row feet each were collected from the NW, SW, center, NE and SE portions of the field. Plant density, MBB life stage and defoliation estimates were taken. Parasites were distributed over the field by releasing 1000 at 150 feet intervals down every 20th row until 600,000 Pf were released. On August 28 a second sample was taken from the same areas of the field.

Results and Discussion

Defoliation in the test field was 14% at the time of release and between the first and second larval generations (Table 1). All of the first instar larvae reported were from newly hatched egg clusters.

Timing of this release was poor (35 days later) and dictated by the availability of excess Pf from the mid-Atlantic MBB demonstration and not the developmental state of the host population. Accordingly, this operationally attractive approach to using parasites for MBB control remains to be properly evaluated.

Defoliation caused by MBB by the second sample period was 30% (Table 1). Pf was not effective in this direct field release. However, the timing of the release was poor, and some of the Pf were in marginal condition. This test should not be repeated unless it has a higher priority of Pf supply. If Pf were available earlier in the season and released at the appropriate host stage, more encouraging results might have been obtained.

Table 1. Mean number of MBB life stages per 3 row feet and percent defoliation.

		Adults	Egg clusters	Larval instars 1	2	3	4	Prepupae & pupae	mummies	% defoliation
$\bar{x}$	July 27	17.6	5	37	2	3	8	10.6	0	14
$\bar{x}$	August 28	3.3	0.3	0	0.4	2.3	4.8	29.6	6	30

Project Number: MBB 1.1.5
Project Title: Control of Mexican Bean Beetle in Truck Farm and Small Plantings of Green Beans with *Pediobius foveolatus*
Report Period: October 1, 1981 - September 30, 1982
Report Type: Final
Project Leader: O. T. Forrester

Introduction

The control of Mexican bean beetle (MBB) with *Pediobius foveolatus* (Pf) is being demonstrated on a large scale in the Middle Atlantic soybean producing area. There is an increasing interest in extending this technology into other areas of the bean producing industry (truck farm, seed production and home gardens). The nurse plot technique being used to establish Pf in soybean production areas is not practical in plantings of snap beans. Therefore, a direct field release would be necessary.

The objective of this project is to develop and demonstrate a MBB biological control strategy suitable for plantings where nurse plots are not practical.

Materials and Methods

This test was executed on a truck farm in East Sandwich, MA. Sample units of 3 row feet were selected on a weekly basis at random from each planting of beans. The number of adults, egg clusters and larvae (by instar) were counted and recorded. A single release of Pf was made when second instar larvae were observed. Following parasite release, the plantings were checked weekly and the number of adults, egg clusters, larvae by instar, mummies, prepupae and pupae for each survey unit were counted and recorded. Plantings were sampled until bean harvest was completed and plants destroyed.

Results

General observations began June 30, nearly 3 weeks later than last season, because of a cold, wet spring. Systematic data collection (in randomly located sample points) began on July 7 and continued through September 29. Mexican bean beetle life stages were not detected in the random survey until July 21. However, second to fourth instar larvae were detected on July 14 in general observation of damage by walking down every 3rd row. On July 15, a single release of 500 Pf was made in the area containing the damage and larvae.

Mummies were observed on July 21 in general observation but not in the random sample units until August 11. The highest mean larval count was on August 11 and the highest mummy count was on September 1. Data are summarized in Table 1.

Discussion and Conclusions

Historically, chemical control has been widely used on this farm, but chemical control measures were not applied to any of the plantings of beans this season. This shift may have been caused by the reduction of overwintering MBB by a high percent of parasitism in the 1981 growing season and a low inoculative Pf release in the 1982 season.

Data collection procedures during the 1981 and 1982 growing seasons were similar. The sampling frequency in 1981 was biweekly and weekly in 1982. The number of samples collected in 1981 and 1982 was 15 and 17 per visit respectively.

MBB population distribution was random in 1981 and reduced sufficiently to be highly aggrigated in 1982.

Maximum larval and mummy counts in 1981 occurred June 26 and July 17 respectively. Peak larval counts in 1982 occurred August 4 and September 1 respectively. Maximum mummy counts in 1981 and 1982 occurred 21 days after maximum larval count. This is due in part to sampling frequency and perhaps degree day accumulation. A second point of interest is that the maximum mummy count equaled the maximum larval count in the 1982 season and no MBB life stages were observed after peak mummy count in the random samples or general observations. The MBB population was either eliminated or below the sensitivity of the sample design.

Mexican bean beetle can be controlled in truck farm situations by using direct field release of Pf. Early plantings of sequentially planted beans should be managed as nurse plots. If insecticides are needed to control first generation MBB they should not be applied before mummies are found in the field.

A two season approach appears to be best. The first season of Pf use will give some benefit but should be considered as a MBB population reduction year. The second season should produce the maximum benefit.

Table 1. A Comparison of mean MBB life stages and mummy counts per 3 row feet during the 1981 and 1982 growing season on a truck farm in East Sandwich, Massachusetts.

		1981						1982			
Date		Adults	Egg Clusters	Larvae-Pupae	Mummies	Date		Adults	Egg Clusters	Larvae-Pupae	Mummies
June	10	2.3	1.6	0.0	0.0						
	12	4.3	2.4	0.0	0.0						
	15	3.3	6.1	0.0	0.0						
	18	3.3	5.0	2.0	0.0						
	23	3.0	6.7	73.0	0.0						
	25	1.7	5.4	50.3	0.0						
	26	2.3	6.1	97.5	0.0						
	26		16000 Pf Released								
Jul	6	0.2	0.6	27.0	0.0	Jul	7	0.0	0.0	0.0	0.0
	8	0.8	0.7	77.8	3.6						
	10		Chemical Control Applied								
	16	0.1	0.7	4.2	7.7		14	0.0	0.0	0.0	0.0
	17	0.3	0.3	5.2	9.0		15		500 P.f. released		
	21	0.2	0.2	5.0	2.4		21	0.0	0.2	1.6	0.0
	24	0.3	0.3	4.1	1.2						
	28	0.9	0.5	7.6	0.4		28	0.1	0.0	2.2	0.0
	30	0.7	0.1	5.4	1.4						
Aug	4	0.5	0.5	2.8	2.1	Aug	4	0.0	0.2	2.8	0.0
	6	0.6	0.9	2.5	2.8						
	10	0.6	0.5	7.2	4.5		11	0.1	0.0	3.1	0.2
	13	0.3	0.4	7.4	3.1						
	18	0.7	1.0	8.1	2.1		18	0.0	0.0	0.0	0.1
	20	0.0	0.3	3.3	0.6						
	26	0.2	0.2	8.2	1.4		25	0.1	0.0	0.0	0.4
Sept	1	0.0	0.2	2.9	1.0	Sept	1	0.0	0.0	0.0	3.3
	3	0.1	0.1	1.1	1.0						
	8	0.1	0.3	3.0	1.9		8	0.0	0.0	0.0	0.0
	10	0.1	0.0	3.1	0.6						
	17	0.2	0.1	1.8	0.3		15	0.0	0.0	0.0	0.0
	22	0.1	0.1	0.5	1.2		22	0.0	0.0	0.0	0.0
	25	0.0	0.2	1.6	1.6						
	28	0.0	0.0	0.2	0.4		29	0.0	0.0	0.0	0.0
Oct	1	0.2	0.0	0.0	1.2						

Project Number: MBB 2.1.1
Project Title: Dispersal of Pediobius foveolatus from Standard Nurse Plots
Report Period: October 1, 1981 - September 30, 1982
Report Type: Interim
Project Leader: Othel T. Forrester

Introduction

Innoculative releases of Pediobius foveolatus (Pf) into established nurse plots have been shown to suppress Mexican bean beetle in soybeans in Maryland. The average percent parasitism by Pf in 3 soybean fields was 70% by July 11 and remained between 60 and 90% during the remainder of the year (Stevens et al 1975).

Rust (1977) evaluated trap crops for control of MBB in soybeans and lima beans. Comparisons of trap crops, trap crops as release sites for Pf and chemical controls were made. Control obtained using Pf released into trap crops was equal to the control using the other methods.

Aldred et al (1980) ran a stochastic model of parasitism of MBB in soybeans by Pf over a 10 week period. Pf host ratios used were 1:20, 1:100 and 1:400. Population levels were reduced to acceptable levels with Pf:host ratios of 1:20 and 1:100 but not with 1:400. Results from their simulations were similar to those from two sets of field data from releases in soybean fields.

Pf has been reported 40 miles from the nearest release point; this did not approximate maximum dispersal distance but only reflected the extent of the survey (Stevens et al 1975).

In the above studies and also in the Mid-Atlantic Bio-Control Demonstration Project an essential piece of information is missing. Bailey et al (1975) covered the point very well. "It is essential to know the distance an insect will disperse before we can determine the density at which a species must be released to adequately cover an area . . ."

The objective of this study is to obtain information on the dispersal of Pf from standard nurse plots (50' x 100') to adjacent soybeans and to soybeans within a 1.5 mile radius from the release. 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 were established in Fayette, Pickaway, Pike and Ross Counties, Ohio during the first two weeks of May. The plots were planted with a mixture of 1/3 soybeans and 2/3 snap beans. The plots were checked at weekly intervals until over-wintered adult MBB and egg clusters were observed and at 3 - 5 day intervals until 50% or more of the larvae were second instar or older. A single Pf release of 80x (80 times the larval count in 15 feet of row) was made in each plot 1-2 days after the release criterion was met. Pf used in this study were supplied by the Niles, Michigan satellite facility and contract no. 54-6395-2-65.

Two weeks after the Pf release, the first 25 mummies encountered in each nurse plot were collected and individually eclosed in no. 000 Lilly gelatine capsules. Yield and sex ratio for each of the collected mummies were recorded. Nurse plot yield was estimated by multiplying the number of row feet in the plot times the average number of mummies in 1 row foot times the average number of Pf per mummy.

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

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. Zones 2 thru 5 were divided into quarters on the North-South and East-West axes. Zone 6 was divided into eighths, and 1 soybean field in each eight 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.

Results

Pediobius release criterion were met in Pickaway, Pike and Ross Counties on June 21. Innoculative Pf releases were made on June 22 in Pike County and on June 23 in Pickaway and Ross Counties. Host counts at "trigger" dates for Pickaway, Pike and Ross counties were 222, 709 and 639 larvae per 15 row feet, respectively. Pf production (mummies/row foot) for Pickaway, Pike and Ross counties were 2.8, 30.6 and 9.3. Estimated Pf yields of Pickaway, Pike and Ross counties were 113,120, 1,733,184 and 496,620, respectively.

