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April 1, 1978 - September 30, 1978

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

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Animal and Plant Health Inspection Service

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Project Number: GM 6.1.5
Project Title: Regulatory Treatments
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: Winfred H. McLane, J. A. Finney

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

A limited amount of testing was conducted during the reporting period with a Dylox® dust formulation.

Petri dishes (1.2 x 9 mm) were dusted with 3 dust formulations of Dylox®, and twenty, 4th instar larvae were infested onto the treated surface for 1 minute, removed and placed on artificial diet. Test insects were held in an environmental chamber at 26° C and 60% RH. Mortality readings were made 1 and 2 days after exposure.

Table 1. Mortality of 4th Instar Gypsy Moth Larvae Exposed to Dylox® Dust.

| Formulation | Grams Formulation Per Dish | Percent Mortality 24 Hours | 48 Hours |
|---|---|---|---|
| 5% Dust | 0.1 | 2 | 2 |
| 10% Dust | 0.1 | 21 | 24 |
| 25% Dust | 0.1 | 78 | 83 |

A prototype apparatus for dispensing uniform dust applications to petri dishes and other surfaces was developed. It is mounted inside the microliter syringe spray chamber and makes use of its ventilation system and air supply. This device has been calibrated using gas chromotography. However, results indicate that design changes are necessary for reproducible applications.

Gas chromatographic analysis was accomplished with a Tracor 222 equipped with a flame ionization detector. The column was 6' x 1/4" glass packed with a mixture of 1.5% 2250 and 1.95% 2401 on 100/120 Supelcon AW-DMCS at oven temperature of 160° C. Dylox elutes 3 peaks under these conditions and the first peak on the chromatogram was used for quantitative calculations.

Linearity tests were done with dylox standards on the peak to ensure reliability of results.

Table 2. Test Results of Calibration of Dusing Device Using Gas Chromotography.

| Initial Dosage (g/dish)1/ | % Recovery2/ |
|---|---|
| 0.5 | 18.70 |
| 0.25 | 9.92 |
| 0.10 | 3.76 |
| 0.075 | 4.98 |
| 0.050 | 8.96 |
| 0.025 | 4.22 |
| 0.0125 | 4.67 |
| 0.0062 | 1.87 |
| Check | 0 |

1/ 25% dust formulation

2/ Average of 5 replicates

Data indicate that little active material was recovered from treated dishes and recovery was not linear with the amount of material applied. This in part was due to the limited amount of solvent used for rinsing and clean-up.

Additional calibration work must be done with the device before it can be used on a routine basis.

Project Number: GM 7.1.0
Project Title: Contact Toxicity of Registered Insecticides Against Gypsy Moth Larvae
Report Period: April 1, 1978 - September 30, 1978
Report Type: Final
Project Leaders: Winfred H. McLane, J. A. Finney

It is important that the stage of larval development is known during an insecticide application program. If larvae are too large, treatments may not be efficacious and additional insecticide may be needed to kill the later instars. Alternatively, adequate control of small larvae may require less insecticide. A series of tests was conducted to determine the $LD_{50}$ of 2nd, 3rd, 4th and 5th instar laboratory-reared gypsy moth larvae when treated topically with the major registered insecticides for gypsy moth control. Dimilin®25W, Dylox®1.5 oil, Dylox®80S, Orthene®75S, Sevin® 4-oil, Sevin®80S and SIR 8514 were tested. Test insects were newly moulted, hand picked larvae from the $NJ_{F16}$ strain.

Chemicals were mixed in acetone prior to testing and were stored in sealed vials under refrigeration when not in use. Dosage-mortality data were obtained by application of one microliter of acetone containing the insecticide to the dorsal metathoracic region. This was done using a 50 microliter syringe equipped with a repeating dispenser calibrated to deliver 1/50th of its capacity with each discharge. Mortality curves were obtained by treating groups of larvae with 7 dilutions of each insecticide. Tests consisted of treating 20 larvae with each of the 7 dosages on consecutive days until a minimum of 100 larvae were exposed to each dilution. Treated 2nd or 3rd instar larvae were held in groups of 20 in plastic dishes (94 mm x 48 mm) containing 60 ml of artificial diet. Treated 4th and 5th instar larvae were held 10 per dish to prevent crowding. With each test two sets of controls were also observed. One was treated with acetone only and the other had no treatment. During testing all insects were held in an environmental chamber at 26°C and 60% RH. Mortality readings were made daily. Criterion for death was no reaction when larvae were prodded gently with a dissecting needle. Results are summarized in the following table.

Table 1. $LD_{50}$ (ug) of 5 insecticides applied topically to 4 instars of gypsy moth larvae.

| Material | INSTAR II | III | IV | V |
|---|---|---|---|---|
| Dimilin 25W | 0.0031 | 0.0251 | 0.014 | 0.964 |
| SIR 8514 (Chemagro) | 0.0060 | 0.0260 | 0.0984 | 9.3020 |
| SIR 8514 (Bayer) | 0.0089 | 0.0366 | 0.1209 | 2.8350 |
| Sevin 4-oil | 0.0326 | 0.0991 | 0.510 | 1.181 |
| Dylox 80S | 0.247 | 0.493 | 0.448 | 2.51 |
| Sevin 80S | 0.277 | 0.3415 | 0.8106 | 2.65 |
| Orthene 75S | 0.349 | 0.535 | 1.41 | 3.67 |
| Dylox 1.5 | 0.454 | 0.275 | 3.760 | 5.540 |
| Check | - | - | - | - |

Dimilin and SIR 8514 $LD_{50}$ was established 10 days after treatment. All others were established 2 days following treatment.

The insect growth regulators Dimilin and SIR 8514 demonstrated excellent contact activity when applied to early instar. Larvae treated as 5th instar were resistant to the treatments, however, most resulting pupae were deformed. It is possible that a number of the 5th instar larvae were male and no additional moulting took place. However, Dimilin was by far the most effective of the materials tested.

It is interesting to note that Sevin 4-oil was more effective than Sevin 80S.

Dosages needed to establish an $LD_{50}$ increased proportionally with size of test insect used. Regulatory personnel should take this into account when treating larger than normal gypsy moth larvae.

Project Number: GM 8.1.1
Project Title: Evaluation of Ultraviolet Screening Agents for Microbials
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: Winfred H. McLane, J. A. Finney

It has been known for some time that ultraviolet radiation plays an important role in the deterioration of pesticides in the field. A number of investigators have reported effects of ultraviolet rays on insecticides and microbials (David, 1968, Krieg, 1975, Cantwell, 1966, and Bullock, 1970). Wavelengths of 290-400 (nm) cause the rapid breakdown and ineffectiveness of several compounds once sprayed on foliage. Materials such as the pyrethroids and microbials are extremely susceptable, whereas, carbamates and organo phosphates tend to be more tolerant.

It is important to identify the most active ultraviolet absorbers and screens so they can be incorporated into pesticide and microbial formulations for field tests. To do this, an active laboratory screening program must be undertaken. Before this can be done, a laboratory ultraviolet radiation source should be available throughout the year.

On March 20, 1978 a series of tests were started using Mylar Type A (10 mil; 0.25 m m) polyester film (DuPont) to filter out radiation emitted by the FS 40 sunlamps below 315 nanometers (nm). Tests were conducted using the sunlamp and a blacklight blue lamp side by side, 7.6 cm above treated foliage. When filters were used they were placed 2.6 cm below lights and 5 cm above foliage. Treated plants were exposed for various times under lamps without filters and under lamps using one and two filters. An equal number of plants were not exposed to ultraviolet light.

The material tested was nucleopolyhedrosis virus, lot number #35 from Hamden, CT. Dosage and rate of application was 1.54 g/2 gal/ac. ($2.54 \times 10^7$ PIBs/ml). After exposure to radiation, 20 newly moulted second instar gypsy moth larvae were exposed to each seedling. Five untreated seedlings were used in each test as a control. After an exposure of 48 hours, larvae were removed from plants to plastic dishes containing artificial diet. Larvae were then held for 16 days at 24°C and relative humidity of 60%. Mortality readings were made each day and terminated after 16 days. All mortality data recorded in this report is based on 16 day readings.

Table 1 presents results of 4 tests all with a 15 minute exposure time. Three formulations were used along with a check.

Table 1. Test results with 3 formulations of virus exposed to ultraviolet radiation, with and without polyester film filters.

| Formulation | PERCENT MORTALITY AFTER 16 DAYS | | | |
|---|---|---|---|---|
| | NO UV | UV | 1 Filter | 2 Filters |
| NPV + $H_2O$ | 45 | 2 | 25 | 28 |
| " " | 70 | 9 | 20 | 46 |
| " " | 64 | 14 | 28 | 53 |
| " " | 63 | 17 | 49 | 57 |
| Average | 60.5 | 10.5 | 30.5 | 46 |
| NPV + Pro Tec® 2 | 46 | 38 | 43 | 63 |
| " " | 48 | 44 | 49 | 62 |
| " " | 61 | 29 | 62 | 61 |
| " " | 84 | 22 | 57 | 71 |
| Average | 59.7 | 33.2 | 52.7 | 64.2 |
| Std.(Shade)® | 44 | 34 | 55 | 53 |
| " " | 64 | 55 | 49 | 24 |
| " " | 54 | 53 | 62 | 43 |
| " " | 74 | 77 | 54 | 54 |
| Average | 59 | 54.7 | 55 | 43.5 |
| Checks | 1 | | | |
| " | 1 | | | |
| " | 2 | | | |
| " | 2 | | | |
| Average | 1.5 | | | |

Data in Table 1 indicate that radiation below 315 nanometers has been filtered out using one Mylar type A (10 mil; 0.25 mm) polyester film (DuPont) filter. If this is so, and if no influential ultraviolet radiation below 315 nanometers reaches the earth surface, results tell us that when screening ultraviolet absorbers one should use a filter such as the polyester film made by DuPont.

This is especially true when using Westinghouse sunlamp, FS 40 and blacklight blue F 40 BLB if no filter is used. Absorbers may be passed over because they were not formulated to arrest radiation below 315 nanometers, a range unlikely to be encountered at the earths surface.

Tests with filters also resulted in the ProTec® formulation looking equally as good as the standard Shade® mixture. This was not true when filters were not used.

The same formulations and lighting combination was also tested with an exposure time of 30 minutes.

Table 2. Test results with 3 formulations of virus exposed to ultraviolet radiation, with and without polyester film filters.

| | PERCENT MORTALITY AFTER 16 DAYS | | | |
|---|---|---|---|---|
| Formulation | NO UV | UV | 1 Filter | 2 Filters |
| NPV + $H_2O$ | 11 | 12 | 3 | 5 |
| NPV + Pro Tec® 2 | 36 | 51 | 41 | 41 |
| Std.(Shade®) | 55 | 55 | 49 | 43 |
| Check | 2 | | | |

The percent of mortality recorded from formulations not treated with ultraviolet radiation was less than expected. It is possible that formulations tested could act as a feeding inhibitor. However, this has not been recorded when the same formulations have been tested in the past.

Two additional tests were designed to test artificial ultraviolet radiation against natural sunlight. The second test was terminated after 10 days because of personnel shortages.

Table 3. Test results with 4 formulations of virus exposed to artificial sunlight and natural sunlight.

| | TEST 1 | | | TEST 2 | | |
|---|---|---|---|---|---|---|
| Formulation | NO UV | UV[1/] | N-UV[2/] | NO UV | UV | N-UV[3/] |
| NPV + $H_2O$ | 76[4/] | 26 | 9 | 27[5/] | 3 | 0 |
| NPV + ProTec® 1 | 65 | 56 | 51 | 30 | 7 | 4 |
| NPV + ProTec® 2 | 89 | 68 | 62 | 31 | 16 | 3 |
| NPV-Std.(Shade)® | 73 | 73 | 74 | 5 | 20 | 10 |
| Check | 2 | | | 1 | | |

1/ Exposed to sunlamp and blacklight blue side by side for 15 minutes. Plants were 7.6 cm below foliage.

2/ Plants exposed to natural sunlight for 2 hours between 1100-1300 hours.

3/ Plants exposed to natural sunlight for 6 hours between 1030-1430 hours.

4/ Percent mortality of exposed test insects 16 days after larvae had disposed of treated foliage.

5/ Percent mortality of exposed test insects 10 days after larvae had disposed of treated foliage.

Results indicate that a 2 hour exposure to natural sunlight in early May has equivalent or greater effects on nucleopolyhedrosis virus than does a 15 minute exposure under a sunlamp and blacklight blue lamp when used side by side. Data also indicate that the standard formulation (Shade®) is somewhat more effective than any of the Pro Tec® formulations. Until research indicates that a more effective lighting system is available to simulate sunlight in the laboratory we will use a Westinghouse FS 40 sunlamp and blacklight blue lamp side by side, 7.6 cm above treated media. The Mylar Type A polyester film (DuPont) will also be used to filter out radiation below 315 nanometers.

Project Number: GM 8.1.2
Project Title: Field Studies of Insecticides and Microbials Applied by Aircraft.
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: W. H. McLane, R. G. Reeves

One of the main functions of the pesticide section of this laboratory is to field test toxic materials against the gypsy moth. Such materials may be registered or experimental. For example, it may be desireable to establish field efficacy on a new material that has already cleared rigorous laboratory testing. Alternatively, work on a registered compound may be warranted to improve a formulation, lower rate and/or dosage or lower the cost of application.

Aerial application work during 1978 was conducted on two registered insecticides, Dimilin® and Sevin® . Dimilin is presently registered for use at 0.03-0.06 lb AI/a and Sevin 4-oil at 1.0 lb AI/a. Previous experiments with Sevin 4-oil (Ford et al, 1975) suggested the material was effective against gypsy moth larvae at dosages well below the registered rate. Therefore tests were originally scheduled using Sevin 4-oil at 0.75 lb/AI/a and 1.0 lb AI/a. However, the lower dosage work was not conducted due to a limited number of suitable spray plots. Dimilin has also been demonstrated effective at lower dosages and rates in the past (Herbaugh and McLane, 1975-1976). Efficacy of Dimilin 25W was therefore tested at a series of dosages and rates lower than label recommendations.

The two main objectives of the program were to establish the most efficient dosages and rates of Dimilin applied by aircraft for regulatory treatments and to determine the dosage, rate and number of applications to use when treating isolated gypsy moth infestations for eradication.

Table 1. Materials, dosages, rates and number of applications used for aerial application in 1978 field tests.(Clinton and Lycoming Co, PA).

| Material | Dosage (lb/a) | Rate (gal/a) | Number Applications | Number Plots |
|---|---|---|---|---|
| Dimilin 25W | 0.0075 | 0.5 | 2 | 3 |
| Dimilin 25W | 0.015 | 0.5 | 2 | 3 |
| Dimilin 25W | 0.015 | 0.25 | 2 | 3 |
| Dimilin 25W | 0.015 | 0.25 | 1 | 6 |
| Dimilin 25W | 0.03 | 0.25 | 1 | 6 |
| Dimilin 25W | 0.03 | 0.25 1/ | 1 | 6 |
| Sevin 4-oil | 1.0 | 40 2/ | 1 | 3 |
| Checks | - | - | - | 6 |

1/ mixed with 50% Savol oil

2/ oz/a

Thirty 20 ha spray plots and 6 check plots were established in Clinton and Lycoming Counties Pennsylvania during the early spring of 1978. Twenty prism points were established on 4 parallel lines 50 m apart within the central 4 ha of each plot. Distance between the 5 sample points on each line was 67 m. Pre spray egg mass counts were made at each point using the prism technique (Wilson and Fontaine, 1978). In addition to the prism point egg mass survey, each plot receiving more than one application had 200 burlap bands, approximately 23 cm wide, attached to host trees within the center 4 ha of the plot. Burlaps were for monitoring larvae, pupae and new egg masses within the sprayed plots. Two check plots also had burlap around 200 trees. All burlaps were checked 2 times for larvae, once for pupae and will be checked in the fall for egg masses.

Treatment of plots was conducted May 27-June 6, 1978. The application schedule was such that material was applied when the majority of larvae were in the late second instar and oak foliage was 50-75% expanded.

A Cessna® Ag Truck aircraft equipped with a hydraulic driven impeller pump and a conventional spray system was used to apply all material. The aircraft dispersed the material in 18 m swaths at 120 mph as near to tree top level as possible. Ground wind velocity did not exceed 3 mph during any treatment.

Table 2. Aircraft spray system data. (Clinton and Lycoming Co. PA, 1978).

| Material | Dosage (lb AI/a) | Rate (gal/a) | No. Flat Fan Nozzles Used | Orifice |
|---|---|---|---|---|
| Dimilin 25W | 0.0075 | 0.5 | 18 | 8004 |
| Dimilin 25W | 0.015 | 0.5 | 18 | 8004 |
| Dimilin 25W | 0.015 | 0.25 | 18 | 8002 |
| Dimilin 25W | 0.015 | 0.25 | 18 | 8002 |
| Dimilin 25W | 0.03 | 0.25 | 18 | 8002 |
| Dimilin 25W | 0.03 | 0.25 1/ | 15 | 8002 |
| Sevin 4-oil | 1.0 | 40 2/ | 15 | 8003 |

1/ mixed with 50% Savol oil

2/ oz/a

Dimilin formulations were mixed and transferred into the aircraft with conventional equipment (mixing tank, pumps, bases, measuring devices and necessary safety equipment). Sevin 4-oil was mixed with kerosene directly in the aircraft spray tank. All mixing and spraying equipment was thoroughly cleaned between each treatment to avoid adulteration. Radio communications were not maintained between the ground crew and aircraft because of a faulty radio in the aircraft. However, the pilot had no trouble locating the treatment blocks. Large yellow balloons filled with helium were used to mark each corner of all spray plots.

following treatments a number of readings were made under burlaps to monitor larval populations. Burlap bands were only used in the double application. Once pupation was completed, burlaps were again checked to record number of pupae per plot. During the fall post spray egg mass survey, burlaps will again be checked for egg masses.