Surprisingly, dispersal from the nurse plot in Pickaway county was not detected during the first sample period (August 7) after the innoculative release; mummies were not observed in the adjacent or surrounding soybean fields (Figure 1a). Defoliation estimates in the adjacent and surrounding fields were below the economic threshold (Et.) to 1 mile (Figure 2a).

Dispersal of Pf during the second sample period (August 31) in Pickaway County was detected at 3/4, 1-1/4 and 1-1/2 miles from the release point, where 9, 2 and 6 mummies per 3 row feet, respectively were observed. Mean yield of field collected mummies was 9.3 Pf, the sex ratio was 4.1:1 female:male.

Dispersal from the Pike County nurse plot, during the first sample period (July 29) after the innoculative release, was detected to 3/4 mile and good dispersal was detected to 1/4 mile (Figure 1b). Defoliation estimates were slightly above Et. at 1/2 mile and exceeded 25% at and beyond 3/4 mile (Figure 2b).

Pf dispersal in Pike county during the second sample period (August 29) was detected to 1-1/2 miles. Peak mummy counts were at 1 mile (23 per 3 row feet). Defoliation estimates were taken but of little real value. Soybeans were in stage R7 and a complex of bean leaf beetle, Japanese beetle, and Mexican bean beetle was difficult to sort out. The mean yield of field collected mummies was 9.5 and the sex ratio was 4.5:1 female:male.

Dispersal from the Ross county nurse plot during the first sample period (August 2) after innoculative release was detected to 1 mile. Peak mummy count was found at 1/8 mile (Figure 1c). Defoliation estimates exceeded economic threshhold at 3/4 mile (Figure 2c). All fields beyond 3/4 mile in Ross county were sprayed with carbamate before the first sample was completed.

Dispersal during the second sample period in Ross county was detected at 1-1/4 miles. Defoliation estimates were taken but are of questionable value because of a complex of bean leaf beetle, Japanese beetle, Mexican bean beetle and R7 developmental stage (Figure 2C). The mean yield of field collected mummies was 12.4, the sex ratio was 1.6:1 female-male.

The Fayette county nurse plots did not produce sufficient larval populations to warrant a Pf release.

Discussion

In looking at the areas individually the Pickaway county nurse plot was sprayed for bean leaf beetle, estimated Pf production was 113,000, and no parasitized MBB were found in the first sample period. But this may have been the most successful nurse plot. The economic threshhold (15% defoliation) was not exceeded at 1/2 mile throughout the season, 65 to 80% of the larvae found were parasitized (Figure 1a) and adult MBB populations were lower in sample 2 (Figure 4a). The dry conditions that occurred this season in Pickaway county and the lower over-wintered MBB population (peak count 13 adults/15 row feet in the nurse plot) are important factors to consider.

The Pike county nurse plot was by far the most productive. An estimated 1.7 million Pf were produced in this plot. While Pf production was high, foliage protection was not. Et was exceeded throughout the study area, even with chemical control in early August (Figure 2b).

The positive side of this study site for Pf is the large reduction in numbers of MBB from 1/16 to 3/4 mile from the nurse plot (Figures 3b and 4b) and the high percent parasitized at 1 to 1-1/2 miles was 55 to 75%, respectively (Figure 1b).

I believe the reduction of MBB numbers in this area is a synergistic effect of Pf and chemical control. All of the mummies collected 3 days after control eclosed. The MBB population did not reestablish within a 1 mile radius of this site. Additional factors to consider are, the Pike county area had a more even rainfall distribution and a higher adult count in the nurse plot (34 per 15 row feet).

The Ross county nurse plot was located in the center of an 80 acre soybean field and was perhaps the best location for a dispersal study. Estimated Pf production was 496,000. Economic threshhold was not exceeded at 1/4 mile during the first sample and was exceeded throughout the area by the second sample period (Figure 2c). MBB populations did not reestablish following chemical control, and the only larvae found within the 1-1/2 mile radius were all parasitized (Figure 1c). As in the other study sites MBB populations were lower in the second sample. Rainfall in this area was slightly less than in Pike county. Peak adult count in the nurse plot was 13 per 15 row feet, the same as Pickaway county but MBB larval counts were higher.

Data from Ross county were used to test dispersal direction because of the central location of the nurse plot. Percent parasitism to the NE was significantly higher (Table 1).

The MBB population trend in all areas (Figures 1, 3 and 4) were similar. The larval population was virtually eliminated to 1/2 mile and highly parasitized to 1-1/4 mile. The adult populations were greatly reduced throughout most of the study sites and remained essentially the same at 1-1/2 miles in Pickaway County. These population trends are similar to those observed in the 1981 truck farm test. Late season adult populations were low and high percentages of parasitized larvae were observed. The low overwintered adult population in 1982 may have been caused by this high percent parasitism in the 1981 season.

Percent parasitism was negatively correlated with distances from the nurse plot in the first sample period to 3/4 mile (R^2 = .61; P 0.001; Y = 1.098X + -.089; n = 61). No significant linear relationship was shown for percent parasitized with distance in the second sample.

The use of Pf to control MBB in soybeans, I think, should be a two season program. In high populations Pf has not multiplied and dispersed rapidly enough to effect foliage protection (15% economic threshhold) throughout the season. Foliage protection can be effected by Pf to about 1/4 mile for the early part of the season.

If a two season approach were used, the first season should be viewed primarily as a population reduction season. If a large area were to be tested the nurse plots could be planted on a 2-1/4 to 2-1/2 mile grid. MBB populations appear to have been reduced within a 1-1/4 mile radius this season. If this is accurate the nurse plots could be planted on a 2-1/4 to 2-1/2 mile grid and reduce MBB populations to a manageable level. If the foliage protection observed in Pickaway County can be used as an example of lower MBB population, then the grid could be 3/4 to 1 mile for foliage protection in the second season. The third season of Pf use could be determined by a fall survey or late plantings of green beans.

The number of insect species defoliating soybeans appears to be increasing. The Mexican bean beetle is the primary defoliator in many areas but Japanese beetle and bean leaf beetle are gaining in importance. When we control MBB with Pf we still have a potential problem with other insects. We should be considering some means of controlling these other insects that is compatible with biocontrol.

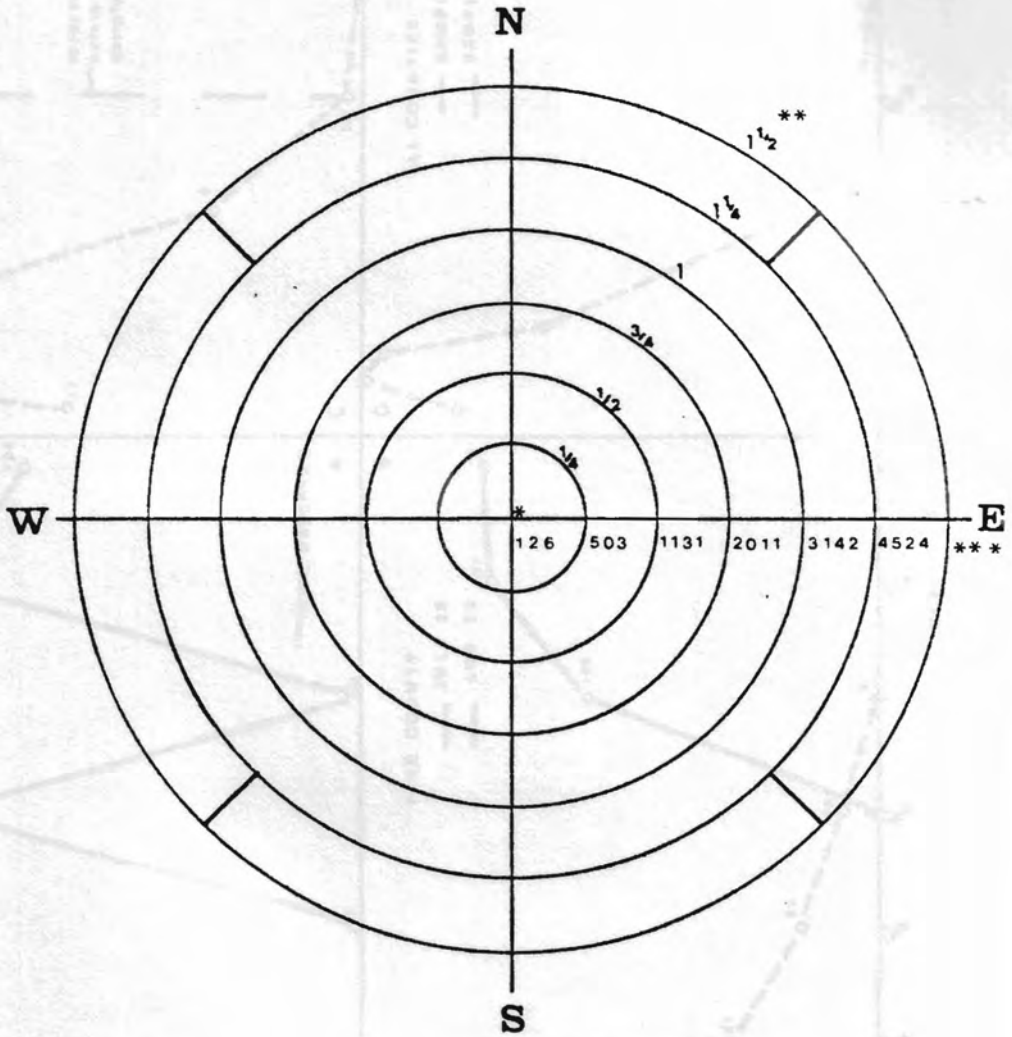
Table 1. Parasitization of MBB larvae in various directions from the Ross county nurse plot.

Direction	Unparasitized/parasitized	% Parasitized [a]	
NE	4/74	94.9	A
SE	75/43	36.4	B
NW	40/15	29.4	B
SW	161/190	35.8	B

a Percent parasitized based on parasitized larvae mummies observed in both sample periods.

Percent followed by different letters are significantly different at the .005 level (Chi square test).

Diagram for selection of sample sites



* Nurse plot

** Radius (miles)

*** Acres (cumulative)

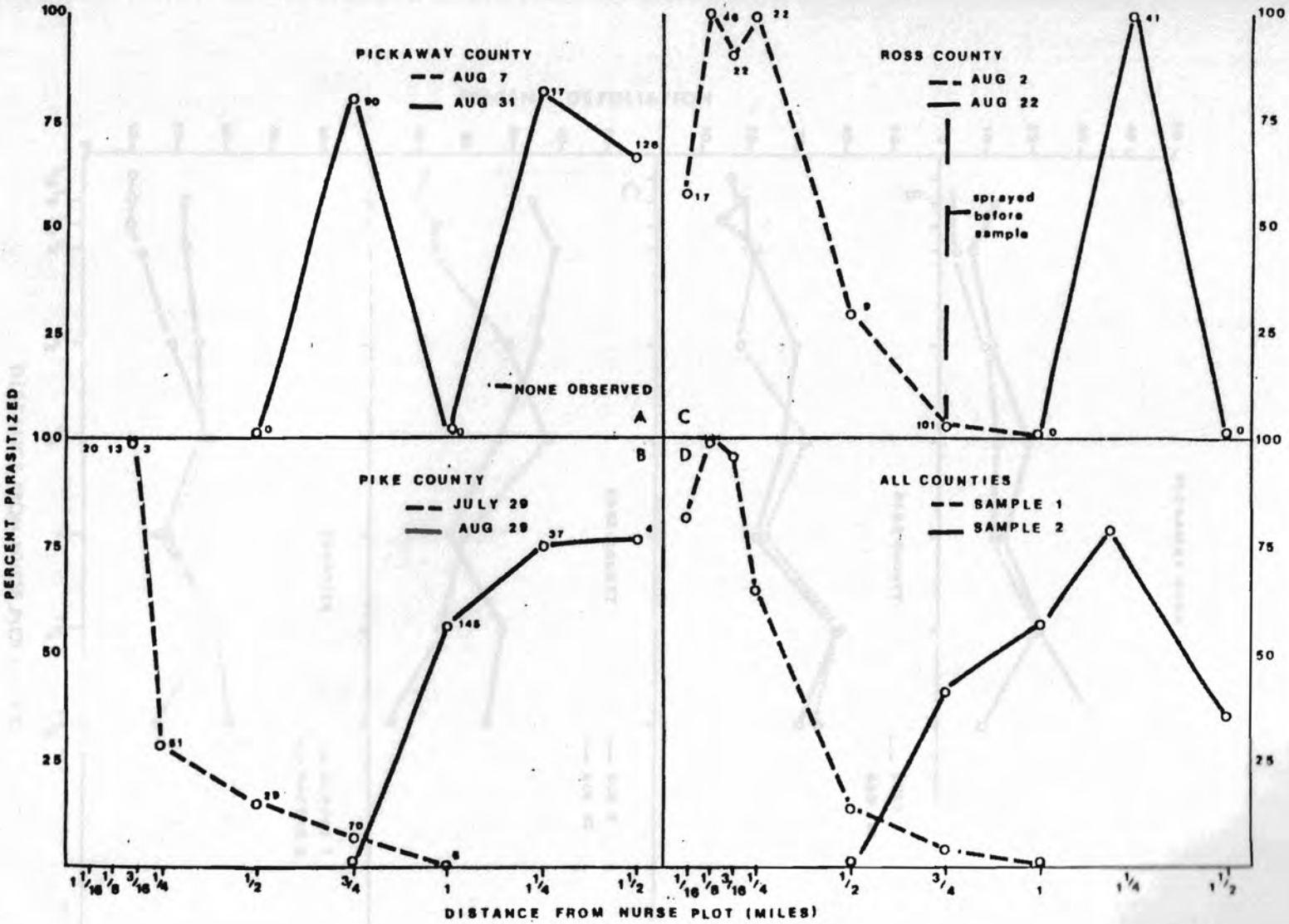


Figure 1. Comparison of percent parasitized in sample periods one and two by county and all counties combined.

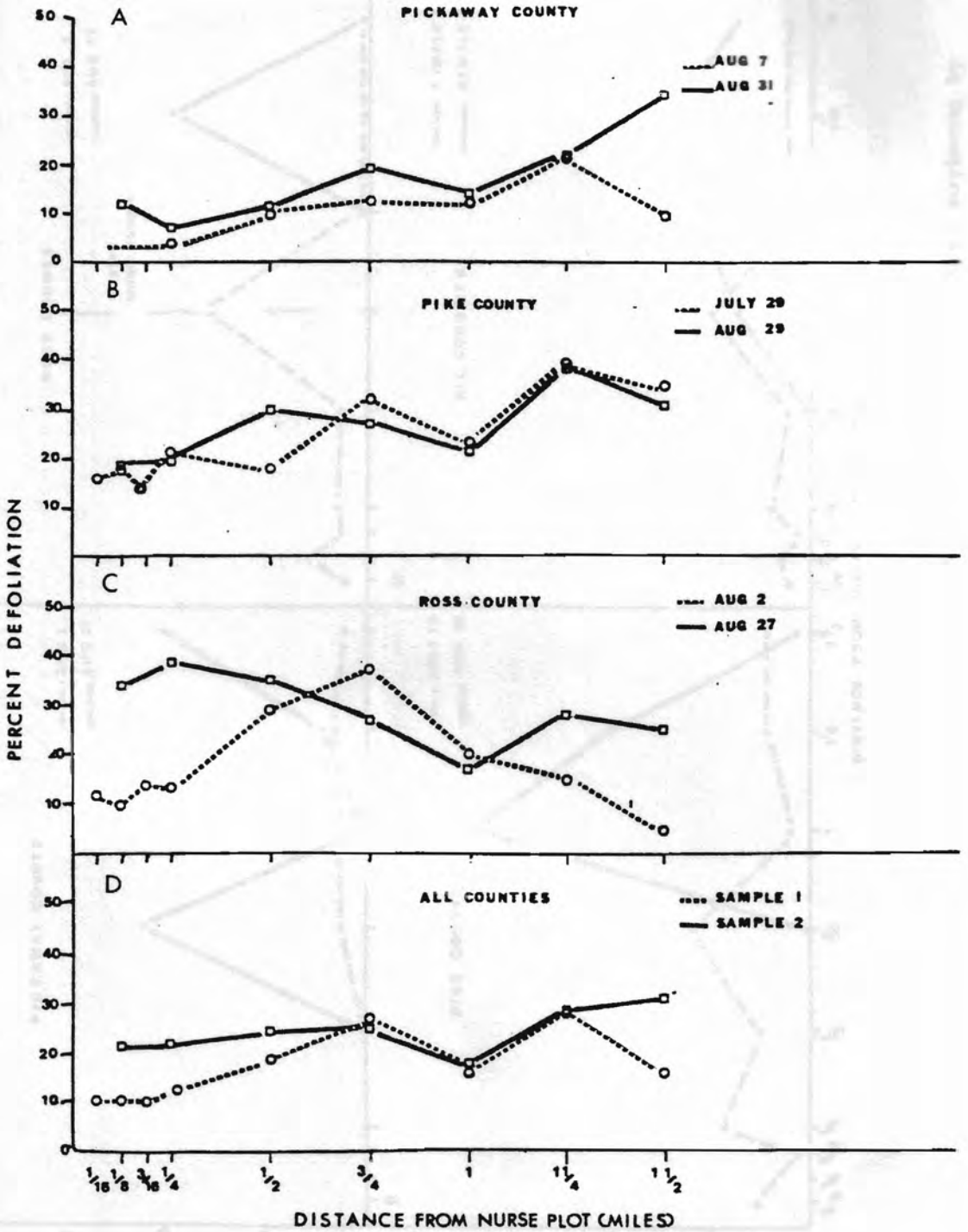


Figure 2. Comparison of percent defoliation in sample periods one and two by county and means of all counties.

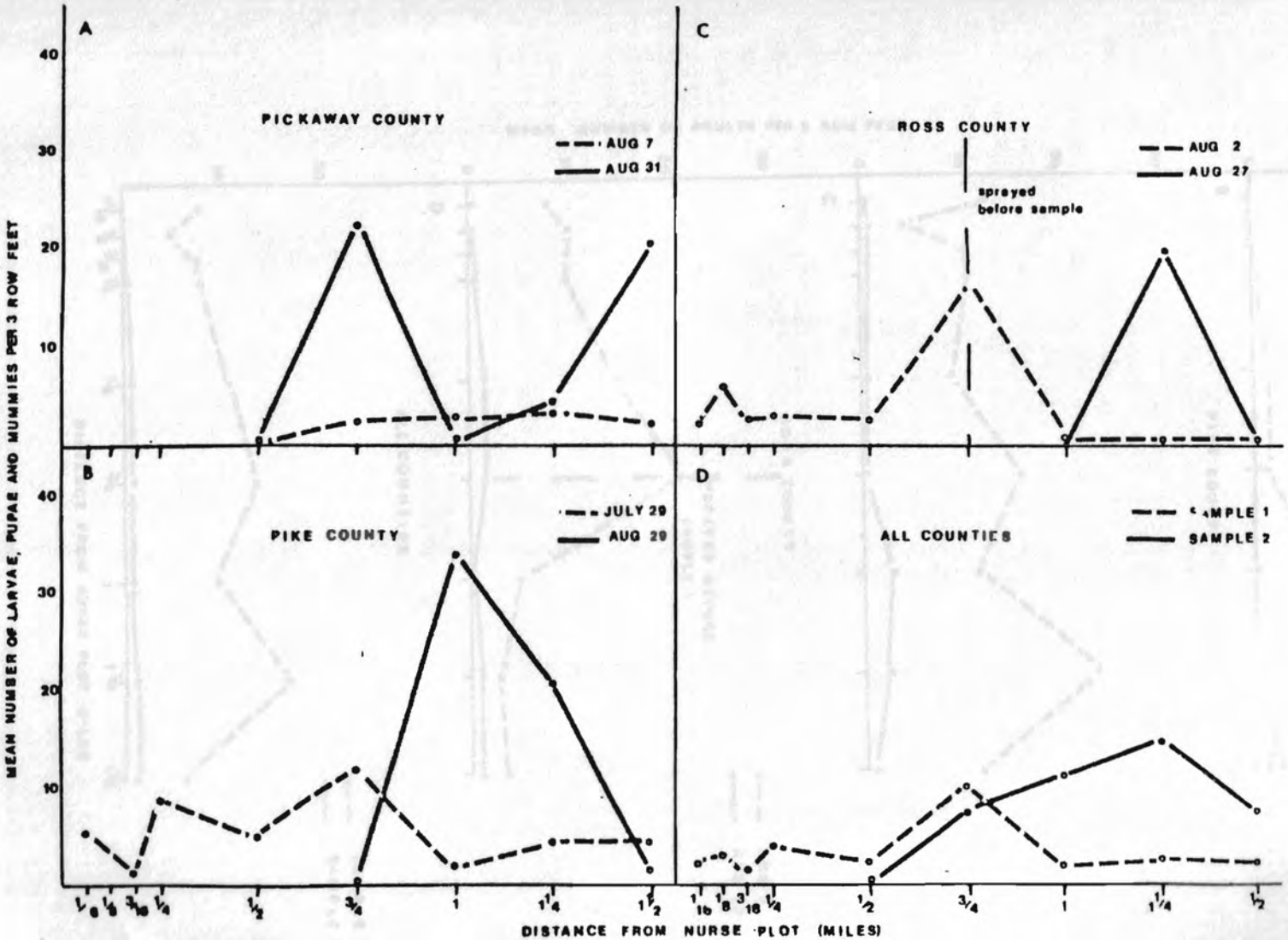


Figure 3. Comparison of the mean number of immature MBB per 3 row feet of soybeans by county and combined.