Table 3. Pre-spray egg mass and post spray larval and pupal counts in plots receiving two applications of Dimilin. Values are the average of 3 replicates. (Clinton and Lycoming Co. PA, 1978).

| | | | Post-Spray Burlap Readings 1/ | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pre-Spray | 19 Days | | 40 Days | | 64 Days | |
| Dosage (lb/a) | Rate (gal/a) | Egg Masses Per Acre | Larvae 2/ | Pupae 3/ | Larvae | Pupae | Larvae | Pupae |
| .0075 | 0.5 | 796 | .7 | 0 | .3 | .1 | 0 | 02 |
| .015 | 0.5 | 1509 | 28.8 | 0 | 2.5 | .8 | 0 | 0 |
| .015 | .025 | 852 | 31.3 | 0 | .2 | .2 | 0 | 0 |
| Check (2 replicates) | | 292 | 79.5 | 0 | 108.5 | 13 | .03 | .8 |

1/ Readings were made at the indicated number of days following the 2nd application.

2/ Average number of larvae under 10 burlaps at each of twenty prism points.

3/ Average number of pupae under 10 burlaps at each of twenty prism points.

Table 4. Pre-spray egg mass density of plots treated with one application of Dimilin or Sevin 4-oil. (Clinton and Lycoming Co. PA, 1978).

| Material | Dosage (lb/a) | Rate (gal/a) | No. plots | Egg masses/a |
|---|---|---|---|---|
| Dimilin | 0.01 | 0.25 | 6 | 754 |
| Dimilin | 0.03 | 0.25 | 6 | 960 |
| Dimilin (50% Savol) | 0.03 | 0.25 | 6 | 722 |
| Sevin 4-oil | 1.0 | 40 oz. | 3 | 799 |
| Check | | | 4 | 463 |

Post spray egg mass counts will be made in late October and all data will be tabulated in final form in the October 1, 1978 - March 31, 1979 laboratory report.

References Cited:

Ford, R. P., P. Snowden and W. E. Andrews. 1975. Evaluation of the effectiveness of 8 ounces of Trichlorfan and Carbaryl in suppression of gypsy moth populations, U. S. Forest Service Report P-75-4, File 5240, pp. 1-20.

New York State Department of Environmental Conservation 1970. Gypsy Moth Trials Testing Sevin 4-oil. New York State Department of Environmental Conservation Report No. 03891, pp. 1-21.

Herbaugh, L. L., W. H. McLane. 1975-1976. 1975 field evaluation of candidate insecticides for gypsy moth control. APHIS, Laboratory Report of September 1975- March, 1976.

Wilson, R. W., G. A. Fountaine. 1978. Gypsy Moth egg mass sampling with fixed and variable radius plots, USDA, Agriculture Handbook No. 523, pp. 1-46.

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides Against the Gypsy Moth
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: Winfred H. McLane, J. A. Finney

The primary objective of this laboratory screening project is to collect mortality data on registered and experimental compounds potentially useful against the gypsy moth, evaluating this data and selecting suitable materials for field testing. All of the tests herein reported were conducted by standard techniques with oak seedlings and 2nd instar larvae unless otherwise stated.

In anticipation of possible field experiments during 1979, laboratory tests were conducted with the new growth regulator SIR 8514, a Chemagro product.

Three formulations of SIR 8514 were tested using 3 dosages.

Table 1. Test Results When 3 Formulations of SIR 8514 were Tested Against 2nd Instar Gypsy Moth Larvae.

| Material | Dosage | Formulation | Percent mortality after 9 days |
|---|---|---|---|
| SIR 8514 | 0.03 lbs AI/g/a | 4 Flowable | 98 |
| SIR 8514 | " | 25 W/P (Bayer) | 97 |
| " | " | 25 W/P (Chemagro) | 100 |
| Dimilin® | " | 25 W/P | 98 |
| SIR 8514 | 0.01 lbs AI/g/a | 4 Flowable | 100 |
| " | " | 25 W/P (Bayer) | 100 |
| " | " | 25 W/P (Chemagro) | 100 |
| Dimilin® | " | 25 W/P | 100 |
| SIR 8514 | 0.004 lbs AI/g/a | 4 Flowable | 91 |
| " | " | 25 W/P (Bayer) | 90 |
| " | " | 25 W/P (Chemagro) | 94 |
| Dimilin® | " | 25 W/P | 97 |
| Check | - | - | 0 |

A test was conducted to determine the contact toxicity of SIR 8514. Petri dishes were sprayed in a seedling spray chamber a 0.01 lbs AI/gal/a using 3 formulations of SIR 8514 and one formulation of Dimilin®. Directly after treatment 20 second instar gypsy moth larvae were allowed to walk on treated surfaces for 1 minute. Another group was allowed on treated surfaces for 5 minutes. After exposure, test insects were held on untreated diet for 16 days in an environmental chamber at 26° C and relative humidity of 60 percent. Mortality readings were made periodically over those 16 days. All treatments were replicated 5 times.

Table 2. Topical Test Results with 3 Formulations of SIR 8514 and 1 Formulation of Dimilin®.

| Material | Dosage per dish | Formulation | Exposure Time | % Mortality after 16 days |
|---|---|---|---|---|
| SIR 8514 | 0.01 lbs. AI/g/a | 25 W/P (Bayer) | 1 | 45 |
| " | " | " | 5 | 95 |
| " | " | 25 W/P (Chemagro) | 1 | 5 |
| " | " | " | 5 | 47 |
| " | " | 4 Flowable | 1 | 13 |
| " | " | " | 5 | 82 |
| Dimilin® | " | 25 W/P | 1 | 15 |
| " | " | " | 5 | 87 |
| Check | - | - | - | 0 |

An additional test was conducted to determine the contact toxicity of SIR 8514 when it was applied directly to the test insects using an applicator syringe. One-hundred larvae were exposed to each dosage with acetone used as a solvent. Table 3 results are also reported under project number GM 8.1.0.

Table 3. LD 50 of SIR 8514 When Applied Toxically to 4 Instars of Gypsy Moth Larvae.

| Instar | Formulation | LD 50 | Upper Limit | Lower Limit |
|---|---|---|---|---|
| II | Chemagro | 0.0060 ug/ul | 0.0082 ug/ul | 0.00396 ug/ul |
| II | Bayer | 0.0089 ug/ul | 0.00113 ug/ul | 0.00667 ug/ul |
| III | Chemagro | 0.0260 ug/ul | 0.03036 ug/ul | 0.02207 ug/ul |
| III | Bayer | 0.0366 ug/ul | 0.04261 ug/ul | 0.03158 ug/ul |
| IV | Chemagro | 0.0984 ug/ul | 0.0118 ug/ul | 0.08109 ug/ul |
| IV | Bayer | 0.1209 ug/ul | 0.1383 ug/ul | 0.1053 ug/ul |
| V | Chemagro | 9.3020 ug/ul | 38.11 ug/ul | 4.378 ug/ul |
| V | Bayer | 2.8350 ug/ul | 6.063 ug/ul | 1.745 ug/ul |

Weathering tests were also conducted using SIR 8514 and Dimilin®.

Table 4. Results of Weathering Tests Using SIR 8514 and Dimilin®.

| Material | Dosage | Formulation | Weather | % Mortality after 7 days |
|---|---|---|---|---|
| SIR 8514 | 0.015 lbs AI/g/a | 4 Flowable | 1" | 99 |
| " | " | 4 Flowable | 3" | 97 |
| " | " | 25 W/P(Chemagro) | 1" | 100 |
| " | " | " | 3" | 100 |
| " | " | 25 W/P(Bayer) | 1" | 100 |
| " | " | " | 3" | 100 |
| Dimilin® | 0.015 lbs AI/g/a | 25 W/P | 1" | 99 |
| " | " | " | 3" | 97 |
| Check | | | | |

All experimental data to date indicate that the Bayer and Chemagro formulations of SIR 8514 perform as well as Dimilin® when used against gypsy moth larvae in the laboratory and weathering characteristics are virtually the same.

An application of pesticide on foliage is normally of little value if the material will not cling to the target area when exposed to adverse weather conditions. This is especially true when the spray material used is a stomach poison and a one or two week residue is needed.

Some spray formulations have built in stickers and others are just natural adhesives. However, when using some materials in the gypsy moth program, stickers have to be added to the formulation being used. This could be a formulation of insecticide, gypchek or pheromone. It is important that the best possible stickers, one which are compatible with the active material being applied be identified.

Table 5 gives test results of five stickers when tested in an Imidan® formulation and exposed to 7.6 cm of rainfall.

Table 5. Laboratory Test Results of Five Stickers Exposed to 7.6 cm of Rainfall.

| Material | Sticker | Percent Mortality 1/ |
|---|---|---|
| Imidan® 50 W/P | No Sticker | 32 |
| " | 3% Pinolene® (New Film 17) | 89 |
| " | 3% Plyac® (Hodgkins) | 96 |
| " | 3% RA-1645® (Monsanto) | 94 |
| " | 3% Chevron Spray Sticker® | 95 |
| " | 3% Rhoplex® | 90 |
| Check | - | 0 |

1/ Mortality to 2nd instar gypsy moth larvae after 6 days.

When used for regulatory treatments, cost can be an important factor when selecting the right sticker. Table 6 gives 1978 retail prices on some of the more effective stickers.

Table 6. Retail Prices of Stickers Tested.

| Material | Company | Retail Price per Gallon | | |
|---|---|---|---|---|
| | | 1 Gal + | 5 Gal + | 55 Gal + |
| Chevron Sticker® | Chevron | $ 4.50 | - | - |
| Pinolene Nufilm 17® | Miller | $19.80 | $19.30 | $19.00 |
| Plyac® | Hopkins | $10.35 | $ 9.40 | $ 8.85 |
| RA-1645® | Monsanto | $ 4.27 | - | - |
| Rhoplex® B15 | Rohm Haas | $ 3.53 | - | - |

Target NL,® Super Target NL with Rhoplex® and Rhoplex® B15 were tested as sticking materials with Imidan®.

Table 7. Laboratory Test Results of 3 Stickers Exposed to Various Amounts of Rainfall.

| Material | Dosage | Sticker | Weather | % Mortality after 7 days |
|---|---|---|---|---|
| Imidan® | 1.0 lbs AI/g/a | 1% Target NL® | 3" | 77 |
| " | 1.0 lbs AI/g/a | 3% Target NL® | 3" | 77 |
| " | " | 5% Target NL® | 3" | 74 |
| " | " | 1% Super Target NL | 3" | 64 |
| " | " | 3% Super Target NL | 3" | 56 |
| " | " | 5% Super Target NL | 3" | 28 |
| " | " | 1% Rhoplex® 15B | 3" | 97 |
| " | " | 3% Rhoplex® 15B | 3" | 100 |
| " | " | 5% Rhoplex® 15B | 3" | 94 |
| Check | - | - | - | 0 |

Table 8. Laboratory Test Results When Idinfos was Screened Against 2nd Instar Gypsy Moth Larvae.

| Material | Company | Dosage | Percent Mortality After 5 days |
|---|---|---|---|
| Idinfos | Montedison | 1.0 lbs AI/g/a | 100 |
| " | " | 0.5 lbs AI/g/a | 100 |
| " | " | 0.25 lbs AI/g/a | 78 |
| " | " | 0.12 lbs AI/g/a | 75 |
| Check | - | - | 0 |

Savol® a crop oil used as a carrier for pesticides was tested on oak seedlings to see if it produced any toxic or repellent effects.

Table 9. Laboratory Test Results Using Savol® Against 2nd Instar Gypsy Moth Larvae.

| Material | Dosage | Percent Mortality after 4 days |
|---|---|---|
| Savol® | 100% at 1.0 lb/g/a | 0 |
| " | 50% at 1.0 lb/g/a | 1 |

Table 9. (Cont'd).

| Material | Dosage | Percent Mortality after 4 days |
|---|---|---|
| Savol® | 25% at 1.0 lb/g/a | 0 |
| " | 10% at 1.0 lb/g/a | 0 |
| Check | - | 0 |

Phytotoxicity or feeding repellency was not observed during the test.

Orthene® 75S, Dimilin® 25 W/P, Sevin® 80S, Sevin® 4 oil, Dylox® 80S and Dylox® 1.5 oil were tested at their registered dosages to compare swiftness of activity.

Table 10. Test Results When 2nd Instar Larvae were Exposed to Seedlings Treated with Insecticides Registered for Gypsy Moth Control.

| | | PERCENT MORTALITY | | | | |
|---|---|---|---|---|---|---|
| Material | Dosage | Day I | Day II | Day III | Day IV | Day V |
| Dimilin® | 0.03 lbs AI/g/a | 0 | 0 | 29 | 65 | 99 |
| Dylox® 80S | 1.0 lbs AI/g/a | 57 | 80 | 93 | 100 | |
| Dylox® 1.5 oil | 1.0 lbs AI/5.3 pt/a | 92 | 100 | | | |
| Orthene® 75S | 0.75 lbs AI/g/a | 92 | 99 | 100 | | |
| Sevin® 80S | 1.0 lbs AI/g/a | 35 | 67 | 83 | 98 | 100 |
| Sevin® 4 oil | 1.0 lbs AI/40 oz/a | 35 | 75 | 92 | 100 | |
| Control | - | 0 | 0 | 0 | 0 | 0 |

A series of residue tests were started using the materials presently registered for use against the gypsy moth. Forty seedlings were sprayed with each material using the recommended field dosage. Plants were then held in an environmental chamber at 26° C and a relative humidity of 60 percent. Each week 2nd instar larvae were introduced to 5 treated plants and 1 control.

Table 11. Residue Tests Results Using Registered Materials Against 2nd Instar Gypsy Moth Larvae

| Material | Dosage | PERCENT MORTALITY AFTER DAYS AGED | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 7 | 14 | 21 | 28 | 35 | 42 |
| Dimilin® 25W | 0.03 lbs AI/g/a | 83 1/ | 94 | 96 | 84 | | | |
| Dylox® 80S | | | | | | | | |
| Dylox® 1.5 oil | 1.0 lbs AI/5.3 pt/a | 100 2/ | 100 | 75 | 96 | | | |
| Orthene® 75S | 1.0 lbs AI/.5 g/a | 100 3/ | 96 | 100 | 93 | 97 | | |
| Sevin® 80S | 1.0 lbs AI/g/a | 98 4/ | 100 | 98 | 88 | 84 | 80 | 99 |
| Sevin® 4 oil | 1.0 lbs AI/40 oz/a | 86 5/ | 89 | 94 | 88 | 86 | 90 | 77 |
| Control | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

1/ Mortality readings were made 8 days after larvae were introduced onto plants.

2/ Mortality readings were made 5 days after larvae were introduced onto plants.

3/ Mortality readings were made 3 days after larvae were introduced onto plants.

4/ Mortality readings were made 3 days after larvae were introduced onto plants.

5/ Mortality readings were made 4 days after larvae were introduced onto plants.

Project Number: GM 8.1.4
Project Title: Spray Deposition Patterns of Water and Oil Formulations Applied by Aircraft
Report Period: April 1, 1978 - September 30, 1978
Report Type: Final
Project Leaders: J. A. Finney, W. H. McLane


## Abstract:

A series of oil, water, and oil + water + emulsifier formulations were sprayed with a Cessna® Ag-Truck over Kromekote spray cards. The rate of application was 1 and 2 pints per acre. An analysis of spray cards was then made by recording droplet size and density (Makaymiuk, 1964). Data indicate that when water or oil formulations are sprayed from an aircraft at 1 pint per acre, little material reaches the target. However, when a 1:1 mixture of oil and water were mixed with an emulsifier, delivery improved.


## Introduction:

Until recent years most insecticide formulations have been applied at the rate of 1 gallon or more per acre to ensure effective coverage. This practice results in increased cost of material and ferry time. It is presently possible to aerially spray as little as 8-16 ounces of formulation per acre. However, controversy remains over what material is the best carrier for applying low volume formulations. At low volume application, the material must be broken up into a large number of droplets. However, small droplets of oil formulations tend to drift excessively. In hot and dry weather, most of the small droplets of a water formulation will not reach the target insect because of evaporation. The main objectives of this study were to determine and compare the spray deposition pattern of water and oil formulations applied at the rate of 1 pint and 1 quart per acre.

## Methods:

During April 3-7, various water and oil formulations were aerially applied to spray cards at the USDA Airport in Beltsville, Maryland. A summarization of the experimental parameters is given in Table 1.

Table 1. Formulations, nozzle types and application rates employed to evaluate spray deposition patterns. Spray system was pressurized to 40 PSI in all applications.

| Formulation | Flat Fan Nozzle | Rate | # Replications |
|---|---|---|---|
| Kerosene | 80015 | Pt/A | 3 |
| Kerosene | 8003 | Qt/A | 4 |
| 50% $H_2O$ + 50% Kerosene + TH-46818 Emilsifier | 8003 | Qt/A | 2 |
| 50% $H_2O$ + 50% Kerosene + *TH-46818 + .01 lb.* Dimilin® 25 WP | 8003 | Qt/A | 2 |
| 50% Savol® + 50 $H_2O$ + .01 lb. Dimilin 25 WP | 8003 | Qt/A | 4 |
| 75% Savol + 25% $H_2O$ | 8003 | Qt/A | 2 |
| 75% Savol + 25% $H_2O$ + .01 lb. Dimilin 25WP | 8003 | Qt/A | 2 |
| $H_2O$ + Rhodamine® B | 80015 | Pt/A | 4 |
| $H_2O$ + Rhodamine® B | 8003 | Qt/A | 4 |

For each run, the Ag-Truck flew at approximately 120 mph and at an altitude of 10-15 feet. It flew over the marked center of the designated spray area which ran parallel to an east-west runway. Before each run, 30 "Kromekote Cover" spray cards attached to flat aluminum plates were laid on the runway at 5 foot intervals. When we observed that the material was drifting beyond the designated area, spray cards were laid at 10 foot intervals beyond the original designated area until the swath encompassed 350 feet. In total, 31 cards were used for a spray area of 150 feet, and 51 cards were used for a spray area of 350 feet. The cards were collected after 5-10 minutes which allowed the spray time to dry. The D-Max method of estimating volume median was used to determine the average droplet size (Maksymiuk, 1964). A plastic template 5" x 8" which had 1 $CM^2$ removed from the center was used to estimate the number of drops/$CM^2$.