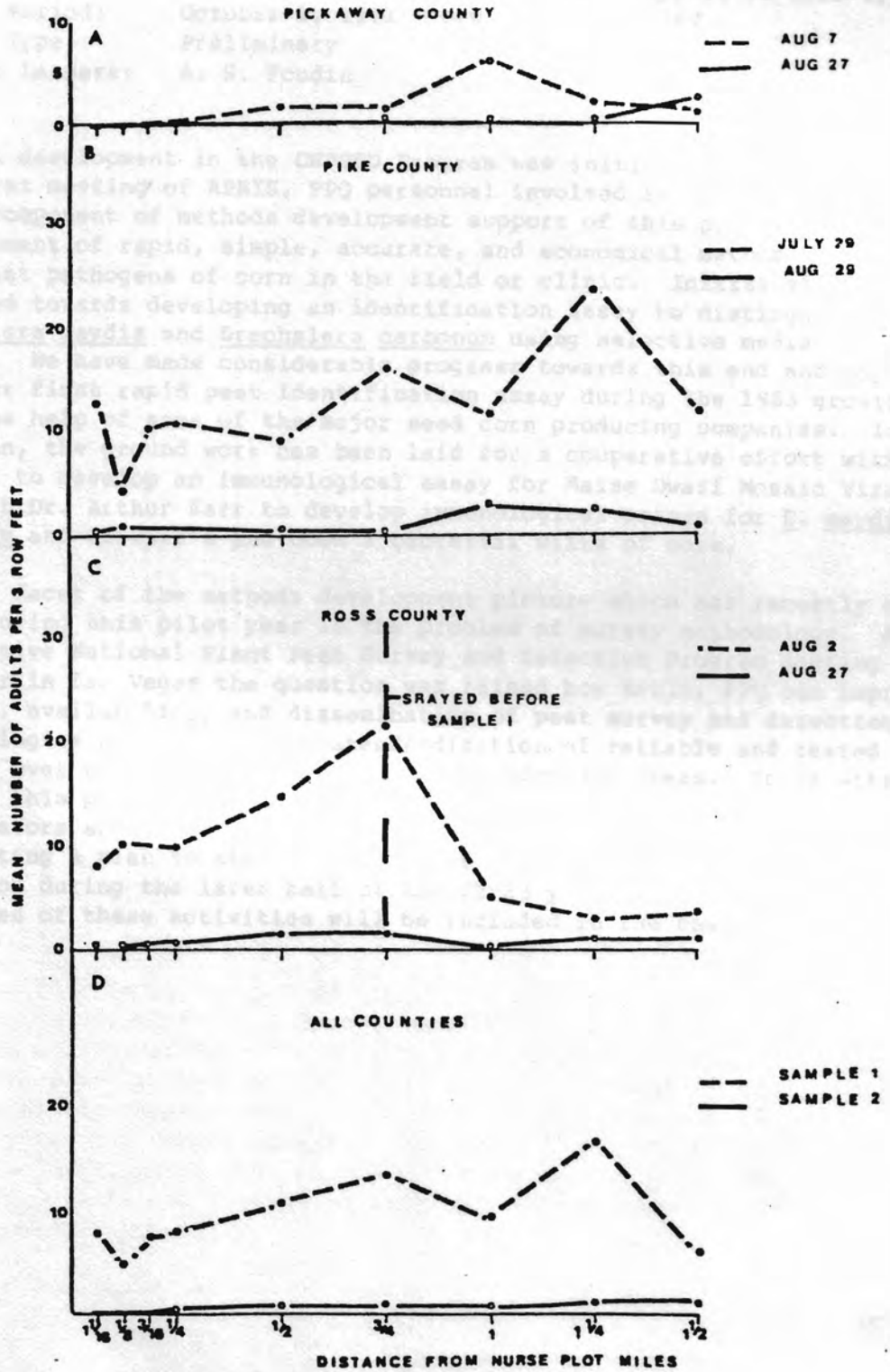


Figure 4. Comparison of the mean number of adult MBB by county and all counties combined.

Project Number: NPSDP 2.1.1
Project Title: Summary of Methods Development Support of NPSD Program
Report Period: October 1, 1981 - September 30, 1982
Report Type: Preliminary
Project Leaders: A. S. Foudin

Methods development in the CNPPSD Program was initiated on April 5, 1982, at the first meeting of APHIS, PPQ personnel involved in the new program. A major component of methods development support of this program was the development of rapid, simple, accurate, and economical methods of identifying important pathogens of corn in the field or clinic. Initial efforts were directed towards developing an identification assay to distinguish between Drechslera maydis and Drechslera carbonum using selective media in multi-well plates. We have made considerable progress towards this end and hope to field test our first rapid pest identification assay during the 1983 growing season with the help of some of the major seed corn producing companies. In addition, the ground work has been laid for a cooperative effort with Dr. Om Sehgal, to develop an immunological assay for Maize Dwarf Mosaic Virus of corn and with Dr. Arthur Karr to develop immunological assays for D. maydis and D. carbonum and Stewart's and Goss's bacterial wilts of corn.

Another facet of the methods development picture which has recently come into focus during this pilot year is the problem of survey methodology. At the Cooperative National Plant Pest Survey and Detection Program meeting in November in Las Vegas the question was raised how APHIS, PPQ can improve the quality, availability, and dissemination of pest survey and detection methodologies and promote the standardization of reliable and tested survey methods over broad geographical or crop production areas. In an attempt to address this problem, methods development, in consort with the APHIS area coordinators and Dr. Paul Teng of the University of Minnesota, are currently formulating a plan to start work on this pressing aspect of pest survey and detection during the later half of the FY-83 year and beyond. Detailed summaries of these activities will be included in the next Center Report.

Literature Review on Rearing *Bathyplectes curculionis* and *Bathyplectes anurus*

Antone C. Felix III
and
Philip C. Kingsley

Introduction

This review was undertaken in preparation for experiments to be conducted dealing with the development and survival of *Bathyplectes curculionis* and *Bathyplectes anurus* on *Hypera postica* larvae reared on various artificial diets. A wide variety of rearing methods was encountered in the literature. Partially due to the differing objectives of researchers, little consistency in rearing procedures could be found among the studies. To assist ourselves and others in designing experiments, a synthesis of rearing techniques is presented in this report. The text is divided into four sections -- Cocoon storage, Parasite emergence, Parasitization of alfalfa weevil larvae, Rearing of parasites -- and is supplemented by a table contrasting the rearing methods used in all literature to date. This report is also viewed as a first step towards the development of a standardized (optimal) rearing procedure.

Bathyplectes curculionis

Cocoon storage

Dysart and Bingham (1976) placed cocoons in 1/2-pint cartons (with transparent lids) which were kept in an air conditioned laboratory (ca. 22.8°C) during the summer. In late September, cocoons were transferred to transparent plastic boxes (with screened side holes and solid transparent lids) containing vermiculite. Boxes were stored for one month at 7.2°C and no light. The temperature was lowered to 1.7°C in late October and remained so until March when cocoon boxes were moved to an outdoor insectary. Dysart and Bingham achieved an emergence yield of 80% (2010 adults from 2522 cocoons).

Yeargan et al. (1978) stored cocoons at 21-24°C in summer, 8-10°C from September to January, and 3.3-4.4°C thereafter until needed. During winter, Miller (1970) and Berberet et al. (1976) maintained cocoons at 4.5°C and 4°C, respectively. Bartell and Pass (1978a) subjected their cocoons to a temperature - photoperiod regime of 23°C - 8 hr photophase and 15°C - 16 hr scotophase. Approximately two months before adult parasites were required for experiments, storage conditions were changed to 10°C and constant darkness. Parrish and Davis (1978) held cocoons at 23-25°C for two months. Temperature was then lowered to 4°C for three months.

To prevent dessication, Dysart and Bingham (1976) set a single layer of cocoons between thin layers of moistened vermiculite; thereafter, the vermiculite was sprayed with water biweekly. Miller (1970) put his 1/2-pint storage containers on moist sponge in one-gallon jars. Storing cocoons at relative humidity levels in excess of 80% should be avoided. Parrish and Davis (1978) reported that cocoons became saturated and most of the parasites died. They also noted that low humidity (10-20% RH) resulted in greater than 50% diapausing cocoons.

Bathyplectes curculionis diapauses as prepupae in stored cocoons. According to Hamlin et al. (1949, as reported by Parrish and Davis 1978) and Brunson and Coles (1968, as reported by Parrish and Davis 1978), B. curculionis does not pupate until spring.

Parasite emergence

Adult emergence was induced by removing cocoons from cold storage and placing them at room temperature (Yeargan et al. 1978). Under a 12-hr photoperiod at 23.9°C, adult parasites emerged in 12 days (Miller 1970). Butler and Ritchie (1967) put cocoons in one-gallon cartons with emergence tubes. Emerged parasites were placed in quart jars, stored at 15°C, and fed sugar water. Bartell and Pass (1978a) used flasks and 2% honey water, whereas Miller (1970) placed a one-pint parasite holding container with a screened hole in the bottom on a piece of sponge moistened with sugar water.