Results:

Test results of water and oil formulations applied using the previously described method are summarized in the following tables:

Table 2. Data of spray deposition, pattern of water and oil formulations tested at Beltsville, MD, 1978.

| Formulation | Rate | Replication | Average Droplet Size (u) | Droplet Size Range (u) | Average No. of Drops/$CM^2$ | Average No. of Drops/$CM^2$ Range |
|---|---|---|---|---|---|---|
| Kerosene | Pt/A | A | 70.06 1/ | 47-103 | 4 | 0-10 |
| | | B | 83.35 | 47-103 | 1.29 | 0-6 |
| | | C | 75.57 | 47-112 | 1.85 | 0-4 |
| | | Average | 76.32 | 47-106 | 2.38 | 0-6.6 |
| Kerosene | Qt/A | A | 79.82 | 47-103 | 2.5 | 0-6 |
| | | B | 62.92 | 40-84 | 6.9 | 0-14 |
| | | C | 81.11 | 56-93 | 1.94 | 0-5 |
| | | D | 86.56 | 75-93 | 2.1 | 0-5 |
| | | Average | 77.60 | 54.4-93.2 | 3.36 | 0-7.5 |
| 50% $H_2O$ + 50% Kerosene + T-H 46818 Emulsifier | Qt/A | A | 87.75 | 75-112 | 6.8 | 0-18 |
| | | B | 80.76 | 18-140 | 4.6 | 0-16 |
| | | Average | 84.25 | 46.5-126 | 5.7 | 0-17 |

Table 2. (Cont'd)

| Formulation | Rate | Replication | Average Droplet Size (u) | Droplet Size Range (u) | Average No. of Drops/$CM^2$ | Average No. of Drops/$CM^2$ Range |
|---|---|---|---|---|---|---|
| 50% $H_2O$ + 50%[2/] Kerosene + T-H 46818 Emulsifier + .01 lb. Dimilin 25 WP | Qt/A | A | 112.0 | 75-140 | 6.1 | 0-14 |
| | | B | 99.38 | 28-140 | 2.5 | 0-6 |
| | | Average | 105.69 | 51.5-140 | 4.3 | 0-10 |
| 50% Savol + 50% $H_2O$ + .01 lb. Dimilin 25 WP | Qt/A | A | 141.68 | 56-195 | 1.1 | 0-9 |
| | | B | 134.45 | 46-213 | 1.9 | 0-5 |
| | | C | 124.52 | 46-186 | 2.2 | 0-8 |
| | | D | 137.60 | 102-186 | 1.4 | 0-5 |
| | | Average | 134.56 | 62.5-195 | 1.6 | 0-6.7 |
| 75% Savol + 25% $H_2O$ | Qt/A | A | 157.36 | 58-286 | 3.5 | 0-17 |
| | | B | 192.89 | 92-263 | 1.6 | 0-4 |
| | | Average | 175.13 | 75-274.5 | 2.5 | 0-10.5 |
| 75% Savol + 25% $H_2O$ + .01 lb. | Qt/A | A | 207.32 | 115-252 | 2.5 | 0-10 |
| | | B | 158.92 | 57-275 | 5.9 | 0-13 |
| | | Average | 183.12 | 86-263 | 4.2 | 0-11.5 |
| $H_2O$ + Rhodamine B | Pt/A | A | 85.83 | 36-132 | 1.080 | 0-5 |
| | | B | 93.85 | 47-132 | .615 | 0-4 |
| | | C | 88.70 | 53-119 | .347 | 0-2 |
| | | D | 89.18 | 53-145 | .545 | 0-3 |
| | | Average | 89.39 | 49.7-132 | .650 | 0-3.5 |
| $H_2O$ + Rhodamine | Qt/A | A | 107.58 | 53-159 | 1.400 | 0-8 |
| | | B | 104.83 | 66-185 | .409 | 0-3 |
| | | C | 110.91 | 66-145 | .590 | 0-2 |
| | | D | 105.69 | 53-132 | 1.920 | 0-5 |
| | | Average | 107.25 | 60-155 | 1.080 | 0-4.5 |

1/ Spread factors used were from USFS publication; (Spread factor and linear relationship between spot and drop diameter for microbial insecticides).

2/ Some formulations were tested with the active material Dimilin® present.

Table 3. Deposition of water and oil formulations within designated 150' swath at Beltsville, MD - 1978.

| Formulation | Rate | Replication | Visual Observation of Spray Deposition + Drift | Wind Velocity (mph) | No. Ft. Within Swath |
|---|---|---|---|---|---|
| Kerosene | Pt/A | A | No drift | | 150 |
| | | B | Off to left | 2-3 | 45 |
| | | C | Off to left | 2-3 | 55 |
| | | | | Average | 81 |
| Kerosene | Qt/A | A | | | 40 |
| | | B | | | 125 |
| | | C | Off to left | | 40 |
| | | D | Off to left | | 40 |
| | | | | Average | 74 |
| 50% $H_2O$ + 50% Kerosene + T-H 46818 Emulsifier | Qt/A | A | Off to left | 0-1 | 90 |
| | | B | No drift | | 150 |
| | | | | Average | 120 |
| 50% $H_2O$ + 50% Kerosene + T-H 46818 + .01 lb. Dimilin | Qt/A | A | Off to left | 0-1 | 95 |
| | | B | | | 135 |
| | | | | Average | 115 |

Table 3. (Cont').

| Formulation | Rate | Replication | Visual Observation of Spray Deposition + Drift | Wind Velocity (mph) | No. Ft. Within Swath |
|---|---|---|---|---|---|
| 25WP 50% Savol + | Qt/A | A | No drift | | 150 |
| 50% $H_2O$ + .01 lb. | | B | Off to right | 5-7 | 85 |
| Dimilin 25WP | | C | Off to right | 5-7 | 85 |
| | | D | Plane off to left | | 80 |
| | | | | Average | 100 |
| 75% Savol + | Qt/A | A | | 0-1 | 120 |
| 25% $H_2O$ | | B | Off to left | | 75 |
| | | | | Average | 98 |
| 75% Savol + | Qt/A | A | Off to left | 0-1 | 90 |
| 25% $H_2O$ + .01 lb. | | B | Off to left | 0-1 | 110 |
| Dimilin 25WP | | | | | |
| | | | | Average | 100 |
| $H_2O$ + Rhodamine | Pt/A | A | Off to right | 3-5 | 65 |
| B Dye | | B | Off to right | 2-4 | 85 |
| | | C | Off to right | 3-5 | 85 |
| | | D | Off to right | 3-5 | 85 |
| | | | | Average | 79 |
| $H_2O$ + Rhodamine B | Qt/A | A | Off to right | 3-5 | 80 |
| Dye | | B | Off to right | 5-8 | 60 |
| | | C | Off to right | 5-8 | 55 |
| | | D | Within card | 3-5 | 100 |
| | | | | Average | 76 |

Summary:

A 100% water formulation gave the poorest spray pattern of all the formulations tested. Because of excessive drift, little of the material reached the ground. With the 100% oil formulation, the material was broken up into finer droplets and therefore, more reached the cards; however, drifting did occur.

The results of tests using a 1:1 formulation of kerosene and water point out that when the 2 materials are combined with an emulsifier, the spray deposition of the material is improved. Brake-up of the formulation is improved and coverage is superior to that obtained when the materials are used individually. The results of this study show that a formulation using a combination of water and oil, plus an emulsifier, results in a more desired spray coverage of small droplets in higher numbers.

Project Number: GM 8.1.5
Project Title: Field Studies of Insecticides and Microbials Applied by Ground Equipment
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: Winfred H. McLane, R. G. Reeves

United States Department of Agriculture regulatory personnel treat thousands of acres of woodlands each year in their fight against the destructive gypsy moth. The majority of the areas are sprayed with modern agricultural aircraft. However, where treatment acreage is small, ground equipment can sometimes be used. For example, areas in and adjacent to campgrounds, trailer parks, water reservoirs and crop land, usually it is advantageous to use ground equipment.

It is important that we continue to search for new materials which can be effectively used with ground equipment. Also, it is equally important that we improve the materials and techniques which are presently available.

Sevin® 80S is the most common insecticide used against gypsy moth larvae with ground application equipment. The present gypsy moth control manual calls for the use of 11.34 kg. (25 lbs) in 189.25 liters (50 gallons) of water + a sticker. Operators use a sufficient volume of the mixture to obtain good coverage (ca. 5 gal/acre). Actual insecticide used is about 2.0 lbs/acre.

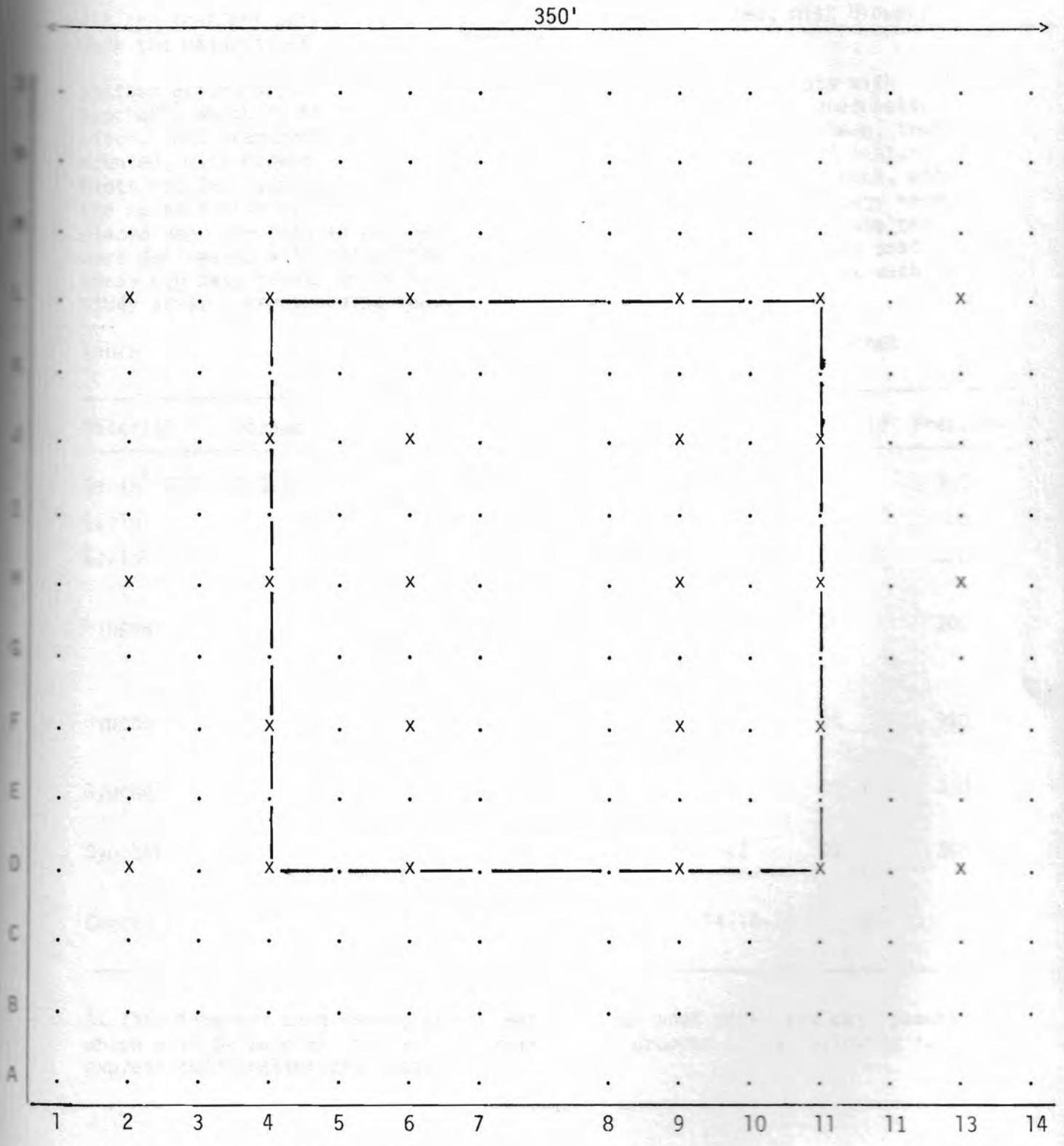
Although ground applications are normally made when spray conditions are suitable, questions of how far the effective spray deposits are distributed from the sprayer have been asked. Insect migration back into treated areas has also concerned regulatory officials. Often spray crews must re-treat heavily infested areas because one application is not effective.

Three 1.1 (ha) spray plots were established in Clinton and Lycoming counties, Pennsylvania for treatment with Sevin® 80S using a truck mounted mist blower. The objective was to determine the extent of larval migration into a .4 (ha) plot and determine deposition and disappearance of the insecticide. Plots were located on state land with predominantly oak trees and a healthy gypsy moth population of 50-500 egg mass/acre. A road ran through the center of each plot, and only the center 0.4 (ha) of each plot was treated. (Figure 1).

Plots were evaluated using a grid system (Figure 1) with sample points 25 feet (7.6 m) apart in each direction except on the road. The first set of points were 25 feet (7.6 m) in from the road on each side. Points were established using a compass and were marked with white surveyors tape except around the boundary of the .4 (ha) spray area which was marked with orange tape. At each point, pre and post spray egg mass counts were made within a 8.33 foot (2.5 m) radius of the center point. One live oak tree was banded with burlap at each of the 210 sample points. All burlap bands were checked for larvae, pupae and egg masses.

Foliage samples were collected at or near established points throughout the plot (Figure 1). These samples are being analyzed by gas chromatography, and a full report will appear in the next laboratory report.

Figure 1.




. - 1 Burlap per sample point

x - Foliage sample points for gas chromatography

N

All applications were made using a John Bean, truck mounted, mist blower, when the majority of larvae were 2nd and 3rd instar.

Another ground application involved spraying sixteen 0.4 ha plots with Gypchek®, which is the registered name for gypsy moth nucleopolyhedrosis visur. All treatments were made in late afternoon using a John Bean, truck mounted, mist blower when the majority of larvae were 2nd and 3rd instar. Plots had two subplots or sampling units totaling 33 square feet each, with one on each side of the road. Orange ribbons and identification tags were placed near the road at each end of the 200 foot plots. The two subplots were delineated with white ribbon and identification tags. Pre and post spray egg mass counts as well as defoliation estimates were made at each study area. Corresponding readings were made in all check plots.

Table 1. Data pertaining to the application of Sevin® 80S and Gypchek Using Ground Equipment.

| Material | Dosage | Date | No.App. | Plot | Nozzle | Pressure |
|---|---|---|---|---|---|---|
| Sevin® 80S | 2.0 lbs AI/5g/a | 6/2/78 | 1 | A | D2 | 300 |
| Sevin® 80S | 2.0 lbs AI/5g/a | 6/2/78 | 1 | B | D2 | 300 |
| Sevin® 80S | 2.0 lbs AI/5g/a | 6/2/78 | 1 | C | D2 | 300 |
| Gypchek | 2x10"PIBS/a | 5/30/78<br>6/6/78 | 2 | 1-3-7 | D2 | 300 |
| Gypchek | 5x10" PIBS/a | 5/30/78<br>6/6/78 | 2 | 2-4-6 | D2 | 300 |
| Gypchek | 5x10" PIBS/a | 6/2/78 | 1 | 5-8-10 | D2 | 300 |
| Gypchek | 2x10" PIBS/a | 6/4/78 | 1 | 9-11-13 | D2 | 300 |
| Checks | - | - | - | 12-14-15-16 | - | - |

At this time all data have been collected except post spray egg mass counts which will be made as soon as most foliage has dropped. The following tables express this preliminary data.

Table 2. Egg mass counts and defoliation estimates in Gypchek® treated plots.

| Dosage-rate | No.App. | Plot | Pre Spray E/M/A | Post Spray E/M/A | Defoliation PRE | Defoliation POST |
|---|---|---|---|---|---|---|
| 2x10" PIBS/5gal/a | 2 | 1 | 480 1/ | | 0-5% | 0-5% |
| | | 3 | 960 | | 0-5% | 0-5% |
| | | 7 | 660 | | 0-5% | 0-5% |
| 5x10" PIBS/5gal/a | 2 | 2 | 420 | | 0-5% | 0-5% |
| | | 4 | 980 | | 0-5% | 0-5% |
| | | 6 | 580 | | 0-5% | 0-5% |
| 2x10" PIBS/5gal/a | 1 | 9 | 1,220 | | 0-5% | 0-5% |
| | | 11 | 740 | | 0-5% | 0-5% |
| | | 13 | 740 | | 0-5% | 0-5% |
| 5x10" PIBS/5gal/a | 1 | 5 | 400 | | 0-5% | 0-5% |
| | | 8 | 640 | | 0-5% | 0-5% |
| | | 10 | 1,320 | | 0-5% | 0-5% |
| Checks | - | 12 | 800 | | 0-5% | 0-5% |
| | | 14 | 240 | | 0-5% | 0-5% |
| | | 15 | 660 | | 0-5% | 0-5% |
| | | 16 | 400 | | 0-5% | 0-5% |

1/ Based on the average of two 1/40th acre subplots, one on each side of the road.

Table 3. Average pre-spray E/M for Sevin ground plots.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 11 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 67 | 200 | 0 | 133 | 733 | 200 | 67 | 133 | 133 | 333 | 467 | 400 | 0 | 133 |
| | 400 | 333 | 333 | 0 | 0 | 0 | 867 | 200 | 267 | 400 | 333 | 333 | 400 | 200 |
| | 0 | 800 | 133 | 67 | 0 | 200 | 0 | 200 | 67 | 133 | 867 | 67 | 467 | 333 |
| | 0 | 200 | 400 | 200 | 200 | 533 | 600 | 533 | 200 | 0 | 67 | 133 | 267 | 0 |
| | 200 | 0 | 400 | 933 | 600 | 133 | 267 | 333 | 133 | 67 | 133 | 533 | 400 | 1000 |
| | 133 | 600 | 0 | 333 | 400 | 133 | 67 | 267 | 400 | 267 | 67 | 67 | 333 | 67 |
| | 67 | 600 | 267 | 0 | 400 | 67 | 467 | 67 | 67 | 133 | 467 | 0 | 267 | 0 |
| | 400 | 67 | 400 | 267 | 67 | 333 | 800 | 67 | 67 | 200 | 67 | 200 | 600 | 267 |
| | 133 | 933 | 0 | 267 | 600 | 133 | 267 | 667 | 0 | 67 | 267 | 333 | 600 | 333 |
| | 133 | 267 | 333 | 800 | 67 | 67 | 0 | 67 | 133 | 333 | 67 | 133 | 67 | 267 |
| | 267 | 267 | 733 | 333 | 533 | 333 | 200 | 133 | 200 | 133 | 67 | 200 | 67 | 133 |
| | 933 | 600 | 600 | 267 | 267 | 67 | 400 | 267 | 800 | 133 | 400 | 0 | 267 | 867 |
| | 133 | 267 | 600 | 67 | 200 | 133 | 200 | 133 | 133 | 133 | 133 | 267 | 267 | 0 |
| | 333 | 667 | 600 | 467 | 0 | 267 | 267 | 467 | 800 | 200 | 800 | 67 | 200 | 0 |
| | 133 | 400 | 1533 | 267 | 67 | 400 | 0 | 200 | 133 | 67 | 467 | 67 | 133 | 67 |

Average of plots A-B-C

. - 1 Burlap per sample point

x - Foliage sample points for gas chromatography

N

Table 4. Post spray larvae counts under burlaps 2 days after treatment.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 11 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H | 0.3 | 1.6 | 2.0 | 3.3 | 1.0 | 0.6 | 1.0 | 5.0 | 1.3 | 3.3 | 2.3 | 1.6 | 2.3 | 4.0 |
| G | 3.0 | 1.6 | 3.0 | 2.3 | 1.0 | 1.3 | 0.3 | 0.0 | 2.0 | 3.3 | 0.0 | 5.3 | 6.6 | 6.0 |
| F | 3.0 | 4.0 | 1.3 | 1.3 | 2.3 | 0.3 | 1.3 | 1.0 | 1.6 | 4.6 | 1.3 | 1.3 | 1.3 | 1.6 |
| E | 2.3 | 7.0 | 0.6 | 1.0 | 0.6 | 1.6 | 2.6 | 0.0 | 3.0 | 0.3 | 0.6 | 2.3 | 2.3 | 0.3 |
| D | 1.6 | 1.6 | 0.0 | 0.6 | 4.0 | 3.6 | 0.0 | 0.3 | 0.0 | 0.3 | 0.6 | 0.6 | 1.6 | 1.6 |
| C | 0.3 | 2.6 | 6.3 | 3.6 | 1.0 | 0.3 | 0.3 | 0.0 | 1.0 | 0.0 | 1.6 | 2.0 | 2.0 | 2.0 |
| B | 3.0 | 2.3 | 2.0 | 3.0 | 6.0 | 0.3 | 0.6 | 0.0 | 0.6 | 1.0 | 1.0 | 0.0 | 3.0 | 1.0 |
| A | 0.6 | 1.6 | 0.0 | 2.0 | 1.0 | 2.3 | 1.0 | 1.0 | 1.0 | 3.0 | 0.6 | 1.0 | 0.3 | 0.3 |
| | 1.0 | 1.0 | 2.0 | 1.6 | 7.6 | 0.3 | 0.3 | 0.3 | 4.0 | 0.6 | 1.6 | 1.3 | 0.6 | 1.3 |
| | 2.6 | 1.3 | 2.0 | 2.6 | 4.0 | 2.6 | 0.0 | 0.0 | 0.0 | 2.0 | 0.3 | 3.3 | 2.3 | 4.6 |
| | 1.6 | 0.0 | 1.3 | 1.6 | 2.0 | 2.0 | 0.6 | 1.3 | 0.6 | 3.6 | 1.0 | 0.3 | 2.0 | 0.6 |
| | 2.0 | 2.0 | 2.3 | 1.6 | 0.6 | 1.0 | 3.0 | 0.6 | 1.0 | 2.3 | 3.6 | 1.3 | 2.3 | 2.0 |
| | 1.3 | 1.0 | 0.6 | 0.3 | 3.0 | 3.0 | 2.0 | 1.0 | 2.6 | 2.0 | 1.0 | 4.6 | 2.0 | 1.0 |
| | 2.0 | 3.3 | 3.6 | 2.3 | 0.6 | 1.6 | 2.0 | 1.0 | 1.0 | 2.0 | 1.6 | 2.0 | 0.3 | 1.6 |
| | 0.6 | 3.6 | 3.0 | 1.6 | 1.3 | 1.0 | 2.6 | 3.0 | 4.3 | 1.6 | 1.6 | 1.3 | 2.6 | 0.6 |

Average of plots A-B-C

. - 1 Burlap per sample point

x - Foliage sample points for gas chromatography

N

Table 5. Difference in Larvae per-burlap when compared to 2 days post spray counts.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 11 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| O | +1.3 | +2.0 | +0.3 | +0.7 | +1.0 | +0.4 | 0.0 | -4.0 | +0.3 | -2.0 | -7.0 | -0.6 | -1.7 | -0.7 |
| N | +2.0 | +0.4 | +1.0 | -1.7 | +1.3 | +0.3 | -0.3 | 0.0 | -1.4 | -2.3 | +2.6 | -3.0 | -1.3 | -0.4 |
| M | +7.0 | -2.4 | 0.0 | +3.4 | -2.0 | +0.7 | -0.7 | -0.4 | -1.0 | -4.0 | -0.7 | +0.3 | +1.3 | +3.7 |
| L | +5.5 | +2.0 | +.03 | +0.6 | -0.3 | +0.7 | -1.3 | +0.3 | -0.7 | +0.3 | -0.3 | -1.0 | -0.3 | +0.3 |
| K | +0.7 | -0.6 | +1.6 | 0.0 | -2.0 | -3.6 | 0.0 | -0.3 | +0.3 | 0.0 | +0.7 | +4.0 | -1.3 | +0.4 |
| J | 0.0 | -2.3 | -4.3 | -3.0 | -0.7 | -0.3 | -0.3 | 0.0 | 0.0 | +1.3 | +0.4 | -0.4 | -0.7 | -0.7 |
| I | -1.4 | -1.3 | -1.4 | -1.7 | -5.4 | +0.3 | 0.0 | +0.3 | -0.3 | -1.0 | +0.6 | +1.0 | -1.7 | -0.7 |
| H | +0.7 | -0.6 | +0.6 | -1.4 | -0.4 | -1.7 | -1.0 | -1.0 | -0.7 | -2.0 | -0.6 | -0.7 | +1.3 | +1.0 |
| G | -0.4 | -0.4 | -1.7 | -0.6 | -0.6 | -3.0 | -0.3 | -0.3 | -4.0 | +0.4 | -1.0 | 0.0 | +1.0 | -0.3 |
| F | -1.6 | -0.7 | -1.0 | -1.6 | -3.0 | -2.6 | 0.0 | 0.0 | +0.3 | -0.4 | 0.0 | -2.2 | -2.2 | -2.3 |
| E | -1.3 | +2.3 | -0.3 | -0.3 | -1.4 | -0.7 | -0.7 | -1.0 | -0.6 | -2.6 | 0.0 | +1.0 | 0.0 | +0.7 |
| D | -1.4 | 0.0 | -1.7 | -1.0 | +0.4 | 0.0 | -1.4 | -0.6 | +0.3 | -1.7 | -2.0 | -0.7 | -2.0 | -1.4 |
| C | +1.3 | +0.6 | +1.0 | 0.0 | -0.4 | -0.4 | -1.0 | -0.4 | -2.3 | -1.7 | -0.7 | -3.0 | -2.0 | -0.7 |
| B | -1.4 | -1.7 | -3.3 | -0.7 | 0.0 | -1.0 | -2.0 | -0.4 | +0.3 | -1.0 | -0.3 | -1.0 | 0.0 | -0.3 |
| A | +0.4 | -3.0 | -1.7 | -0.6 | +0.3 | -0.7 | -1.0 | -3.0 | -1.7 | -1.0 | -0.6 | +0.3 | -1.0 | 0.0 |

Burlap per sample point

7 day post spray larvae counts

. - 1 Burlap per sample point

x - Foliage sample points for gas chromatography

N

Table 6. Difference in larvae per-burlap when compared to 2 day post spray counts.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 11 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| O | +4.7 | +6.7 | +7.3 | +3.3 | +2.6 | +4.0 | +2.0 | -1.7 | +1.3 | +4.0 | +2.3 | +4.7 | +0.7 | +11.0 |
| N | +8.3 | +4.4 | +6.0 | +1.7 | +2.0 | +3.3 | +3.0 | +1.6 | +0.3 | +0.3 | +4.3 | +10.3 | +5.0 | +42.3 |
| M | +12.3 | +4.3 | +5.0 | +4.0 | +2.0 | +2.3 | +1.7 | -0.4 | -1.0 | -3.3 | +11.7 | +2.0 | +10.0 | +11.4 |
| L | +10.7 | +19.3 | +2.4 | +1.3 | +0.4 | -1.0 | -0.3 | 0.0 | -2.0 | 0.0 | +8.4 | +1.7 | +18.7 | +1.0 |
| K | +6.4 | +3.4 | +5.6 | +3.4 | -1.0 | +0.4 | +0.3 | -0.3 | 0.0 | +0.7 | +1.0 | +6.7 | +3.4 | +13.7 |
| J | +5.3 | +3.7 | -2.0 | +1.4 | +5.3 | +4.7 | -0.3 | 0.0 | -1.0 | +1.0 | +0.7 | +0.6 | +4.3 | +0.3 |
| I | +12.6 | +19.7 | +5.0 | +8.3 | +1.6 | +7.0 | 0.0 | 0.0 | -0.6 | -0.4 | +0.6 | +2.3 | +1.6 | +1.3 |
| H | +6.4 | +13.4 | +3.6 | -1.0 | -1.0 | -2.0 | -0.7 | -1.0 | -1.0 | +0.3 | +0.4 | +2.0 | +14.0 | +2.7 |
| G | +3.0 | +9.6 | +2.0 | +2.7 | -4.3 | -0.3 | 0.0 | +0.3 | -3.7 | +0.7 | +0.4 | +3.3 | +1.7 | +1.0 |
| F | +27.4 | +1.7 | +3.0 | +1.0 | +1.3 | -2.6 | 0.0 | 0.0 | +0.3 | +2.0 | +2.3 | +4.3 | 4.3 | -1.0 |
| E | +3.4 | +21.0 | +5.3 | +6.4 | +9.0 | -1.7 | -0.3 | -1.3 | +1.0 | -0.6 | +4.6 | +2.7 | +2.0 | +8.7 |
| D | +3.0 | +5.6 | +9.7 | +9.0 | +1.0 | 0.0 | -2.7 | -0.3 | +2.3 | -0.3 | +3.4 | +3.3 | +3.0 | +3.3 |
| C | +8.3 | +4.3 | +5.4 | +2.7 | +8.6 | +2.3 | +4.3 | +1.3 | +16.4 | +6.6 | +7.0 | +1.7 | +2.6 | +0.6 |
| B | +1.0 | +7.7 | -0.6 | +3.7 | +6.0 | +4.4 | +0.3 | +2.6 | +7.0 | +5.0 | +7.4 | +2.6 | +4.7 | +4.3 |
| A | +1.4 | +0.4 | +1.6 | +5.0 | +4.3 | +3.3 | +5.4 | +8.0 | +2.0 | +3.0 | +6.4 | +5.0 | +2.0 | +6.4 |

Average of plots A-B-C

14 day post spray larvae counts

N

Table 7. Difference in larvae per-burlap when compared to 2 day post spray counts.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 11 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | +5.0 | +5.7 | +7.3 | +6.0 | +6.0 | +3.0 | +2.6 | -3.0 | +4.0 | -0.7 | +7.6 | +0.7 | -1.3 | +9.3 |
| | +3.6 | +5.0 | +8.3 | +5.7 | -1.0 | +5.7 | +1.3 | +1.6 | -0.7 | +0.7 | +1.3 | +5.3 | +5.7 | +21.0 |
| | +7.6 | +2.0 | +7.3 | 0.0 | -0.7 | +1.3 | +2.0 | 0.0 | -1.0 | -3.3 | +11.0 | +10.0 | +9.0 | +4.0 |
| | +11.7 | 0.0 | +2.7 | +3.0 | +1.7 | -0.3 | -1.3 | 0.0 | -3.0 | -0.3 | +13.7 | -1.0 | +15.0 | +0.3 |
| | +3.7 | +2.7 | +12.3 | +12.7 | -2.4 | -3.0 | 0.0 | -0.3 | 0.0 | +0.3 | 0.0 | +7.0 | +34.4 | +12.0 |
| | +1.7 | +1.4 | -3.3 | +3.7 | +3.0 | +13.0 | -0.3 | 0.0 | -1.0 | +0.3 | -1.0 | -0.7 | +2.3 | +0.3 |
| | +0.6 | +12.3 | +0.3 | +1.6 | +2.3 | +0.7 | -0.6 | 0.0 | -0.3 | -1.0 | +3.3 | +3.3 | -1.7 | -0.4 |
| | +10.0 | +7.0 | +5.3 | 0.0 | -0.3 | -2.0 | -0.6 | -1.0 | -1.0 | +4.3 | +0.7 | +0.7 | +19.0 | +5.7 |
| | +3.6 | +6.0 | +3.3 | +7.1 | -4.9 | +0.3 | +0.3 | -0.3 | -3.4 | -0.6 | +0.1 | +2.0 | +22.1 | +0.3 |
| | +23.4 | +7.3 | +11.0 | +7.4 | +20.7 | +1.3 | 0.0 | 0.0 | 0.0 | +0.7 | +5.0 | +4.0 | +4.0 | -2.6 |
| | +6.6 | +4.0 | +5.7 | +11.0 | +9.0 | +1.3 | -0.6 | -0.3 | -0.3 | +0.3 | +3.6 | +7.7 | +1.0 | +4.7 |
| | +3.3 | +4.0 | +7.3 | +27.4 | +3.0 | +0.6 | -1.4 | +2.7 | +7.0 | +1.3 | -0.3 | +3.0 | +3.0 | +2.3 |
| | +7.3 | +0.3 | +11.7 | +1.7 | +3.0 | +3.3 | +8.0 | +0.6 | +27.7 | +13.6 | +3.6 | +1.0 | +4.1 | +2.0 |
| | +2.6 | +8.7 | +3.0 | +1.3 | +2.4 | +2.0 | +4.6 | +5.3 | +7.6 | +2.3 | +18.4 | +1.0 | +4.3 | +4.3 |
| | +1.7 | +1.0 | -1.7 | +4.7 | +1.3 | +0.3 | +5.7 | +7.0 | -0.3 | +4.0 | +6.7 | +2.3 | +12.4 | +9.7 |

1 Burlap per sample point

Average plots A-B-C

21 day post spray larvae counts

N

It should be noted that as test insects become larger they have a natural tendency to migrate more. This is especially true on hot days when they migrate to the lower parts of trees to seek out shade. It is therefore natural to recover larger numbers of larvae under burlap bands as the season progresses. One should keep this in mind when evaluating the results of this study.

A final report will be made on this work in the October, 1978 - March, 1979 report.

Project Number: GM 6.2.3
Project Title: Periodicity of Pheromone Release from Female Gypsy Moths
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leader: R. G. Reeves

A manuscript is being prepared for publication in the "Journal of Chemical Ecology". Upon finalization of the manuscript, it will be included as a Final Report in the next Laboratory Report.

Project Number: GM 7.2.1
Project Title: Efficiency of (+) Disparlure-Baited Traps in Arrays for Estimating Male Moth Density and Delimitation of Infestations
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: C. P. Schwalbe, E. C. Paszek

In 1977 it was found that the patterns of capture of male moths in arrays of traps were highly indicative of the points of origin of the insects. Larger proportions of insects were captured in traps closest to the release sites. It was also noted that rates of capture decreased as trap density decreased and that many more insects were recovered in traps baited with the (+) enantiomer of disparlure than in those baited with the racemic mixture. These tests led to the conclusion that systematic use of traps baited with (+)-disparlure would improve the precision of delimitation surveys and increase the sensitivity of detection surveys. Consequently, a formulation development program was initiated with SEA-AR to develop a slow-release dispenser of the costly lure. An effective dispenser which required only ca. 1 mg of attractant to charge and which was active for 8 weeks in the field was developed (GM 7.2.7). In the 1978 survey program, ca. 20,000 traps were placed which were baited with 1 mg (+) disparlure dispensed from Hercon strips measuring 5x25 mm.