In field studies in Illinois, adult parasite males were observed to emerge sooner than females, but females attained higher densities (Barney et al. 1978).

Parasitization of alfalfa weevil larvae

Parasite mating:

Before introducing adult parasites to parasitization chambers, Miller (1970) left the parasites in one-pint containers for 24 hrs to ensure that mating had occurred. Berberet et al. (1976) and Bartell and Pass (1978a) waited 24-48 hrs and several days, respectively.

Selection of alfalfa weevil instars:

Based on dissections of field collected weevil larvae, Barney et al. (1978) concluded that B. curculionis prefers to oviposit into 2nd and 3rd instar hosts. In contrast, laboratory studies of Miller (1970), Duodu and Davis (1974b), and Parrish and Davis (1978) showed that the first three instars of H. postica were equally preferred (attacked). The 4th instar was the stage least attacked by parasites. In an experiment conducted by Bartell and Pass (1978a), only 44% (1156/2600) of parasitized 1st instars (1-2 days old when exposed to parasites) completed development as opposed to 68% for larvae not exposed to parasites. Duodu and Davis (1974a) used 1 day-old host larvae in their tests because newly-hatched parasitized larvae exhibited excess mortality and erratic behavior. For tests involving 2nd, 3rd, and 4th weevil instars, they chose only newly-molted larvae.

Parasitization methods:

At one day post-emergence, Miller (1970) transferred anesthetized parasites to 1/2-pint cartons (with a screened hole in lids) containing alfalfa bouquets and weevil larvae. Each container held one female parasite and 25 host larvae. Parrish and Davis (1978) used the same weevil to parasite ratio with a 9 hr photophase at 24°C and 15 hr scotophase at 7°C.

The parasitization units of Bartell and Pass (1978a) and Berberet et al. (1976) consisted of alfalfa within a funnel supported in a container of water. Weevil larvae were placed on the alfalfa and 24 hrs elapsed before parasites [three females and one male (Bartell and Pass 1978a); one 24-48 hr old female (Berberet et al. 1976)] were inserted. Bartell and Pass (1978a) used 65 host larvae. Funnels were sealed with plastic wrap. After 24 hrs parasites were removed (Miller 1970, Bartell and Pass 1978a, Parrish and Davis 1978). Environmental conditions for the Bartell and Pass (1978a) experiment were 21 ± 1°C and 16:8 L:D photoperiod.

Yeargan and Latheef (1977) and Yeargan et al. (1978) placed a pair of male and female parasites (less than 3 hrs old) in one-gallon cartons with alfalfa bouquets and 40 weevil larvae. A new group of host larvae was introduced every 24 hrs until the female parasite died. As in other studies, the parasitization chamber was covered with plastic wrap; however, small holes were made in the plastic to allow parasites access to a cotton wick soaked with 10% honey solution. Parasite fecundity was determined at two temperature regimes, 21.1°C constant and 6.7/18.3°C (18.3°C during photophase, 12:12 hr photophase:scotophase) -- there was no significant difference (a higher ovipositional rate was attained using 21.1°C constant, but parasite longevity was reduced). A similar set-up was used by Duodu and Davis (1974b); with the exceptions that 50 weevil larvae and 2 female parasites were held for 12 hrs under continuous light.

In experiments conducted by Butler and Ritchie (1976), larvae of Hypera brunneipennis were placed on alfalfa terminals within covered petri dishes. A female parasite was added only after the larvae had begun to feed. Tests showed that a 10:1 host:parasite ratio, was best in minimizing multiple parasitism yet ensuring that virtually all larvae were attacked (Butler and Ritchie 1967). Weevil larvae with multiple ovipositor punctures were more susceptible to mortality than those with a single puncture (Duodu and Davis 1974b). Parasitization was conducted at room temperature and continuous light (Butler and Ritchie 1967).

As female parasites became older, oviposition decreased (Barney et al. 1977, as reported by Barney et al. 1978) as did the percentage of nondiapausing offspring (Parrish and Davis 1978).

Rearing of parasites

Rearing environment:

Rearing conditions were usually the same as those for parasitization. Parrish and Davis (1978) found that their temperature-light regime resulted in about 95% nondiapausing B. curculionis. Light and temperature conditions right after mating but before oviposition strongly influenced parasite development, i.e., diapausing or nondiapausing. Continuous light resulted in few nondiapausing parasites. They also noted that the incidence of diapause was increased somewhat by extremes in relative humidity, older adult parasites, and using 4th instar host weevil larvae.

Development time:

Development time from oviposition to emergence from host larvae at 21 1°C was 13-18 days for most nondiapausing and 18-21 days for a few nondiapausing and all diapausing parasites (Bartell and Pass 1978b).

Bathyplectes anurus

Cocoon storage

Dysart and Bingham (1976) left cocoons in an air conditioned laboratory (ca. 22.8°C) until mid-September. At that time, the cocoons were removed from 1/2-pint cartons (with transparent lids) and placed in 1/2-pint inverted screened lids. Each lid contained a layer of cocoons covered with moistened sphagnum. Lids were set on the screened bottom of a transparent plastic cylinder which had been positioned over a one-gallon jar of water. The cylinder was covered with a paper towel and capped with a screened lid. The jar-cylinder combination was transferred to an outdoor insectary in late September, and then to a 1.7°C refrigerator (no light) at the end of December. In March, cocoons were taken from the jar-cylinder and put on the bottom of a transparent plastic box (with screened side holes and a solid transparent lid). Vermiculite was added to the cocoon-sphagnum mixture. Although the box was placed in an outdoor insectary for parasite emergence, the cocoons could have been incubated indoors. This method of storing cocoons and eliciting parasite emergence resulted in an emergence yield of 74% (1369 adults from 1838 cocoons).

Instead of inverted lids, Flessel (unpubl.) put the cocoon-sphagnum mixture in an aluminum foil "boat" which was set within a crisper box (7-1/2 x 10-1/2 inches). The box contained a bed of water-saturated perlite and accommodated 3,000 cocoons. It was covered with a paper towel and sealed with a screen lid. Unlike Bingham and Dysart who maintained cocoons in moist vermiculite and/or sphagnum (checked biweekly), Flessel kept his cocoon-sphagnum mixture dry but in a very moist atmosphere (bottom layer of perlite-water). Crisper boxes remained in an outdoor insectary until late October. At that time, a sample of 10 cocoons was taken from each box. If 75% or more of the sampled cocoons contained adult parasites, the boxes were moved to a 1.7 - 4.4°C chamber (no light). Two or three months under such conditions were adequate for breaking diapause. According to Brunson and Coles (1968, as reported by Bartell and Pass 1978a), B. anurus diapause as prepupae in summer, pupae during fall, and adults over the winter months.

Yeargan and Latheef (1977) buried small screen cages containing cocoons in the debris of an alfalfa field. The cages were retrieved in February and stored at 3.3 - 4.4°C until adult parasites were required.

Parasite emergence

To initiate emergence from cocoons, Yeargan and Latheef (1977) raised the temperature to 21-24°C. Using a temperature of 21.1-23.9°C, 50-70% RH, and a photoperiod of 12-16 hrs, Flessel (unpubl.) reported that adult emergence began in 2-4 days. The first adults to emerge were mostly males. Since adults are positively phototropic, Flessel used a back-lighted box for collecting the emerging parasites. Newly gathered adults were fed a 5% honey-water solution. Some were stored at 4.4°C for a week or more; however, every two days they were removed and allowed access to honey water and plain water (free choice).

Parasitization of alfalfa weevil larvae

B. anurus parasitization methods used by Yeargan and Latheef (1977) and Bartell and Pass (1978a) were identical to those used for B. curculionis. In the Bartell and Pass (1978a) study, of 2600 parasitized first instar weevil larvae, 1009 (39%) completed their development in contrast to 68% for unparasitized host larvae.

Rearing of Parasites

Bartell and Pass (1978a) used the same rearing procedure for B. anurus and B. curculionis.

Development Time:

Development time (at 21 ± 1°C) from oviposition until parasites spun cocoons ranged from 21-25 days (Bartell and Pass 1980).

Bathyplectes curculionis		Butler & Ritchie 1967	Miller 1970	Duodu & Davis 1974 a,b	Berberet et al. 1976
Cocoon Storage	environmental conditions	—	4.5°C	—	4°C during winter.
Parasite Emergence	environmental conditions	Emerged adults held at 15°C until needed.	12 hr photophase:12 hr scotophase at 23.9°C; RH 50%.	—	23 ± 3°C
Parasitization	parasite mating period	—	24 hrs	—	24-48 hrs
	selection of host instars	—	1st 3 instars preferred.	1st 3 instars preferred.	2nd and 3rd instars.
	ratio of host instars:parasites	Best parasitism with 10 host larvae:1 female parasite.	25 host larvae:1 female parasite	50 host larvae:2 female parasites	1 host larva:1 female parasite
	parasite introduction	Parasites introduced when host larvae began to feed.	1 day after parasites had emerged.	—	Host larvae allowed 24 hrs to establish on alfalfa before parasite (24-48 hrs old) introduction.
	parasite removal	After 5 hrs.	After 24 hrs.	After 12 hrs.	As each host larva was parasitized, the parasite was removed.
	environmental conditions	Continuous light, room temperature.	—	Continuous light.	23 ± 3°C
Rearing	environmental conditions	15 hr photophase:9 hr scotophase at various temperatures.	—	8 hr photophase:16 hr scotophase at 25-26°C; RH ca. 40%.	—

Bathyplectes curculionis		Dysart & Bingham 1976	Yeargan et al. 1978 and Yeargan & Latheef 1977	Bartell & Pass 1978 a	Parrish & Davis 1978
Cocoon Storage	environmental conditions	1) 22.8°C, air conditioning in summer. 2) 7.2°C, no light for 1 mo. 3) 1.7°C, no light for 4 mos.	1) 21-24°C in summer. 2) 8-10°C for 4 mos. 3) 3.3-4.4°C until adults needed.	1) 8 hr photophase at 23°C : 16 hr scotophase at 15°C; RH 60-80%. 2) 10°C, no light ca. 2 mos. before adults needed.	1) 2 mos. at 23-25°C. 2) 3 mos. at 4°C; RH 50-80%. (For nondiapausing cocoons, used 9 hr photophase at 24°C: 15 hr scotophase at 7°C.)
Parasite Emergence	environmental conditions	Outdoor insectary.	21-24°C	—	9 hr photophase at 24°C: 15 hr scotophase at 7°C; RH 50-80%.
Parasitization	parasite mating period	—	—	Several days.	—
	selection of host instars	—	2nd or early 3rd instars.	1st instars (within 24 hrs of eclosion).	1st 3 instars.
	ratio of host instars:parasites	—	40 host larvae:1 male and 1 female parasite	65 host larvae:1 male and 3 female parasites	25 host larvae:1 female parasite
	parasite introduction	—	Host larvae allowed 21 hrs to establish on alfalfa before parasites (less than 3 hrs old) introduced.	Host larvae allowed 24 hrs to establish on alfalfa before parasites introduced.	—
	parasite removal	—	After 24 hrs.	After 24 hrs.	After 24 hrs.
	environmental conditions	—	12 hr photophase:12 hr scotophase at 21.1°C or 12 hr photophase at 18.3°C:12 hr scotophase at 6.7°C.	16 hr photophase:8 hr scotophase at 21 ± 1°C; RH near saturation.	Same as parasite emergence.
Rearing	environmental conditions	—	—	Same as parasitization.	Same as parasite emergence.