The tests reported herein were conducted to evaluate the effectiveness of the newly designed Hercon dispenser and to obtain additional information on the capture patterns of moths in arrays of traps.

In the 1977 tests, inter-trap distances (ITD's) were 88, 175 and 350 m. Because 88 m appears to be impractical on an operational scale, it was dropped from further testing. In 1978 ITD's were 175, 262 and 350 m. All traps were baited with Hercon dispensers. Male moths (from field collected pupae) were marked and released from the center of each plot. Eight separate releases were made in each plot and traps were checked and emptied every other day. Results of these releases are given in Table 1.

Table 1. Recovery of feral moths in arrays of traps baited with (+)-disparlure. Fall River, 1978.

| ITD(m) | # Traps | # ♂♂ Released | # % Captured |
|---|---|---|---|
| 175 | 100 | 1425 | 21.2 |
| 262 | 36 | 1600 | 9.1 |
| 350 | 36 | 1535 | 7.8 |

As before, increasing the ITD decreased moth recovery. Some comparisons can be made with the 1977 tests since the Hercon dispenser is similar in attractiveness to the 100 μg cotton wicks used in those tests. Traps placed 350 m apart only recovered 7.8% of the released males (vs 5.1% in 1977). It is noteworthy that the "compromise" ITD of 262 m only captured 9.1%, indicating that this increase in density results in only minor improvement of survey precision. However, 21.2% of the released males were captured in traps placed 175 m apart. It is expected that this placement pattern will significantly increase the precision with which delimitation boundaries can be estimated.

Distribution of males within the arrays was also examined. Distances of various trap locations from the release sites were calculated and ranked and the numbers of insects caught at those locations were tabulated. Those results are summarized in Table 2.

Table 2. Distribution of male moths captured in arrays of traps with ITD = 175, 262 and 350 m Fall River, 1978.

| Distance from Release (m) | No. Traps at distance | Percentage of released moths captured at various distances from release sites | | | Percentage of captured moths recovered at various release sites | | |
|---|---|---|---|---|---|---|---|
| | | 175 | 262 | 350 | 175 | 262 | 350 |
| 127 | 4 | 8.4 | | | 39.4 | | |
| 189 | 4 | | 4.4 | | | 49.0 | |
| 246 | 4 | | | 3.6 | | | 46.6 |
| 278 | 8 | 6.3 | | | 29.8 | | |
| 373 | 4 | 1.3 | | | 6.0 | | |
| 418 | 8 | | 2.6 | | | 29.0 | |
| 444 | 8 | 1.3 | | | 6.0 | | |
| 516 | 8 | 1.3 | | | 6.0 | | |
| 557 | 4 | | 0.5 | | | 6.5 | |
| 546 | 8 | | | 2.4 | | | 30.8 |
| 619 | 12 | 1.0 | | | 4.6 | | |
| 667 | 4 | 0.3 | | | 1.3 | | |
| 672 | 8 | | 0.9 | | | 10.3 | |
| 738 | 8 | | | 0.7 | | | 9.2 |
| 754 | 8 | 0.2 | | | 1.0 | | |
| 762 | 8 | | 0.4 | | | 0.5 | |
| 794 | 8 | 0.4 | | | 1.6 | | |
| 841 | 8 | 0.4 | | | 1.6 | | |
| 873 | 4 | 0.1 | | | 0.7 | | |
| 877 | 8 | | | 0.5 | | | 6.7 |
| 913 | 8 | 0.1 | | | 0.3 | | |
| 934 | 4 | | 0.1 | | | 0.7 | |

Table 2. (Cont'd).

| Distance from Release (m) | No. Traps at distance | Percentage of released moths captured at various distances from release sites | | | Percentage of captured moths recovered at various release sites | | |
|---|---|---|---|---|---|---|---|
| | | 175 | 262 | 350 | 175 | 262 | 350 |
| 1008 | 8 | 0.3 | | | 1.3 | | |
| 1016 | 8 | | | 0.5 | | | 5.8 |
| 1127 | 4 | 0.1 | | | 0.3 | | |
| 1230 | 4 | | | 0.1 | | | 0.8 |
| | | 21.5 | 8.9 | 7.8 | 99.9 | 96.0 | 99.9 |

The data in the left-hand portion of Table 2 are the percentages of the released insects which were captured at various distances from the release points. The data have not been normalized to equal numbers of traps; and individual trap distances may have had 4, 8 or 12 traps. Normalization would increase linearity of the data.

Generally, as distance from the release site increases, fewer insects are captured. It is noteworthy that recovery at various distances is comparable among the 3 plots. For example, 4 traps at 246 m in the 350 m plot captured 3.6% of the released population (0.9%/trap). Eight traps at 278 m in the 175 m plot recovered 6.3% of the released population (0.8%/trap).

In the right-hand portion of Table 2 the percentages of captured moths at various distances are tabulated. When expressed this way, data indicate that larger proportions of captured insects are found in "distant" traps in the widely spaced 350 m plots than in the tighter (175 m) grids. This illustrates that delimitation precision improves with closer trap spacing. Presumeably, this is because more insects are removed from the dispersing population by the central traps in the 175 m ITD plots. While only 6.0% of the captured insects were recovered in traps 516 m from the center of the 175 m ITD plot, a total of 71% were captured in all traps between the center of the plot and the 516 m distance. In the 350 m ITD plot 30.8 of the captured males were in traps 546 m from the release point whereas 77% were captured in all traps between the center of the plot and the 546 m distance.

It remains unclear whether most insects are captured near the point of release because they normally do not disperse far or whether they are "intercepted" by the nearest traps before long distance dispersal can occur. To investigate this, all traps were removed from the 175 m ITD plot and a single release of 408 moths was made. After 48 hours, baited traps were replaced. The traps were checked 3 days after placement. Results are summarized in Table 3.

Table 3. Distribution of male gypsy moths 48 hours after release into plots without traps.

| Distance from release | # traps | % ♂♂ captured when released into trap grid | % ♂♂ captured in traps placed in grid 48 hr after release |
|---|---|---|---|
| 127 | 4 | 39.4 | 45.0 |
| 278 | 8 | 29.8 | 27.5 |
| 373 | 4 | 6.0 | 6.0 |
| 444 | 8 | 6.0 | 8.7 |
| 516 | 8 | 6.0 | 4.7 |
| 619 | 12 | 4.6 | 4.0 |
| 667 | 8 | 1.3 | 1.3 |
| 754 | 8 | 1.0 | .7 |
| 794 | 8 | 1.7 | 0 |
| 841 | 8 | 1.7 | 1.3 |
| 873 | 4 | .7 | .7 |
| 913 | 8 | .3 | 0 |
| 1008 | 8 | 1.3 | 0 |
| 1127 | 4 | .3 | 0 |

Results of this test indicate that dispersal of male moths is similar in trapped and untrapped areas. If insects captured in traps are representative of the total population, it can be estimated that only ca. 25% of the population disperses further than 278 m in 2 days and only a very small proportion (ca. 3%) move beyond 619 m This type of information is useful in evaluating the efficacy of detection survey programs.

Two pilot tests were conducted in Wisconsin in 1978. In Oconomowoc, a suspect infestation was surveyed at 20 traps/$mi^2$ (350 m ITD) and another in Appleton was trapped at 84 traps/$mi^2$ (175 m ITD). Both surveys resulted in moth capture patterns that indicated the centers of infestation. Also, in Michigan, 9 sq. mi. that were presumed to be infested were surveyed with 84 traps/$mi^2$. That survey indicated that only ca. 1 $mi^2$ was actually infested. These tests will be covered in more detail in the next laboratory report.

We propose the following plan for gypsy moth survey:

| Year | Survey Type | Density |
|---|---|---|
| 1 | Detection | 1 trap/3 $mi^2$ |
| 2 | Detection Confirmation and Delimitation | 20 traps/$mi^2$ |
| 3 | Delimitation and treatment Evaluation (if intervention in year 2) | 84 traps/$mi^2$ |
| 4 | Detection and Treatment evaluation | 84 traps/$mi^2$ |
| 5 | Detection and Treatment evaluation | 84 traps/$mi^2$ |

Project Number: GM 7.2.4
Project Title: Development of Laboratory Evaluation Techniques for Gypsy Moth Male Competitiveness Comparisons
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: V. C. Mastro, M. Waldvogel

## Introduction:

Currently, two laboratory techniques are being evaluated for male competitiveness comparisons. These techniques, if found to be reliable indicators of male field performance, will be used as standard monitors of laboratory male quality. These techniques are a free flight tunnel where males can be evaluated for response to various pheromone concentrations, and an actograph for comparing activity rhythms for various strains of males. If development of these techniques is feasible, and the results of bioassays are correlated with field performance, they will serve to identify problems in colony, such as changes in rearing procedures or genetic changes in a laboratory strain that result in production of somewhat less than competitive males. Forwarned, corrective actions can be taken before resources are committed to an unproductive field program.

Flight tunnel construction was completed in May thanks to the talents of R. Tardif and C. Owens. Testing was begun in early June by M. Waldvogel, a PhD candidate from the Department of Entomology at Pennsylvania State University. Mike spent the summer with us in a cooperative effort to refine the use of the flight tunnel and to test the response of different strains of males to pheromone. The specific objectives of this series of trials were to:

1) identify minimum pheromone bait concentration needed to elicit a behavioral response from feral males.

2) establish and compare the bait concentration required to stimulate laboratory reared males (untreated and irradiated - 3 age groups).

3) and correlate field performance of males with flight tunnel bioassays.

Components for construction of an actograph have now been delivered by the manufacturer. Final assembly of the unit will be completed, and calibrating and preliminary testing will begin by late November. Objectives of initial trials will be to determine what mechanisms control adult activity periodicity and explore how these mechanisms can be manipulated to the competitive advantage of sterile males. Nearly all studies of insect circadian rhythms have shown that light and/or dark cues act as the primary setting mechanish(s). Although temperature or thermoperiod have often been shown to modify circadian rhythms, they in themselves do not generally control the cycle.

Results from field studies suggest that some component of the photoperiod cycle is the gate that regulates adult male activity in the gypsy moth. To be determined are proper light and/or dark cues necessary for setting the internal clocks of laboratory insects and the proper timing of these cues in the proper developmental stage(s).

Once the proper conditions for rearing have been documented the actograph will act as a quality control technique for monitoring laboratory male quality. Continuous testing of the laboratory colony will determine if activity is diminishing in intensity, or if rhythms are shifted because of genetic drift or funneling.

Methods and Materials:

Flight tunnel bioassays were conducted using feral and laboratory reared males (irradiated and untreated) from June through August. Males used in these trials were taken from groups of males used in sterile male field trials (see GM 8.2.4). Environmental conditions for testing were held constant at 26°C, 45-50% RH, and a wind velocity of 4 cm/sec. Testing was carried out with different groups of males from 6:00 a.m. through 4:00 p.m. In all, 10 treatment groups were bioassayed; 5 feral strains, 2 laboratory strains ($NJ_{F16}$ and $YC_{P1}$) and 3 irradiated strains (6-7, 8-9 and 10-11 day old pupae exposed to 15 krads).

Five males were included in each replicate. Males, held in a small hardware cloth cage, were placed inside of the flight chamber and allowed to acclimate for a few minutes prior to introduction of a pheromone source upwind. Once the bait was in place, elapsed time to male response was recorded. Also the type of response was recorded (i.e. walking, wing fanning, or initiation of upwind flight. After 10 minutes of exposure males and bait were removed and the chamber allowed to clear of latent pheromone before a second bioassay was started. The different treatment groups were exposed to baits containing from 0.001 ng to 50 ng of (+) disparlure.

Results and Discussion:

Analysis of flight tunnel data is not complete. However, some preliminary findings follow. Minimum amount of pheromone required to elicit a behavioral response is dependent on the time of day. When bioassays were done in the early morning large amounts of pheromone were required for positive responses (25-50 ng baits). However, bioassays carried out in the early afternoon hours, resulted in positive behavioral responses at much lower concentrations (5-10 ng baits). These results appear to agree with field trial data which demonstrates males are normally not active during the early morning hours. In other words, there appears to be a dose-response periodicity that closely agrees with the males normal activity periodicity and large amounts of pheromone are required during normally quiescent periods to activate a male. This pattern of response was noted in all groups of males tested.

Significant differences between feral and laboratory reared males response to pheromone baits are not evident. Both laboratory reared strains tested ($NJ_{F16}$ and $YC_{P1}$) responded to as low as concentrations of pheromone as did feral strains. Furthermore when $NJ_{F16}$ males were irradiated as 8-9 or 10-11 day-old pupae and bioassayed as adults they responded in a manner similar to untreated laboratory reared and feral males. However, one group of males bioassayed showed a marked reduction in behavioral response to minimum concentrations of pheromone. $NJ_{F16}$ males when irradiated as 6 and 7 day old pupae did not respond to minimum pheromone bait concentrations in a manner similar to other treatment groups. Even when males in this group were activated, average response time was longer.

Although it was not quantified, anemotatic flight of the various groups did provide some additional information. All displayed the upwind undulating flight typical of male gypsy moths orienting to a pheromone source. When the youngest irradiated age group (6 and 7 day old pupae) were bioassayed, longer times for flight initiation were required and flight appeared uncoordinated and undirected.

Results of these trials indicate that the minimum age for irradiation of pupae should be no less than 8 days at the 15 krad treatment level. The possibility of treating with lower dosages of radiation to produce partially sterile male may allow treatment of less mature pupae. Also, standardized testing for quality control should be carried out only during that part of the day when males are normally active and respond to minimum pheromone concentrations of pheromone. If testing were to be done with different treatment groups at different times of the day results would be misleading. Further testing may indicate that temperature modifies response to different pheromone concentrations. If so, studies will be needed to determine if different strains respond in the same manner at lowered or elevated temperatures.

The flight tunnel appears to present an acceptable measure of male quality. However, results of the trials described above remain to be fully analyzed and compared to 1978 field trial results before any correlations can be drawn between laboratory and field male competitiveness. Also, further testing will provide standard testing conditions and procedures which provide quality measurements which are most meaningful in terms of field performance.

Project Number: GM 8.2.2
Project Title: Radiological Sterilization of Male Gypsy Moths
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leader: C. P. Schwalbe

This project was undertaken to determine the appropriate insect age: radiation dosage most suitable for sterilizing male pupae. Details of techniques were described in the October-March, 1978 Laboratory Report.

In the last report it was noted that male pupae irradiated at 6-11 days of age with 2-20 krads yielded normal adults. The average duration of the pupal stage was 11-12 days. Four-5 day old pupae treated with 15 or 20 krads experienced increased pupal mortality and resultant adults were deformed with "crumpled" wings. Sperm transfer was also normal except in the 4-5 day:15-20 krad class. In those treatments, very few males transferred sperm to normal females.

Table 1 is a summary of observations on egg masses resulting from irradiated ♂ x normal ♀ matings. All pairings that yielded fewer than 100 eggs/female were eliminated since it is likely that mating did not occur. In all cases (with the exception of the 4-5 day old:15 and 20 krad treatment) this was a very small proportion (less than 10%).

No dramatic differences in egg mass size and embryonation were observed between the treated and control group except at the higher dosages administered to 4-5 day old pupae. Females mated with those males laid about 1/2 as many eggs as their control counterparts. However, in nearly all cases, hatch was depressed in the treated groups. Interestingly, the age at which irradiation is administered does not seem to affect the incidence of hatch when sperm is transferred. This is advantageous in that accurate aging of pupae may not be required in large scale use of the SMT. However, 4-5 day old pupae treated with 15 or 20 krad do not mate well, as evidenced by low rates of embryonation.

Table 1. Size, embryonation and hatch of egg masses produced by females mated with irradiated males.

| | Age Class (days) | TREATED n | TREATED No. Eggs/ Mass | TREATED % Embryo-nation | TREATED % Hatch | CONTROL n | CONTROL No. Eggs/ Mass | CONTROL % Embryo-nation | CONTROL % Hatch |
|---|---|---|---|---|---|---|---|---|---|
| 2 krads | 4-5 | 56 | 846 | 95 | 47 | 17 | 869 | 99 | 58 |
| | 6-7 | 33 | 660 | 94 | 21 | 35 | 808 | 87 | 55 |
| | 8-9 | 46 | 812 | 97 | 52 | 23 | 785 | 98 | 58 |
| | 10-11 | 71 | 836 | 99 | 40 | 29 | 763 | 99 | 61 |
| 8 krads | 4-5 | 47 | 765 | 92 | 25 | 18 | 868 | 99 | 57 |
| | 6-7 | 72 | 841 | 96 | 30 | 48 | 907 | 99 | 74 |
| | 8-9 | 89 | 803 | 97 | 21 | 69 | 710 | 93 | 58 |
| | 10-11 | 71 | 670 | 96 | 17 | 22 | 674 | 99 | 53 |
| 10 krads | 4-5 | 39 | 731 | 91 | 28 | 18 | 773 | 99 | 83 |
| | 6-7 | 80 | 852 | 96 | 26 | 34 | 820 | 95 | 84 |
| | 8-9 | 44 | 705 | 95 | 5 | 35 | 808 | 87 | 55 |
| | 10-11 | 65 | 700 | 97 | 18 | 16 | 785 | 98 | 65 |
| 15 krads | 4-5 | 23 | 320 | 9 | 0.17 | 35 | 808 | 87 | 55 |
| | 6-7 | 57 | 831 | 92 | 8.0 | 24 | 861 | 95 | 61 |
| | 8-9 | 86 | 750 | 94 | 6.0 | 51 | 820 | 95 | 53 |
| | 10-11 | 50 | 903 | 90 | 4.0 | 13 | 802 | 98 | 81 |

Table 1. (Cont'd)

| | Age Class (days) | TREATED n | TREATED No. Eggs/ Mass | TREATED % Embryo-nation | TREATED % Hatch | CONTROL n | CONTROL No. Eggs/ Mass | CONTROL % Embryo-nation | CONTROL % Hatch |
|---|---|---|---|---|---|---|---|---|---|
| 20 krads | 4-5 | 21 | 488 | 0 | 0 | 35 | 808 | 87 | 55 |
| | 6-7 | 65 | 859 | 87 | 1 | 25 | 861 | 95 | 60 |
| | 8-9 | 50 | 751 | 92 | 1 | 23 | 847 | 94 | 58 |
| | 10-11 | 23 | 649 | 93 | .22 | 13 | 802 | 98 | 81 |

Based upon these data, we have decided to use 8-11 day old males irradiated with 15 krad in field evaluations. Matings by insects thus treated yield large egg masses (ca 800 eggs/mass) with high embryonation (ca 93%) and low incidence of hatch (6-8%). If necessary, it may be desireable to increase the age class at the time of irradiation to 6-11 days.

Larvae that hatched from egg masses tested in Table 1 were infested onto diet. Survival and developmental rates were monitored. Those results are summarized in Table 2.

A number of conclusions can be drawn from the data. Generally, the time required for development to the pupal stage is longer when larvae are from a treated ♂ x normal ♀ mating. Interestingly, the developmental time seems to be longer when the fathers were treated at a younger age class. For example, male developmental times to pupation ranged from 34.4-41.5 days for 15 krad treatments at 10-11 to 4-5 days, respectively. A similar pattern exists in the female insects at the 15 krad treatment. However, this trend was not observed at lower irradiation levels (2-10 krads). Greater mortality in both the larval and pupal stages was observed in the treated group than in the control. This also was dependent upon the age and dosage administered. Generally, survival was lower when irradiation was administered to young pupae and survival also decreased at high dosages.

Finally, the $F_1$ sex ratio is strongly distorted in favor of males in the treated group. This is particularly important if sterile $F_1$ males are expected to further interact with a population the year following release. As mentioned earlier, we have chosen the 15 krad:8-11 day treatment for further evaluation in the project. Only 4-6% hatch is expected from males thus treated (Table 1). Those larvae that hatch develop only slightly slower than those from untreated fathers and survival to adult is good (47-66%) (Table 2). Furthermore, the sex ratio is distorted. Presumeably, if these $F_1$ progeny are sterile, further effects on the target population can be expected.

The resultant $F_1$ adults (from Table 2) were back-crossed to determine the degree of $F_1$ sterility. T♂ x T♀, T♂ x N♀, N♂ x T♀ and N♂ x N♀ matings were done. The results of these studies will be included in the next laboratory report.

Table 2. Survival and development of larvae from egg masses resulting from treated ♂ x normal ♀ matings.

| | Age Class | Number Infested Tr. | Number Infested Con. | ♂ Time to Pupation Tr. | ♂ Time to Pupation Con. | ♀ Time to Pupation Tr. | ♀ Time to Pupation Con. | % Survival Larva ->Pupa Tr. | % Survival Larva ->Pupa Con | % Survival Larva ->Adult Tr. | % Survival Larva ->Adult Con. | Adult Sex Ratio (♂:♀) Tr. | Adult Sex Ratio (♂:♀) Con. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 krad | 4-5 | 300 | 302 | 31.5 | 32.4 | 33.7 | 35.6 | 82 | 85 | 80 | 81 | 1:1 | 1:1 |
| | 6-7 | 250 | 285 | 35.8 | 32.8 | 41.7 | 32.1 | 87 | 92 | 83 | 88 | .75:1 | 0.8:1 |
| | 8-9 | 250 | 250 | 30.3 | 28.9 | 33.9 | 32.5 | 84 | 90 | 78 | 83 | 1:1 | 1:1 |
| | 10-11 | 250 | 500 | 31.8 | 32.8 | 35.4 | 32.1 | 96 | 94 | 84 | 87 | 0.5:1 | 0.6:1 |
| 8 krad | 4-5 | 300 | 302 | 32.8 | 32.4 | 36.0 | 35.6 | 78 | 85 | 75 | 81 | 1.4:1 | 1:1 |
| | 6-7 | 270 | 300 | 33.5 | 32.1 | 36.3 | 34.7 | 74 | 86 | 71 | 86 | 0.8:1 | 0.5:1 |
| | 8-9 | 250 | 500 | 32.1 | 32.8 | 35.4 | 32.1 | 92 | 94 | 71 | 87 | 0.8:1 | 0.6:1 |
| | 10-11 | 250 | 500 | 33.2 | 32.8 | 36.6 | 32.1 | 84 | 94 | 71 | 87 | 1:1 | 0.6:1 |
| 10 krad | 4-5 | 250 | 300 | 33.5 | 32.1 | 36.4 | 34.7 | 72 | 86 | 58 | 86 | 2.2:1 | 0.5:1 |
| | 6-7 | 256 | 300 | 34.2 | 32.1 | 37.2 | 34.7 | 70 | 86 | 65 | 86 | 1.1:1 | 0.5:1 |
| | 8-9 | 250 | 285 | 34.1 | 32.8 | 37.1 | 32.1 | 80 | 92 | 49 | 88 | 3.1:1 | 0.8:1 |
| | 10-11 | 73 | 500 | 33.7 | 32.8 | 38.8 | 32.1 | 62 | 94 | 49 | 87 | 2.6:1 | 0.6:1 |
| 15 krad | 4-5 | 8 | 285 | 41.5 | 32.8 | - | 32.1 | 50 | 92 | 12 | 88 | 1:0 | 0.8:1 |
| | 6-7 | 270 | 500 | 37.0 | 32.8 | 41.2 | 32.1 | 47 | 94 | 37 | 87 | 3.6:1 | 0.6:1 |
| | 8-9 | 250 | 300 | 35.5 | 32.1 | 38.7 | 34.7 | 69 | 86 | 47 | 86 | 1.9:1 | 0.5:1 |
| | 10-11 | 35 | 250 | 34.4 | 30.0 | 34.5 | 33.1 | 83 | 83 | 66 | 80 | 6.7:1 | 0.5:1 |
| 20 krad | 4-5 | 0 | 285 | - | 32.8 | - | 32.1 | - | 92 | - | 88 | - | 0.8:1 |
| | 6-7 | 114 | 285 | 37.6 | 32.8 | 41.1 | 32.1 | 32 | 92 | 25 | 88 | 2.5:1 | 0.8:1 |
| | 8-9 | 3 | 250 | 33.7 | 30.0 | - | 33.1 | 100 | 83 | 100 | 80 | 3.0:1 | 0.5:1 |
| | 10-11 | 0 | 250 | - | 30.0 | - | 33.1 | - | 83 | - | 80 | - | 0.5:1 |

Project Number: GM 8.2.3
Project Title: Gypsy Moth Mating Behavior
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leader: C. P. Schwalbe

In the last report, it was noted that insemination generally occurs within 10 minutes of copulation. Translocation of sperm from the vulva to the bursa copulatrix began after 10 minutes of copulation and appeared in the spermatheca after 30 minutes of mating. There was preliminary evidence that male involvement beyond insemination was required for translocation of sperm to the bursa and spermatheca.

The following test was conducted to determine if the duration of copulation affects the rate of translocation of sperm from the vulva to the spermatheca. The time of copulation was controlled by forcibly separating the moths after the desired time *in copulo*. Results are presented in Table 1.

Table 1. Distribution of sperm in female gypsy moths after various durations of mating.

| n | Duration of Copulation(min) | Time Dissected(min) | % of ♀♀ with sperm | | | % Ovipositing | No Eggs per Mass | % Embryonation |
|---|---|---|---|---|---|---|---|---|
| | | | vulva | bursa | spermatheca | | | |
| 10 | 5 | 5 | 0 | 0 | 0 | | | |
| 10 | 10 | 10 | 70 | 0 | 0 | | | |
| 10 | 10 | 60 | 100 | 40 | 30 | | | |
| 10 | 10 | 120 | 100 | 100 | 90 | | | |
| 10 | Uninterrupted | 60 | 90 | 90 | 100 | | | |
| 10 | Uninterrupted | 120 | 100 | 100 | 100 | | | |
| 10 | 5 | 2 days | 10 | 10 | 0 | 10 | 654 | 99 |
| 10 | 10 | 2 days | 70 | 70 | 70 | 70 | 733 | 98 |
| 10 | Uninterrupted | 2 days | 100 | 90 | 100 | 90 | 848 | 98 |

In nearly every dissected preparation, both apyrene and eupyrene sperm were observed together. As before, it was noted that 5 minutes of copulation is inadequate for sperm transfer to take place. However, 70-100% of females are inseminated after 10 minutes of mating, as evidenced by sperm in the vulva. It again appears that translocation of sperm cells to the spermatheca is delayed when mating is interrupted after 10 minutes. Only 30% of the females mating 10 minutes had sperm in the spermatheca 60 minutes later. However, 100% of those that mated uninterruped for 60 minutes contained sperm. This indicates that the male moth imparts factors during extended copulation which facilitates translocation of sperm to the spermatheca. It appears that short periods of mating (10 min) is sufficient, however, for effective insemination. After 120 minutes, 90% of the mated females had sperm in the spermatheca and 100% of those mated for 10 minutes laid fully embryonated egg masses within two days. It is also interesting that females which mated for 5, 10 or ca. 60 (uninterruped) minutes laid respectively more eggs/mass (654, 733 and 848), indicating that the length of time in copulation regulates the number of eggs laid.

Project Number: GM 8.2.4
Project Title: Assessing Male Competitiveness Traits of Various Strains of Gypsy Moths
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leader: V. C. Mastro

Introduction:

Successful application of the sterile male technique (SMT) against the gypsy moth, as with any insect, necessitates that males be competitive with those in a target population. Projected applications of the SMT are in isolated, outlying infestations and in low density fringe areas along the leading edge of the generally infested area. Even though the exact distribution of insects in some of these infestations has not been clearly defined and no universal definition of a "sparse population" exists, there is some information available about "low density" populations. Campbell, working in sparse populations, found the majority of egg masses in hidden situations (i.e. under rocks, in or on stumps and under bark flaps). He attributed this distribution to differential pupal survival; insects pupating in the litter had the highest incidence of mortality. Therefore, egg mass distribution was not uniform but was clustered in these niches.

Major emphasis this past field season (1978) was directed toward comparing the competitiveness of male gypsy moth strains in a simulated "target" population. Given that the effects on male performance of variables such as;female density, distribution and niche location are unknown, it is desirable to compare males in a situation as close to the target population as possible. To achieve an approximation of a natural sparse population, females were placed in artificial niches. These niches were small wooden enclosures which provided a hidden location similar to where feral females are found. Other parameters were standardized; female density was set at 10 females/ha and female distribution was in a uniform pattern. Several components of competitiveness were isolated and quantified. These included periodicity of mate location, mating success, vertical and horizontal dispersal and distribution and adult male longevity. In addition, field cage studies were carried out to characterize periodicity of female eclosion and calling behavior. Studies were also conducted to investigate crepuscular male and female behavior.

Complementary laboratory studies were conducted throughout the flight season to compare the response of strains of males to various pheromone levels. The trials were done in cooperation with M. Waldvogel, a PhD candidate at Pennsylvania State University. Trials were run in a free flight chamber at Otis AFB, and are described in Section 7.2.5.

## METHODS AND MATERIALS

### Male Competitiveness Trials:

Competitiveness trials were conducted in 6.15 ha circular plots, with 140 m radius. Sixty-six females were placed in each plot to simulate a density of 10.7 females/ha. Females were placed at 20 m intervals along the circumference of two concentric circles (70 m and 140 m radii). To simulate natural low density populations, where females are found in hidden situations, females were placed in small wooden A-frames (Fig. 1) at the sites. Two different placement strategies were used; female sites were alternately on the ground farther than 1 m from the nearest tree or 1.5 m from the ground on the bole of a tree. Although this design did not randomize female placement it did serve to quantify horizontal male dispersal and provided two specific niches for male competitiveness comparisons.

Male pupae were placed in the center of circular plots and allowed to eclose and disperse naturally. All laboratory male pupae were held in an outdoor insectary prior to release. When males were expanding their wings shortly after eclosion, they were dusted with Dayglo® fluorescent powder to identify the strain and day of release. Eclosion and dispersal were monitored continuously and summarized every hour. Males were usually released for 1 day. However, if eclosion rates were low, release was carried on for a second day to provide enough numbers for strain comparisons.

Points monitored with females in A-frames were checked every 1/2 hour from ca. 9:30 a.m. to 4:00 p.m. When a mating pair was found, the male was collected for laboratory determination of strain and age. Mated females were replaced with virgin 1-2 day-old females. Feral females were used in trials throughout the major portion of the flight season. However, pre- and post-season tests were conducted with laboratory-reared females.

Using the test design described above, 2 laboratory strains of males were compared with 4 feral strains. The laboratory strains were $NJ_{F16}$ (laboratory reared - 16 generations) and $YC_{P1}$ (collected from the field as eggs). The $YC_{P1}$ strain was selected for field testing from 5 possible wild strains on the basis of the following laboratory rearing criteria: developmental times, developmental weights, fecundity, survival rates and incidence of virus.

In addition to the above strain comparisons $NJ_{F16}$ pupae were irradiated (15 krads) in two age groups (8-9 and 10-11 day old pupae) and compared with untreated $NJ_{F16}$ males (Table 1). Fifteen krads was selected as an irradiation dose from results of laboratory studies by Schwalbe (See section 8.2.2).

Table 1. MALE COMPETITIVENESS TRIALS 1978

| PLOT | STRAINS COMPARED | TREATMENT | MONITORING |
|---|---|---|---|
| A | $NJ_{F16}$ - Laboratory colony (LC) | None | Laboratory reared Females |
| | $YC_{P1}$ - (LC) | None | |
| | Yards Creek, NJ-feral | None | |
| B | $NJ_{F16}$ - (LC) | None | Feral females |
| | $YC_{P1}$ - (LC) | None | |
| | Dighton, MA-feral | None | |
| | Abington, MA-feral | None | |
| C | $NJ_{F16}$ - (LC) | None | Feral females |
| | $YC_{P1}$ - (LC) | None | |
| | Bridgewater, MA-feral | None | |
| | Abington, MA-feral | None | |
| D | $NJ_{F16}$ - (LC) | None | Feral females |
| | $YC_{P1}$ - (LC) | None | |
| | $NJ_{F16}$ - (LC) | 8-9 day old pupae (15 krads) | |
| E | $NJ_{F16}$ - (LC) | None | |
| | $YC_{P1}$ - (LC) | None | |
| | $NJ_{F16}$ - (LC) | 8-9 day old pupae (15 krads) | Laboratory reared Females |
| | $NJ_{F16}$ - (LC) | 10-11 day old pupae (15 krads) | |
| F | $NJ_{F16}$ - (LC) | None | |
| | $YC_{P1}$ - (LC) | None | |
| | $NJ_{F16}$ - (LC) | 8-9 day old pupae (15 krads) | Laboratory reared Females |
| | $NJ_{F16}$ - (LC) | 10-11 day old pupae (15 krads) | |

Male Dispersal:

Tests were also conducted to characterize dispersal patterns of adult moths. It is essential to the sterile male program that the dispersal patterns (vertical and horizontal) of sterile males compare favorably with those of wild males. Also, horizontal dispersal patterns must be quantified so that release points can be spaced for effective and efficient flooding of a target area.

Vertical dispersal patterns of wild and laboratory reared males were compared by placing modified delta traps, baited with Hercon®wicks (+ disparlure) at three heights on trees within the test plot; ground level, 1.5 m height and mid-crown in trees. In all 7 traps were placed at each height on the circumference of a 70 m radius circle. Alternately trap sites (20 m between sites) received one of the above three trap treatments.

Males were released over a 3-day period at the center of plots in the manner previously described. Traps were monitored on an hourly basis (10 a.m. to 6 p.m.) from the first day of release to 2 days post release. Traps were replaced when the tacky trap lining became congested with wing scales.

In a second series of dispersal tests designed to determine the effects of pheromone sources on male dispersal patterns, two plots were established and monitored simultaneously. Both plots were circular (140 m radius) and chosen for similarity of topography and vegetation type. In one plot, 2 concentric circles (70 m and 140 m radii) of traps (20 m spacing between traps) were established. Traps were only placed on the outer 140 m radius circle on the second plot. Males (irradiated and untreated laboratory-reared) were released from the center of both plots and allowed to disperse.

Delta traps used in these trials were modified with an additional entry port and were baited with Hercon wicks which contained (+) disparlure. Traps were monitored hourly from 10:00 a.m. to 6:00 p.m. for two days after the release. At each check all males were removed and identified and sorted according to strain and age.

In a separate trial designed to further explore dispersal, males were released in a plot with no pheromone sources. Two days after release pheromone baited traps were placed in the plot and trap catch was monitored daily.

Female Activity Periodicity:

Female eclosion patterns and calling periodicity may affect the success of a sterile male release program. If sterile males are not sexually active when feral females are available for mating, competitiveness would be impaired. Field trials in 1977 characterized the activity periodicity of feral males. However, little is known about female eclosion, calling and mating patterns. The objective of this study was to characterize eclosion and calling patterns of feral females.

Female pupae for this study were collected from a natural population (Abington, MA) and placed in 16 oz. squat cups (ca. 25/cup). Pupae were held in a field cage until eclosion. Continuous observation of the pupae provided exact eclosion times for individual females. As females eclosed they were individually placed in numbered cups and observed for initiation of "calling" behavior. Once calling had begun, females were removed from cups and placed on the sides of a field cage and observed for calling behavior throughout the day. At dusk and dawn females were checked for calling behavior and also female dispersal was observed and quantified. Concurrently males (newly eclosed and 1 and 2 days of age) in field cages were observed for dispersal activity.