Bathyplectes anurus		Flessel Unpublished	Dysart & Bingham 1976	Yeargan & Latheef 1977	Bartell & Fass 1978 a
Cocoon Storage	environmental conditions	1) Outdoor insectary until Oct. 2) 1.7-4.4°C, no light for 2-3 mos.	1) 22.8°C, air conditioning in summer. 2) Outdoor insectary for 3 mos. 3) 1.7°C, no light for 2 mos.	1) Buried in debris in alfalfa field until Feb. 2) 3.3-4.4°C until adults needed.	———
Parasite Emergence	environmental conditions	12-16 hr photoperiod at 21.1-23.9°C; RH 50-70%. Emerged adults held at 4.4°C until needed.	Outdoor insectary.	21-24°C	———
Parasitization	parasite mating period	———	———	———	Several days.
	selection of host instars	———	———	2nd instar.	1st instars (within 24 hrs of eclosion).
	ratio of host instars:parasites	———	———	40 host larvae:1 male and 1 female parasite	65 host larvae:1 male and 3 female parasites.
	parasite introduction	———	———	Host larvae allowed 21 hrs to establish on alfalfa before parasites (less than 3 hrs old) introduced.	Host larvae allowed 24 hrs to establish on alfalfa before parasites introduced.
	parasite removal	———	———	After 24 hrs.	After 24 hrs.
	environmental conditions	———	———	12 hr photophase:12 hr scotophase at 21.1°C or 12 hr photophase at 18.3°C: 12 hr scotophase at 6.7°C	16 hr photophase:8 hr scotophase at 21 ± 1°C; RH near saturation.
Rearing	environmental conditions	———	———	———	Same as parasitization.

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Appendix II

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Field Experiment to Optimize *Pediobius foveolatus* Production from Nurse Plots for Biological Control of the Mexican Bean Beetle in Soybeans[1]

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ABSTRACT

A field experiment in Maryland during 1981 involving 60 nurse plots was conducted to determine optimum parasite release ratios at different host densities for maximizing parasite production. If parasite supplies are adequate for all nurse plots in the biological control program, then maximum net parasite production is attained with release ratios of 10-20 (number of parasites released per host counted in 4.57 row-meters) at host counts of 25-150 (number of hosts/4.57 row-meters on release date), but this increases to 80 or more at host counts greater than 200. When parasite supplies are limited, the lowest release ratios provide the desired maximum individual parasite reproductive performance. Parasites showed little increase in reproductive performance as host density increased 10-fold and may also show little functional response to host density.

INTRODUCTION

Pediobius foveolatus (PF) (Crawford) (Hymenoptera: Eulophidae) was imported from India (Angalet et al. 1968) and has been used to suppress Mexican bean beetle (MBB) populations, Epilachna varivestis Mulsant (Coleoptera: Coccinellidae), in soybeans in Maryland (Stevens et al. 1975a). Since PF does not overwinter in the Middle Atlantic region, MBB biological control programs have depended on inoculative releases into nurse plots. Nurse plots are early season, 0.05 ha mixed plantings of snap beans and soybeans which (1) attract overwintered MBB adults, (2) create a readily accessible concentration of host larvae, and (3) facilitate colonization and increase of PF populations. Since 1980, the USDA has provided additional support to these programs in Maryland, Delaware, New Jersey, and Virginia with the objective of evaluating and improving current program methodology. This paper reports the results of an extensive field experiment to optimize PF production from a single inoculative release into a standard nurse plot. Parasite production was analyzed in terms of the numbers released per host present, the density of hosts within each plot, and the general availability of parasites.

MATERIALS AND METHODS

Nurse plot locations and sampling

The experiment was conducted in nurse plots in 5 counties on the Delmarva Peninsula in Maryland during the spring of 1981. Nurse plots were planted during April and May with a mixture of 3.2 kg snap bean (var. Burpee Stringless) and 1.4 kg soybean (var. Essex) seed in rows spaced 0.76 m apart. Nurse plots were sampled twice weekly after the first larvae were observed. Sampling consisted of thorough examination of five randomly selected 0.914 row-meter units of foliage (4.57 row-meters total). Larvae of all four instars were counted and recorded.

The "trigger" used to call for the parasite release was the date when 50% of the MBB larvae in the 4.57 row-meter sample were in the 2nd, 3rd, and 4th instar. PF parasitizes 2nd through 4th instar larvae but prefers the later stages (Stevens et al. 1975b). The actual releases were made within a few days of the trigger date and coincided with the time for maximum parasite reproduction/parasite released (G. P. Dively and W. K. Mellors, unpublished information). The number of 2nd+3rd+4th instar larvae in the 4.57 row-meter sample on the trigger date for a nurse plot is referred to as the host count for that plot and is a measure of host density in the plot. The number of parasites to be released was calculated as the product of the host count per 4.57 row-m and the experimental release ratio. The release ratio is the number of adult parasites (combined sexes) to be released per host larva in the 4.57 row-m sample. This was a convenient method for rapid calculation of release numbers for the ca. 600 nurse plots in the four states involved in MBB biological control programs.

As plots reached trigger date (as determined by host counts), one of the five experimental release ratios was randomly assigned without replacement to one plot within each of the 4 selected host count ranges. After all ratios were assigned once, then this procedure was repeated with the remaining plots. In this way, approximately three nurse plots in each of the four host count ranges (25-75,

100-150, 200-250, and 500-600) received each of the five experimental release ratios (10, 20, 40, 80, and 160). However, due to limitations on the supply of parasites, the highest release ratio could not be used at the highest host count range.

Altogether, the 19 release ratio - host count combinations were applied to a total of 60 nurse plots. These plots averaged 722.0 ± 357.7 (SD) row-meters of foliage and received parasites which averaged 13.3 ± 5.9 (SD) days in age. All releases consisted of mated, honey and water fed adults which were transported within cooled, insulated containers. Stevens et al. (1975b) found PF adults to remain reproductive for 24 days when provided with honey and stored at 13°C.

In each plot, all parasites were released on the same day by distributing the release containers throughout the plot, opening them, and allowing the parasites to emerge. Since there were about one hundred and sixty 4.57 m sections of row per nurse plot, a release ratio of 10 meant that one parasite was released for every 16 hosts in the entire plot and a ratio of 160 meant one parasite was released for every host. In the laboratory, Stevens et al. (1975b) found that one PF female could parasitize an average of 20.3 host larvae.

After the release, plots were sampled twice a week; all mummies (parasitized hosts) in a total sample of 4.57 row-meters of foliage were counted. From these counts, the maximum number of mummies produced per 4.57 row-meters was determined. In addition, the first 10 mummies encountered 10 days after release were collected and reared individually at 25°C until PF adults emerged. All adults were counted and the average number produced per mummy was calculated for each plot. The timing of this sample corresponds approximately to the occurrence of the peak number of mummies (G. P. Dively and W. K. Mellors, unpublished information).

Data analysis

The number of PF adults produced/4.57 row-m in each plot was calculated as the product of the peak number of mummies/4.57 row-m and the average number of PF adults that emerged per mummy. For each plot, this was multiplied by the actual (measured) number of 4.57 row-m sections in that plot (plot size varied somewhat) to give the total parasite production (sexes combined) from that plot. Usually, only one parasite generation occurs in nurse plots after release on the "trigger" date used (W. K. Mellors, unpublished information).

Parasite production per nurse plot was then examined in terms of two measures of efficiency. (1) Net production was calculated as total production per nurse plot minus the number of parasites released into the plot. Net production provides an economic index of nurse plot profitability in terms of parasite yield. (2) Relative production was calculated as total production divided by the number of parasites released into the plot. Relative production (expressed as parasites produced/parasite released) provides a biological index of average parasite reproductive performance. When parasite supplies are adequate for all nurse plots in a program, net production is useful in determining the release ratio which maximizes total parasite yield for each host count range. However, when parasite supplies are inadequate for all nurse plots, then overall parasite production is maximized by optimizing average parasite reproductive performance (= relative production) by using those parasites that are available in the most profitable way

Multiple linear regression was utilized to test for ascending or descending trends between each of the two production indices (y) and release ratio (x_1),

host count (x_2), and their interaction (x_3). For each index, two regressions were conducted: one with release ratio entered first and host count second and one with these two entered in the reverse order. The interaction term was entered last each time. This process was used to determine whether these independent variables accounted for the same variability in each production index. All partial regressions were tested at the 5% level of significance.

For each of the 19 release ratio - host count combinations, the average net and relative production was calculated from the nurse plots which had had that combination. For each host count range, means were plotted as a function of release ratio to show the shapes of the underlying relationships and to permit economic evaluation of the results.

From data provided by the Maryland Department of Agriculture (R. Tichenor, unpublished information), the host count was determined for each of the 232 nurse plots in the MBB biological control program in Maryland in 1981. The percentage of these with host counts in each of the 4 ranges was calculated and plotted to indicate the potential applicability of the results based on the 60 nurse plots.

RESULTS AND DISCUSSION

The significant results of the multiple regressions for both net and relative production were the same regardless of the order in which release ratio and host count were entered. Therefore, the statistics reported are only for the regressions in which release ratio was entered first.