## RESULTS AND DISCUSSION

### Male Competitiveness:

Results of 1978 field trials are currently being key punched for tabulation and analysis. Tabular summaries will be completed by early November and analysis finished by January. Preliminary sorting of a portion of the data however, indicates that several components of laboratory male competitiveness are comparable to feral males. In addition, preliminary findings have not identified any large deficiencies in the quality or performance of irradiated males.

In contrast to results of 1977 field trials, 1978 results indicate that activity periodicity of laboratory males appears to be synchronous with feral males. Generally the greatest proportion of laboratory males were found mating with females during the same time periods as wild males. One exception was during the first week of studies (Table 1 - Plot A). In this trial peak activity for feral males occurred earlier in the day than their laboratory counterparts (Fig. 2). However, in following trials the activity of the strains appears to be in concert (Fig. 3, 4, 5). There are several possible explanations for these observed differences. Males in the first trial (Fig. 2) were harvested from rearing as several day old pupae "hardened pupae" and held in an outside insectary until field release. In subsequent trials males were harvested as spins or less than 1 day old pupae and held in an insectary. It is possible that a hardened pupae is insensitive to light cues or incapable of adjusting its circadian rhythm; a very late stage larvae or newly formed pupae may still be sensitive to light and able to reset its internal clock. A second change which occurred and which could have effected male activity was a change in the light regimen in the rearing laboratory. Beginning in early July the light requirement for rearing areas was adjusted weekly to coincide with the natural outside conditions. The group of males used in trial 1 would not have experienced this change in photoperiod while in the larval stage; however, those larvae used in subsequent trials would have been exposed to adjusted light cues and possibly reset their biological clocks. If one or both of the factors acted to adjust male activity periodicity it appears that such an adjustment can be made very late in larval development. However, at the present time it is impossible to determine if one of the above or some other factor(s) correct the asynchronous behavior observed in trial 1 and in 1977. Presently, tests are being initiated to determine what the controlling mechanisms are and how best to manipulate these to the competitive advantage of sterile males.

Male Dispersal:

Horizontal dispersal and the resulting male distribution of the different strains appear to be similar. Laboratory-reared males, irradiated and non irradiated, were found mating in proportions similar to feral males at distances of 70 and 140 m from the release point. Also, there does not appear to be any real difference in the vertical distribution of male strains. This dispersal data when analyzed not only will provide a comparison of the various male strains but will give an estimate for spacing release points in an operational program. Males appear to disperse well to at least 140 m, indicating release points can be spaced 250 m - 300 m apart.

Male mating success is a summation of many components of competitiveness. It includes; dispersal, response to a pheromone gradient, mate location and components of precopulatory behavior. It has been used in many sterile male programs as the sole measure of male quality and provides an overall determination of success or failure. No obvious differences in the performance of the strains tested are apparent from a preliminary examination of the data. Laboratory reared males (sterile and fertile) appear to locate pheromone sources (virgin females) as well as their wild counterparts. Moreover, the age groups of laboratory and feral strains of males mating with females appears to be similar.

Female Activity Periodicity:

Female pupal eclosion patterns were similar to those exhibited by males. Eclosion began in the early morning hours and usually reached a peak before mid-day. The rate of eclosion tapered off in the afternoon hours and few insects eclosed in the evening or during the night. Onset of photophase appears to provide the cue for the initiation of eclosion, however, the rate appears to be mediated by temperature. When night and early morning temperatures were high, peak eclosion occurred at 11:00 a.m. (Fig. 6, 7 and 9). However, when night and early morning temperatures were very low, even though daytime temperatures increased rapidly, peak eclosion was delayed until the afternoon hours (Fig. 8).

Females initiate "calling behavior" shortly after eclosion. In all, 251 females were timed from eclosion to initiation of calling. The mean time to begin calling was 54 minutes. Once calling behavior started, females continued to call throughout the day and into the night hours. Female mating periodicity was not studied, however, the observations recorded on calling and eclosion periodicity indicate that greater than 50 percent of females eclosing on any one day are calling, and presumably available for mating, by 11:00 a.m. on that day. Feral male activity periodicity on warm days peaks at or before 11:00 a.m. If sterile male peak activity were shifted to the afternoon hours (See Fig. 2) they would be at a competitive disadvantage. Also, if some females are not mated on the day they eclosed the disadvantage for sterile males would be compounded. On the first full day of female life (the day after eclosion) ca. 90 percent of all females begin calling before 10:00 a.m.

An evening dispersal episode for newly eclosed males was noted in 1977 field trials. Males eclosing in the late afternoon remained sedentary until dusk at which time they became active and dispersed. In 1978, male pupae were placed in field cages and allowed to eclose. Observations were made on males for periods at dusk and dawn for the first three days of adult life. Female pupae were placed in separate field cages and allowed to eclose. However, in order to characterize the eclosion and calling patterns, observations were made throughout the day and at dusk and dawn.

Males (0, 1 and 2 days of age) were found to have dispersal episodes (15-20 minutes in length) at both dusk and dawn. Normally males are active throughout the day with little activity in the early morning or late evening hours. However, there is a "burst" of activity for short periods at the onset of photophase and scotophase.

Females, normally sedentary throughout the day, surprisingly become active and "disperse" at both dusk and dawn. At dusk, females (while still calling) begin to walk around rapidly; a portion of these females exhibit wing fanning behavior. Mapping of females three times a day demonstrated that movement is generally in a downward direction and averaged ca. 50 cm/dispersal period but in a few instances females moved as much as 280 cm.

The behavioral significance of these dusk and dawn redistribution periods is undetermined. Possibly they are adaptations to predator pressure, and movement is directed to sites which are more protected; or it may be these are vestigial remnants of a long abandoned behavioral pattern. Unless female downward movement is an artifact of test design, such as the absence of preferred calling sites on the sides of a field cage, it would serve to move females away from pupation sites and lower in the forest strata.

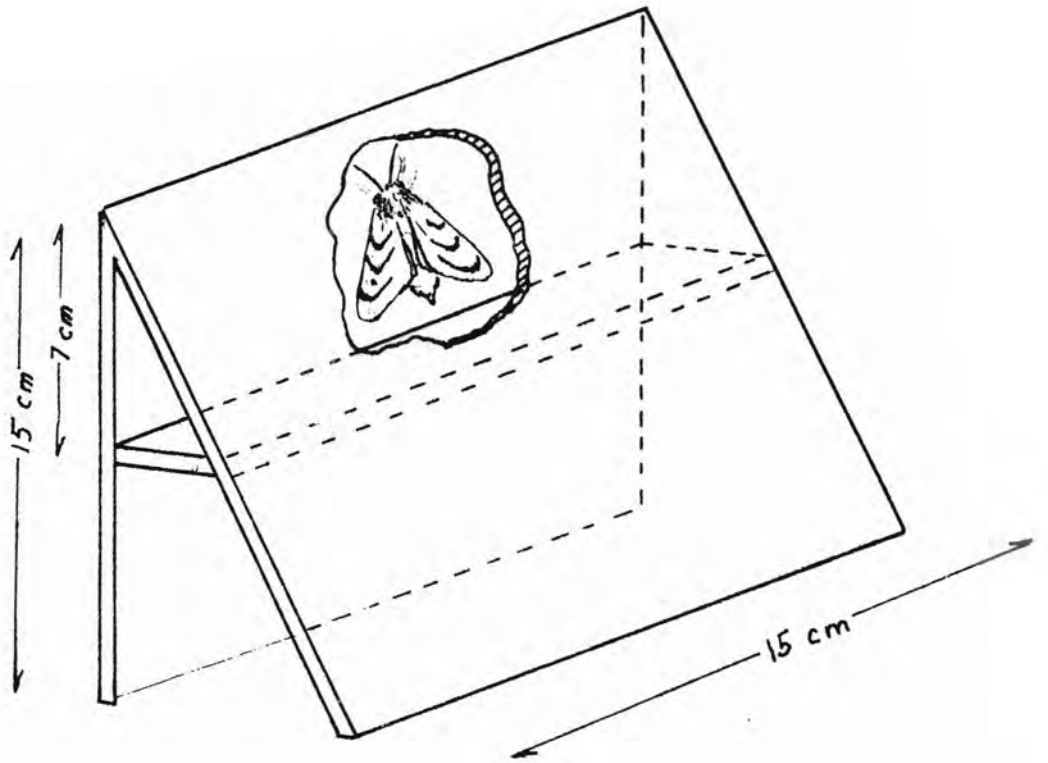
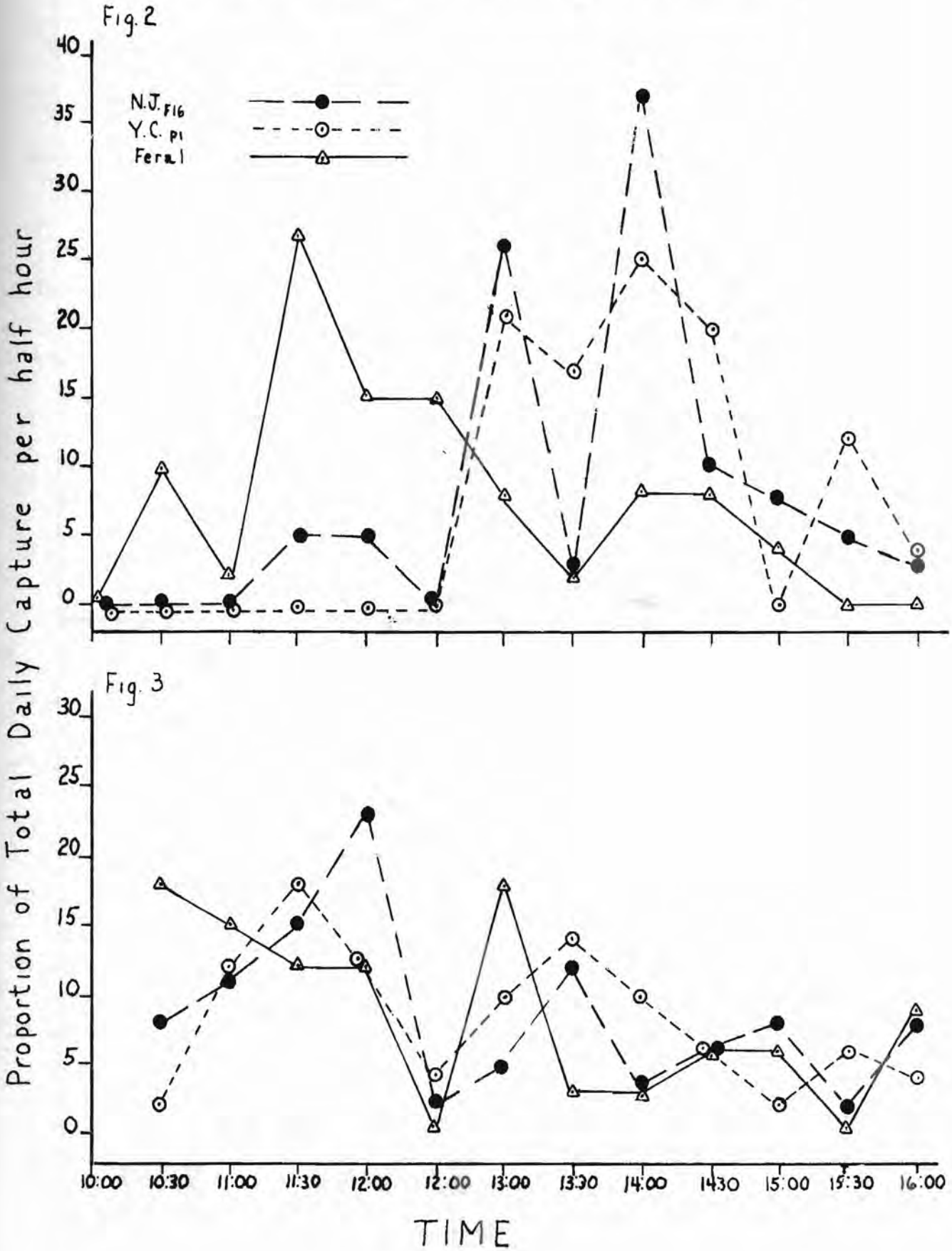
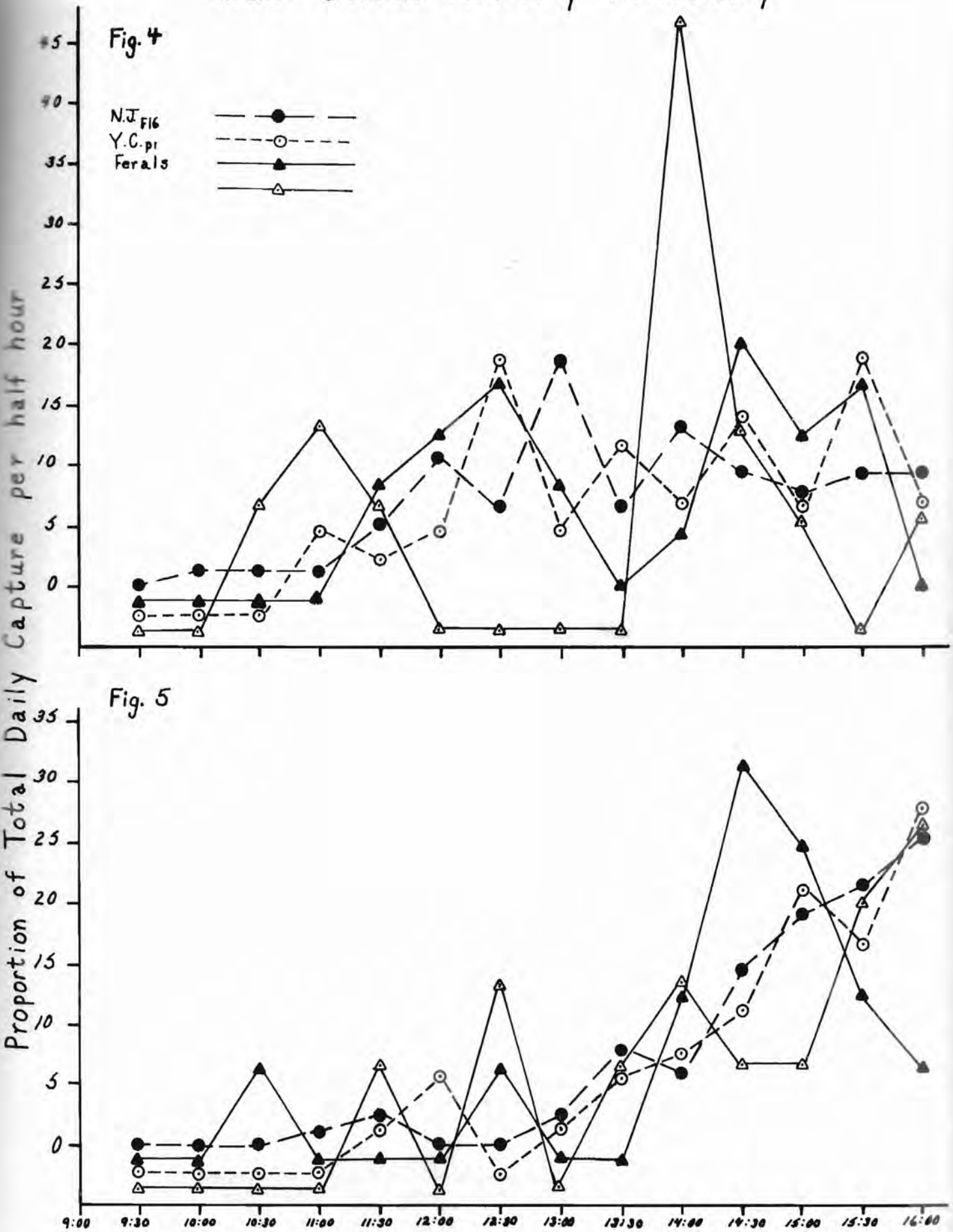