Net production increased significantly with both host count ($F = 22.39$, $b_2 = 70.31$) and the release ratio - host count interaction ($F = 10.52$, $b_3 = 8.605$), but was not significantly related to release ratio ($F = 0.153$). This increase in net production with host count does not imply that released parasites were more efficient at greater host counts but probably results from the greater total numbers released at greater host counts. Net production will increase with host count even if each parasite produces the same average number of progeny regardless of host count. The fact that net production increased with host count indicates that nurse plots with high host counts were the most profitable with respect to parasite production. The significant release ratio - host count interaction further contributed to this trend.

The relationship between average net production/nurse plot and release ratio within each host count range is plotted in Fig. 1. The significant interaction term is evident in those plots because the curves have considerably different shapes for each host count range. Although release ratio did not have a significant linear relationship to net production, these plots show some distinct patterns between net production and release ratio. For the 25-75 host count range, there was only a slight overall trend toward increasing net production with increasing release ratio. From a practical standpoint, the most profitable ratio would be the lowest one for this range. Except for the very high production at a ratio of 20, the same type of pattern occurs for the for the 100-150 host count range. For the 200-250 host count range, net

Fig. 1

production reaches a maximum at a release ratio of 80 and remains the same at 160. This suggests an economically optimum release ratio of 80 for the 200-250 host count range. For the 500-600 host count range, the pattern increases sharply with increasing release ratio and suggests that maximum production may occur at a ratio greater than 80. Altogether, these results indicate that the optimum release ratio increases with increasing host count and that an MBB biological control program should utilize a variable release ratio approach (based upon host density) if parasite supplies are adequate for all nurse plots.

Relative production decreased significantly with increasing release ratio ($F = 5.658$, $b_1 = -0.1978$), but was not significantly related to either host count ($F = 1.029$) or the release ratio - host count interaction ($F = 0.4150$). The decrease in relative production with increasing release ratio implies a declining individual reproductive performance as more parasites are released per host. Such a trend might be expected if increasing release ratios lead to increased intraspecific parasite competition, interference, dispersal, or superparasitism, for example.

The relationship between average relative production and release ratio within each host count range is plotted in Fig. 2. Within each host count range, relative production was highest at the lowest release ratios. If parasite supplies were inadequate for all nurse plots, then a program's strategy should be to maximize reproduction by each parasite available for release. This could be accomplished by making releases at only the lowest ratios regardless of host count.

Fig. 2

The lack of a significant relationship between relative production and host count suggests that PF shows no functional response to host density. The relationship between average relative production and host count for each release ratio is plotted in Fig. 3 to show the shape of the underlying functions. Over a 10-fold increase in host density, there was only a slight increase in reproductive performance at most release ratios. Although progeny production

Fig. 3

is only partially related to the number of hosts attacked/parasite as used to describe a functional response (Holling 1959), the relative production data do not indicate a strong density-related response of PF to MBB larvae in nurse plots. In cage studies with MBB larvae on snap bean foliage, Gale and Shepard (1978) also found little response of PF females to host density. If PF does not show a density response in its host killing ability, then successful biological control using PF must depend on (1) adequate founding populations from released parasites and (2) adequate time for a numerical response over generations. Thus, the level of parasite production from nurse plots could be a major factor contributing to MBB biological control programs.

For the 232 nurse plots in the MBB program in Maryland in 1981, the percentage with host counts in each of the experimental and intervening ranges are plotted in Fig. 4. The four host count ranges used in this experiment included 46.98% of the nurse plots, and 97.40% of the nurse plots had host counts of less than 600 which was the highest included in this study. Clearly, the results of this field experiment are applicable to the great majority of the nurse plots in that program.

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FOOTNOTES

[1] Scientific Article No. A-3299, Contribution No. 6371 of the Maryland Agricultural Experiment Station.

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FIGURE LEGENDS

Fig. 1. Relationship between average net parasite production/nurse plot and release ratio within each host count range. The economic optimum release ratio for each range is the point of maximum net production. Each data point is accompanied by the number of nurse plots that contributed to the calculation of that mean.

Fig. 2. Relationship between average relative parasite production (parasites produced/parasites released) and release ratio within each host count range. Parasite reproductive performance is greatest at the points of maximum relative production. Each data point is accompanied by the number of nurse plots that contributed to the calculation of that mean.

Fig. 3. Relationship between average relative parasite production (reproductive performance) and host count range (host density) for each of the release ratios. No strongly increasing trend occurs as would be indicative of a significant functional response to host density. Each data point is accompanied by the number of nurse plots that contributed to the calculation of that mean.

Fig. 4. Percentage of the 232 nurse plots in Maryland during 1981 with host counts in each range. Arrows indicate the four host count ranges included in this experiment.

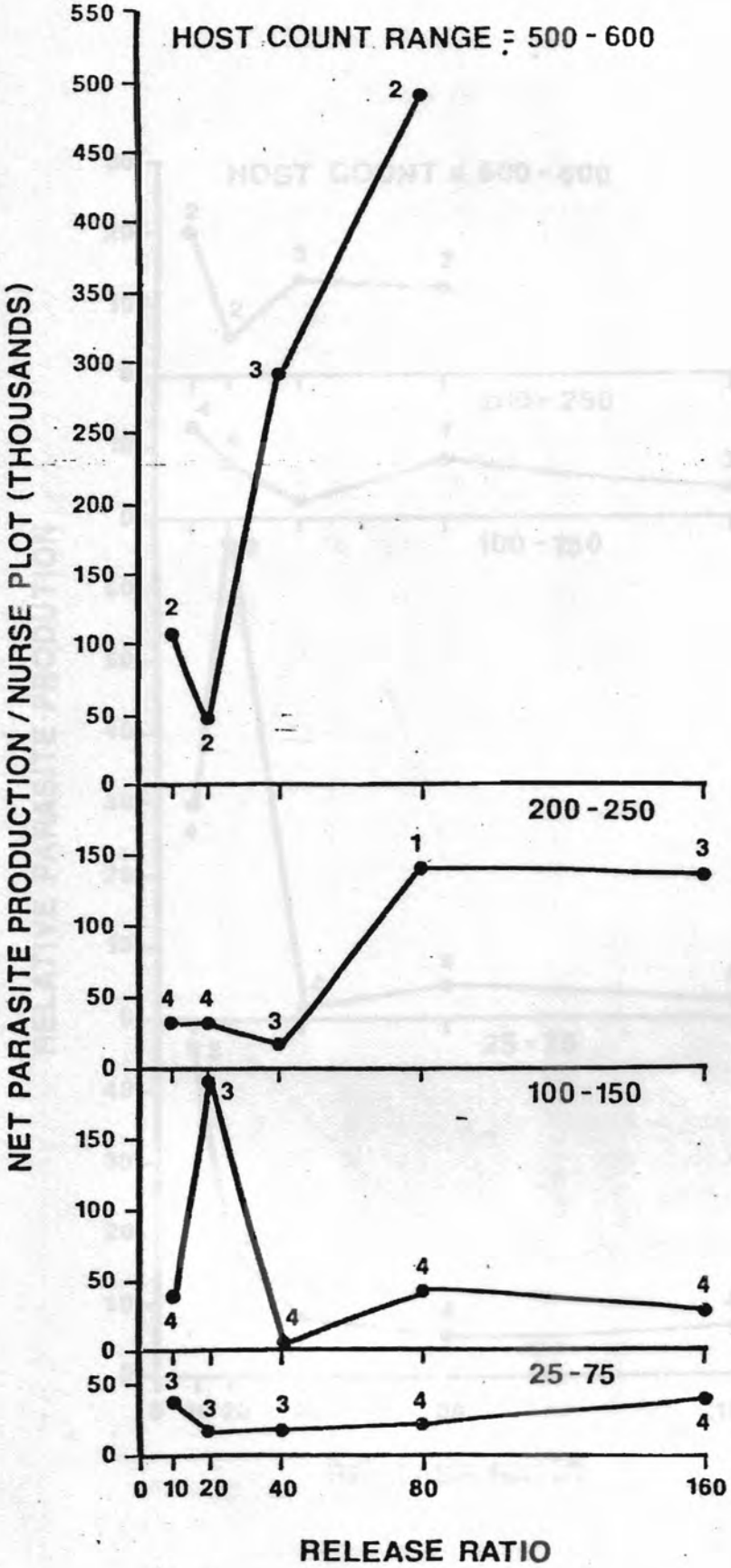

HOST COUNT RANGE = 500 - 600
200 - 250
100 - 150
25 - 75
NET PARASITE PRODUCTION / NURSE PLOT (THOUSANDS)
RELEASE RATIO
0
10
20
40
80
160

Fig. 1

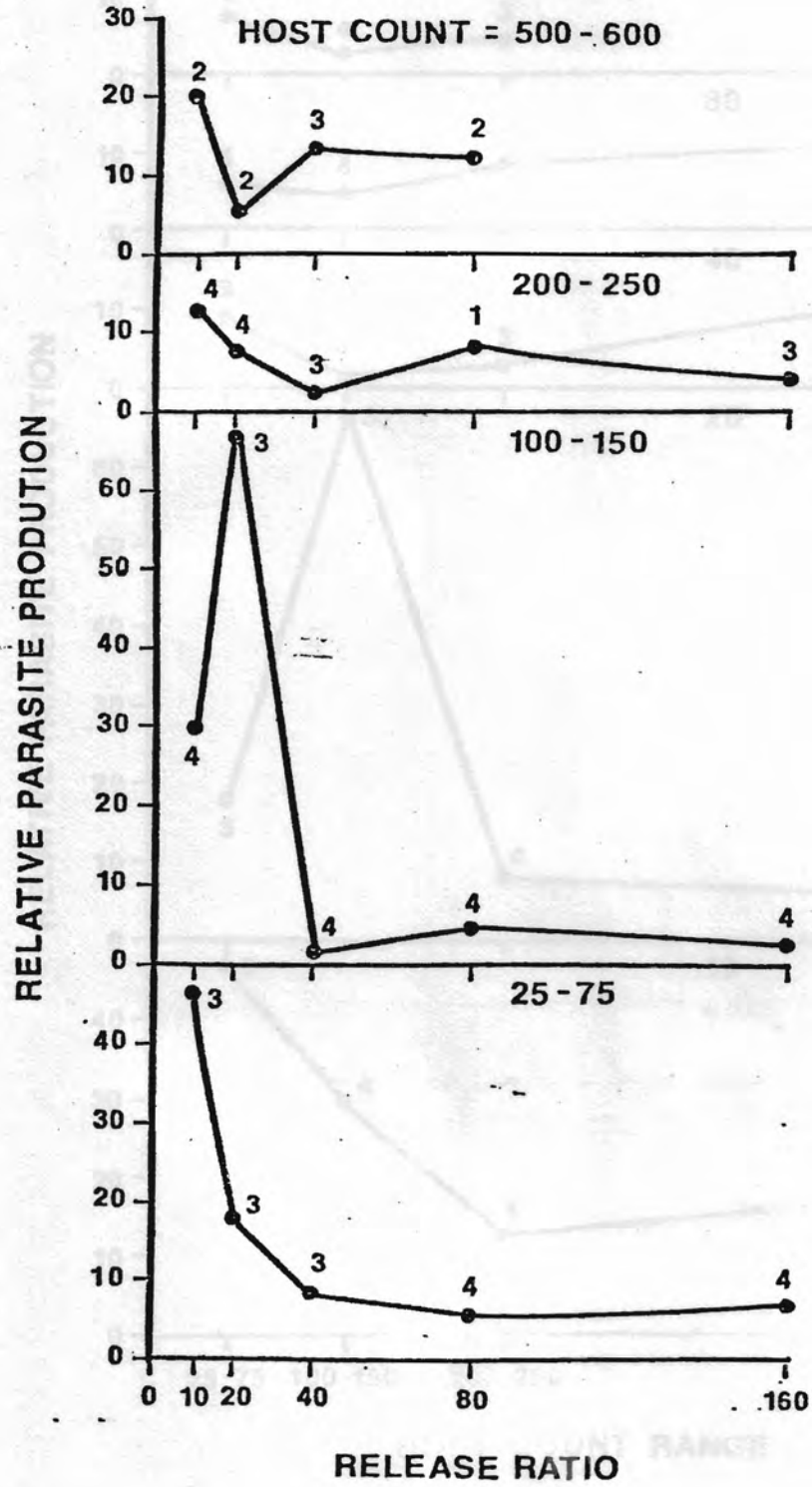

HOST COUNT = 500 - 600
200 - 250
100 - 150
25 - 75
RELATIVE PARASITE PRODUTION
RELEASE RATIO
0
10
20
40
80
160

Fig. 2

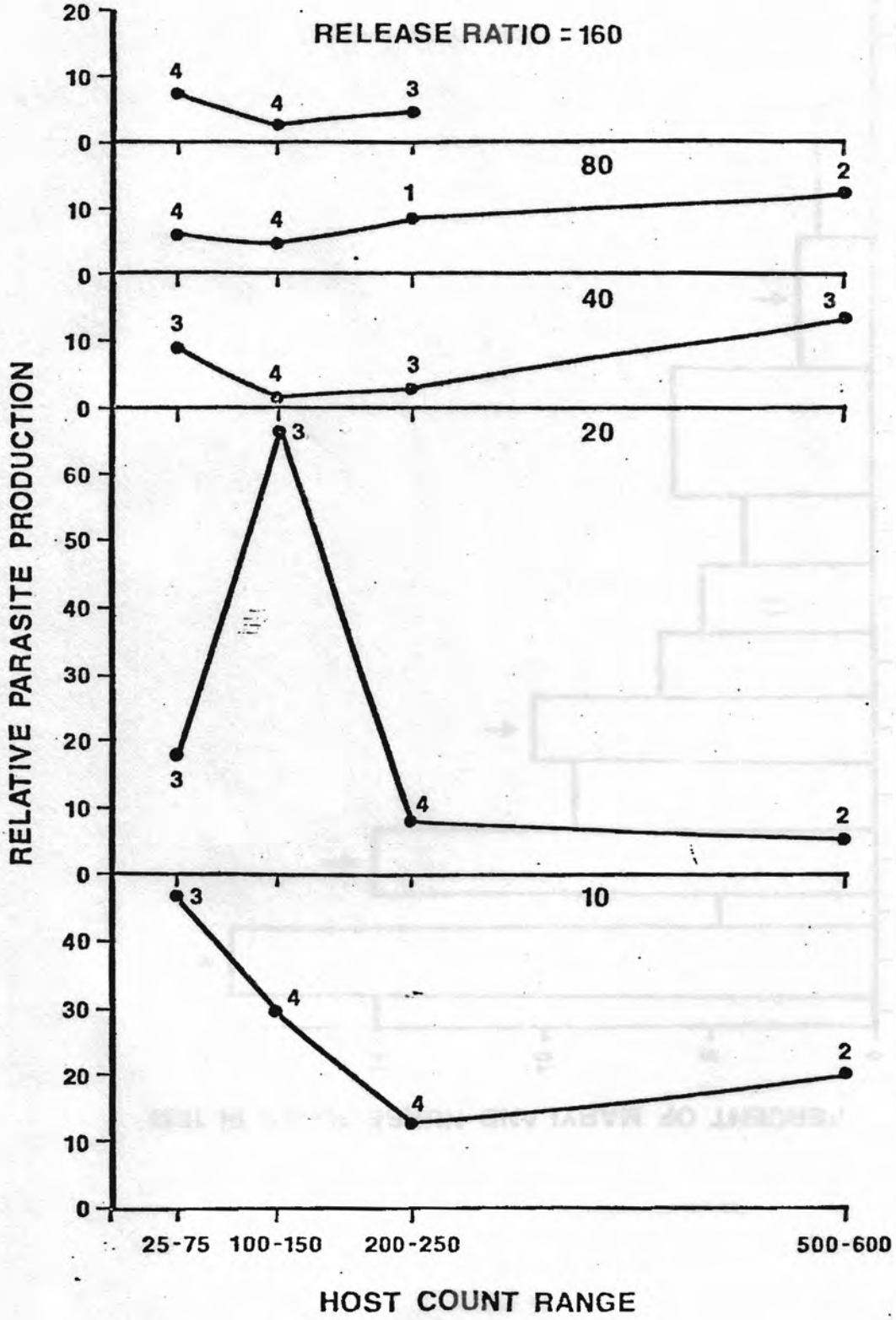
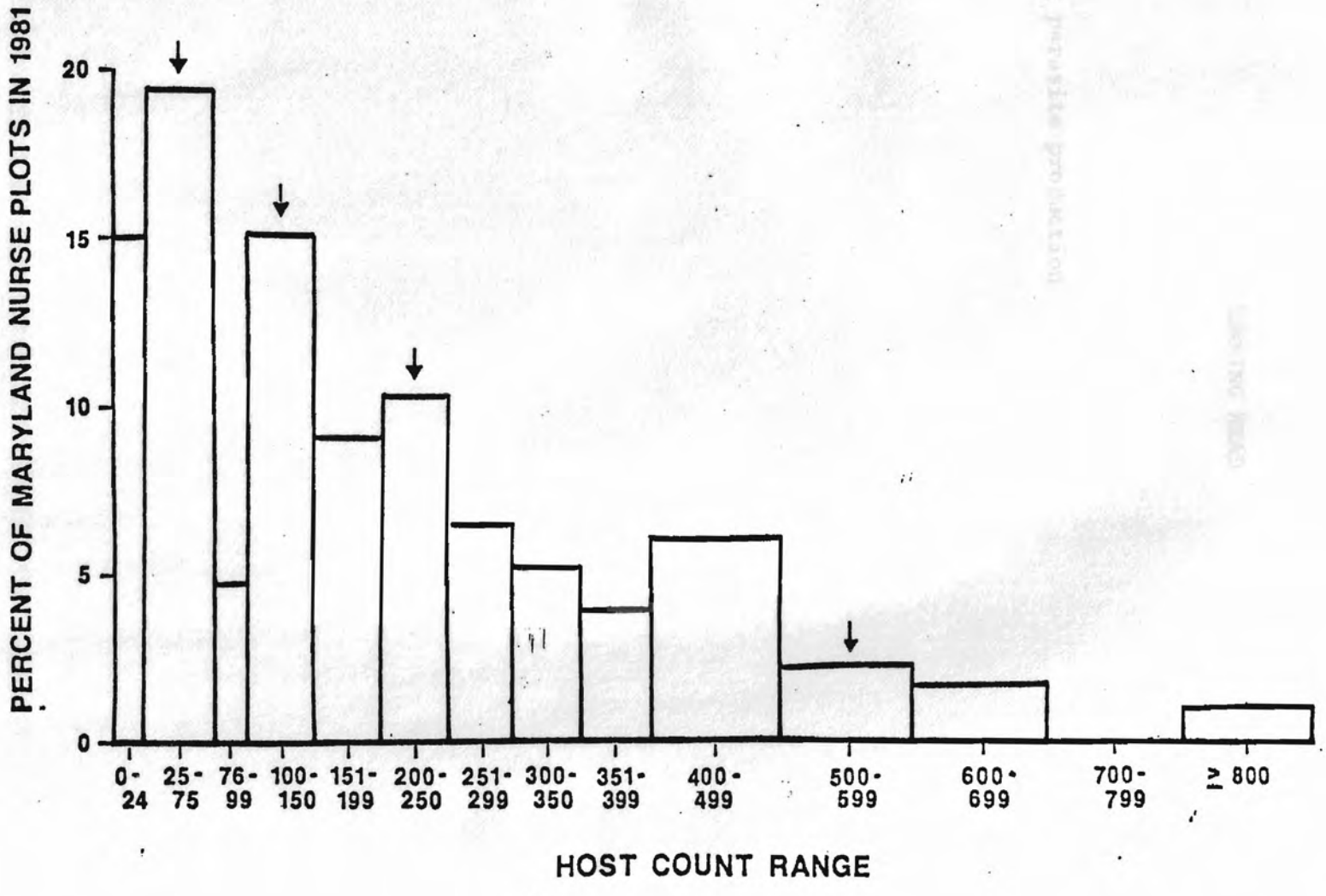

RELEASE RATIO = 160
80
40
20
10
RELATIVE PARASITE PRODUCTION
25-75
100-150
200-250
500-600
HOST COUNT RANGE

Fig. 3


PERCENT OF MARYLAND NURSE PLOTS IN 1981
20
15
10
5
0
0-24
25-75
76-99
100-150
151-199
200-250
251-299
300-350
351-399
400-499
500-599
600-699
700-799
≥ 800
HOST COUNT RANGE

RUNNING HEAD

Optimizing parasite production