Fig. 1 - Standard Female Placement Niche for Male Competitiveness Comparisons

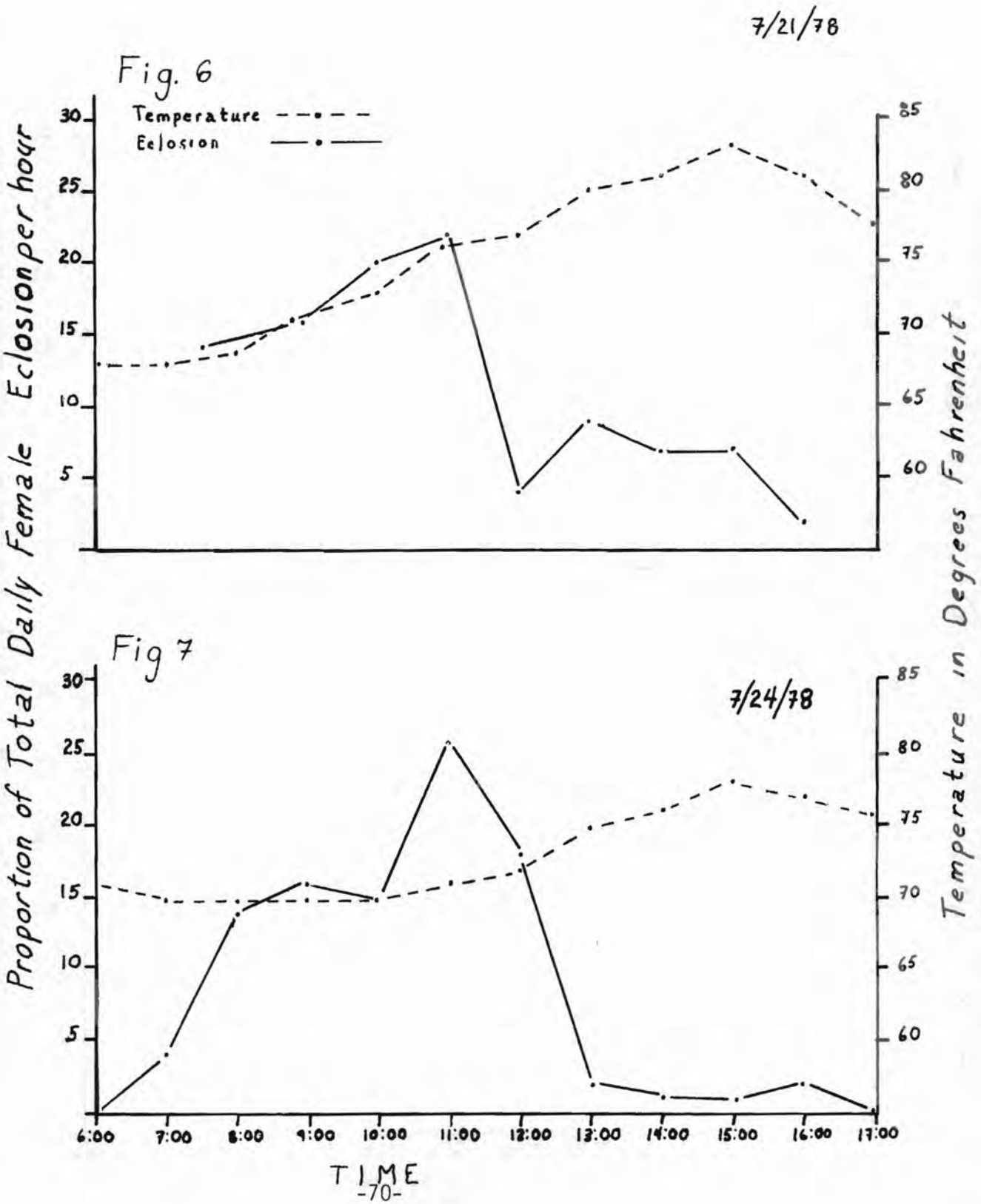
# Male Sexual Activity Periodicity



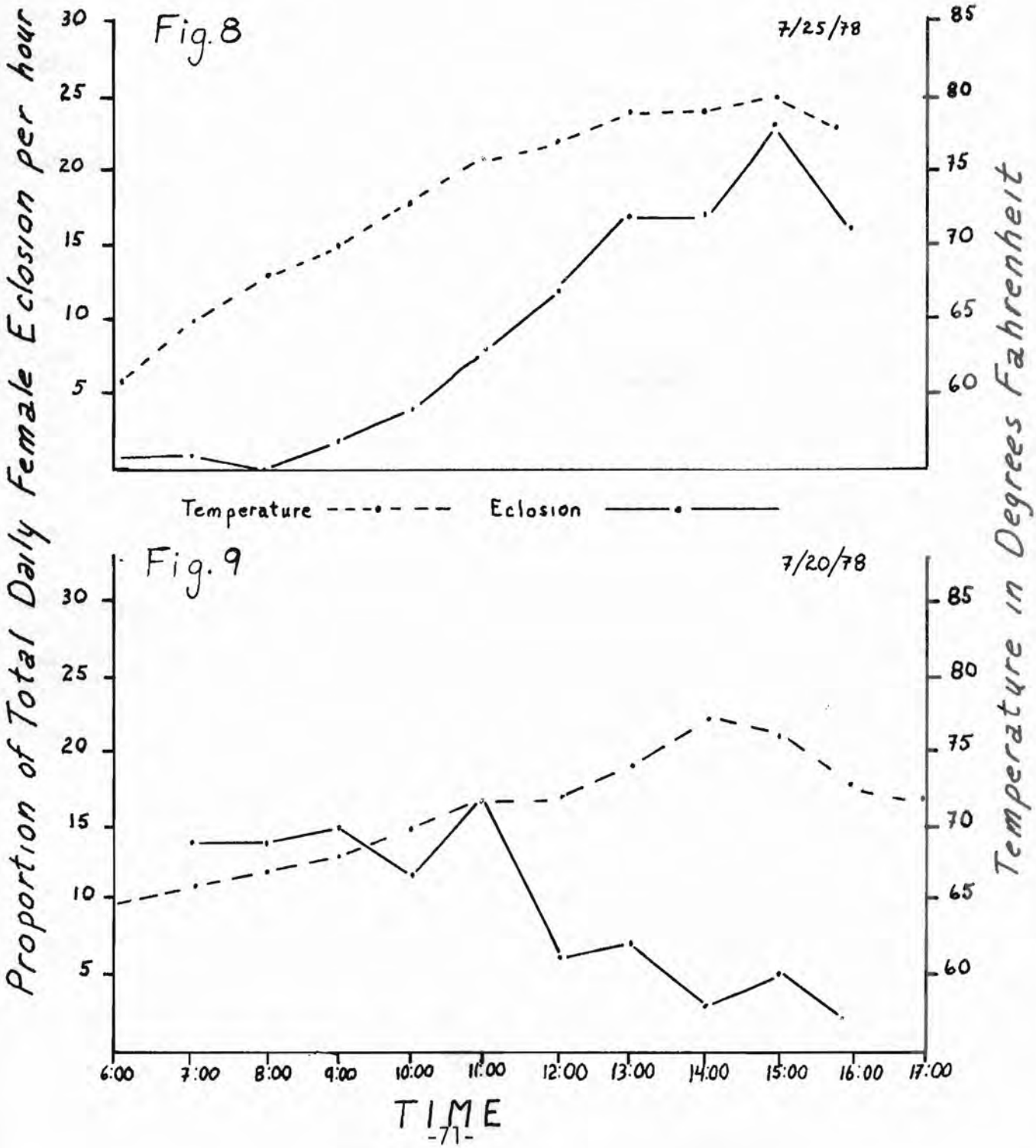
# Male Sexual Activity Periodicity



# Eclosion Rates of Feral Female Pupae



# Eclosion Rates of Feral Female Pupae



Project Number: GM 8.2.5
Project Title: Preliminary Evaluation of Large Capacity Traps
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: C. P. Schwalbe and E. C. Paszek

This project was undertaken to evaluate various designs of traps which have the capacity for capturing large numbers of gypsy moths. Because of limited surface area covered with Tack Trap, the delta trap only has capacity for 15-20 moths. Large capacity traps would be useful for research purposes and for monitoring adult density in the field. Six trap designs were tested:

Graham standard - These traps were included in the test as standards. Moths are captured by ensnarement in a Tack Trap liner and, therefore, capacity is limited to ca 50 moths.

Graham modified - The conical entry ports were modified with unidirectional baffles that retarded exit once males entered the trap. A 1" x 1" square of Vapona killed moths that entered.

Gerberg square - This is a commercially available trap which utilizes heavy unidirectional entry ports. Vapona was used to kill moths entering the trap.

Gerberg Crystal - This trap is basically the same size as the square version above. The geometry is slightly different.

Milk carton - This trap is constructed from 1/2 gallon milk carton. The design is discussed in more detail in GM 8.2.6. Vapona was used as a killing agent.

Quadra trap - This design is similar to an enlarged version of the delta trap. Vapona serves to kill insects entering.

Ten of each of the above traps were baited with a standard 1978 Hercon dispenser containing (+) disparlure. Traps were placed ca 20 m apart in a randomized complete block design near Taunton, MA. Traps were placed on 7-16 and checked on 7-19, 7-22 and 7-29.

Results of the test are summarized in Table 1.

Table 1. Numbers of male gypsy moths captured in traps of various designs. All traps were baited with the standard 1978 Hercon dispenser of (+) disparlure. Taunton, MA, 1978.

| Trap Design | Replicate 1/ 1 | 2 | 3 | Total |
|---|---|---|---|---|
| Graham Standard | 2/ | - | - | - |
| Graham Modified | 257 | 467 | 398 | 1022 |
| Gerberg Square | 440 | 828 | 626 | 1894 |
| Gerberg Crystal | 394 | 728 | 887 | 2009 |
| Milk carton | 408 | 812 | 744 | 1964 |
| Quadra | 135 | 214 | 212 | 561 |

1/ Values are total males captured in 10 traps

2/ These traps were excluded from the evaluation because they filled with moths between readings.

Results of this test indicate that both Gerberg designs and the milk carton trap captured similar numbers of male moths. Other designs were less effective. Because of the low cost of the milk carton traps, this design was selected for further development and improvement of efficiency. Those tests are covered in GM 8.2.6.

Project Number: GM 8.2.6
Project Title: Large-Capacity Milk Carton Traps - Design Improvements
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leader: E. C. Paszek

Introduction:

In the summer of 1978 a large capacity 1/2 gal milk carton was tested for trapping large numbers of moths in dense infestations. The prototype trap had 1-1/4" diameter plastic baffled entrance ports inserted into holes punched in each of the 4 side panels. The ports were protected by 6x6x8-1/2" heavy waxed paper overhangs that were glued to the sloping top of the milk carton above each entrance port. This feature also tended to concentrate male searching in the vicinity of the entry port. Mylar dispensers containing (+) disparlure and 5.25 g sections of vapona (no pest strip) were wired to a twist-em plant tie, suspended inside the trap and attached to the pouring spout. The trap was serviced through the bottom which was sealed with 1" masking tape. This trap has a capacity of at least 800 moths; the delta survey trap becomes saturated with 25 moths.

This large capacity trap had design deficiencies. For example, the plastic baffled popped out in the field allowing captured moths to escape. Some moths had difficulty in entering through the baffled ports and had to wedge themselves between the stiff prongs of the baffles. Furthermore, the traps required too much time to assemble and were somewhat fragile. Experiments were set up late in the flight season in which the delta trap and several modifications of the milk carton (MC) trap were compared.

In the first test, delta traps were compared with the prototype plastic baffled MC traps (described above). Both trap designs were baited with (+) disparlure in Mylar dispensers. The MC traps had 5-1/4 g vapona strips as a killing agent and the delta traps contained tack trap as the moth holding agent. The MC baffled traps were hung on the branches of small saplingsin order to expose the 4 entrance ports. Delta traps were alternately hung on branches or other attached to tree trunks with ca 30" dbh. In these tests, the delta traps were replaced with new traps after each count and the MC baffled traps were emptied of their moths and reused. Results are summarized in Table 1.

Table 1. Comparison of MC baffled trap with delta survey trap. Values are the average of 12 replicates. Dighton Rock, MA. August 3-16, 1978.

| | Date Examined | | | |
|---|---|---|---|---|
| Trap Design | 8-3 | 8-4 | 8-9 | 8-16 |
| MC | 46.5 | 14.4 | 4.3 | 11.3 |
| Delta stapled to trunk | 25.1 | 9.8 | 5.0 | 12.3 |
| Delta suspended from limb | 22.5 | 9.6 | 3.3 | 12.0 |

In the first reading on 8-3 the MC baffle trap captured more moths than the delta because the flight was heavy and the delta's became saturated with moths. In the remaining three bioassays 8-4, 8-9 and 8-9 to 8-16 the MC compared favorably with the delta trap. Similar numbers of moths were captured in the delta traps hung on the tree trunk and those hung away from the tree trunk on a branch.

A preliminary test was conducted to determine if capture would change with increased pheromone concentration. Twelve traps were baited with one Mylar dispenser and 13 were baited with 4 dispensers. Traps were placed on a 5x5 grid with 20 m between traps. On 8-30-78,700 laboratory reared moths were released within the trap grid.

Two hundred and twenty six moths were captured (32.5% recovery). The MC baffled traps baited with 1 dispenser captured 129 moths (18.4%) and those baited with 4 dispensers captured 97 moths (13.8%). It appears that traps with lower disparlure dosage are more efficient in capturing moths than the traps with the higher dosage. This is consistent with other studies where effectiveness decreases with increasing release rates.

Another test compared efficiency of traps with 12 slotted entry ports (1"x5/16") (3 per side) with the standard baffled design. Also, in this test, the hood was constructed from a single piece of cardboard which slips over the trap. (See Figure 1). Six replicates of each design were placed on a 3x4 grid (20 m between traps) and 1200 laboratory reared moths were released on 9-7-78. Of the 1200 moths released, 587 (48.0%) were recovered in the 12 traps. The modified MC trap with the 12 slotted 5/16x1" entrance ports captured 409 moths (34%). The baffled MC trap with the four 1-1/4" baffled entrances captured 178 moths (14.8%).

The modified MC trap with the 12 slotted entrances is 2.3 times more efficient than the MC baffled trap. This trap can be serviced through its pouring spout allowing the bottom to be sealed or taped. Its hood can be cut out in one piece, creased, stapled and nested for storage. Further testing was a comparison of the following 3 traps: 1/2 gal baffled MC with four 1-1/4" baffled entrance ports; 1/2 gal MC with 12 unbaffled 5/16"x1" slots; and a MC with four 1" diameter pie shaped openings each one having its 8 partially cut section (prongs) bent into the trap in order to act as a unidirectional baffle to prevent moth escape. Traps were baited with Mylar dispensers and placed in a 3x4 grid and spaced 20 m apart; 900 moths were released.

Two hundred and sixty moths were captured (29% recovery). The MC slotted trap captured 144 moths (16%), the MC pit shaped 8 prong design captured 69 moths (7.6%) and the MC baffled trap captured 47 moths (5.2%). The MC slotted trap outcaptured the MC baffled trap 3 to 1 and the MC pie shaped 8 prong trap 2 to 1. These traps were left in the field for 72 hours and the prongs of the pie shaped entry ports retracted back and blocked entry. The MC slotted trap is the most efficient in capturing moths in this bioassay. The following 3 traps were compared: 1/2 gal baffled MC with four 1-1/4" baffled entrance ports, 1/2 gal MC with 12 unbaffled 5/16"x1" slots, and a MC with 4 long slots 2-3/4x5/16". Traps were placed in a 4x4 trid and spaced 20 m apart. Nine hundred moths were released on 9-21-78. Of the 900 moths released 313 (35%) were recovered. The modified MC with 12 unbaffled 5/16x1" slots captured 175 moths (19.4%) The modified MC with 4 long slots (2-3/4"x5/16") captured 86 moths (9.5%) and the MC baffled trap captured 52 moths (5.7%). The modified MC with the 12 unbaffled slots outcaptured the MC baffled trap 3 to 1 and the modified MC with 4 long slots 2 to 1. The modified MC trap with the 12 unbaffled slots is the most efficient trap in this bioassay.

A series of escape tests were conducted in the greenhouse to determine escape of moths from the following traps: 1/2 gal modified MC trap with 12 slotted entrances 1" x 5/16" and baited with (+) disparlure treated with d-phenothrin (10% aerosol) and DDVP. The d-phenothrin treatment was a 3 second spray from the aerosol can into the trap. DDVP treatment was a 5.25 g piece of Shell No-Pest-Strip. Additionally, a number of variations in the hood were tested. Twelve male moths were periodically introduced into the traps. After 3 days, the percent escape was determined. Results are summarized in Table 2.

Table 2. Percentage of moths escaping from traps treated with d-phenothrin and DDVP.

| Trap Design Hood Variation | Untreated | 0-day | 3-day | 7-day | 17-day | 25-day | 33-day |
|---|---|---|---|---|---|---|---|
| Hood underside shaded green with d-phenothrin | 29 | 15 | 15 | 30 | 20 | 0 | 0 |
| Hood underside white with d-phenothrin | 58 | 50 | 27 | 65 | 45 | 0 | 0 |
| Hood removed with d-phenothrin | 92 | 46 | 38 | 60 | 10 | 7 | 4 |
| Standard trap with DDVP | - | 15 | 0 | 0 | 0 | 4 | - |

In the first 5 tests, moths were introduced into the traps when temperatures were over 80°F; consequently, escape was high. In subsequent tests they were introduced outdoors into the traps where the cool temperature sedated them. It takes from 1 to 3 hours for d-phenothrin to knock down the moths and they continue to thrash and beat their wings on the bottom of the trap until their wings become skeletonized. The DDVP immobilizes moths in 5 to 7 minutes. They fall to the bottom of the containers with most insect scales attached. The trap design with the shaded hood somewhat darkens the entrance ports and seems to minimize escape.

Another insecticide test began 12/11/78 with a larger sample of 10 traps, 15 moths per trap were used. This test compared 3 sizes of DDVP (5.5, 2.7 and 1.3 g). The average length of time to knockdown was determined. Results of this test are in Table 3.

Table 3. Length of time required for knockdown of male moths in milk carton traps containing various amounts of DDVP. (Average of 3 replicates).

| WT. of DDVP | Length of Time to Knockdown (min) | % Escape |
|---|---|---|
| 1.3 | 17.6 | 0 |
| 2.7 | 11.3 | 0 |
| 5.5 | 7.0 | 0 |

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| Standard trap with DDVP | - | 15 | 0 | 0 | 0 | 4 | - |

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|---|---|---|
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| 2.7 | 11.3 | 0 |
| 5.5 | 7.0 | 0 |

It took the 1.3 g insect strips an average of 17 minutes to knock down the moths, the 2.7 g strips 11 minutes and the 5.5 g strips 7 minutes. No moths escaped from the traps. Since no escape took place in any of the treatments, there doesn't seem to be an advantage to using larger quantities of DDVP.

In another test moths were introduced outdoors into untreated traps baited with (+) disparlure. Two entry port sizes were tested: 1) 4 ports (2.75 x 5/16") and 2) 12 ports (1 x 5/8"). Two hood colors were also evaluated.

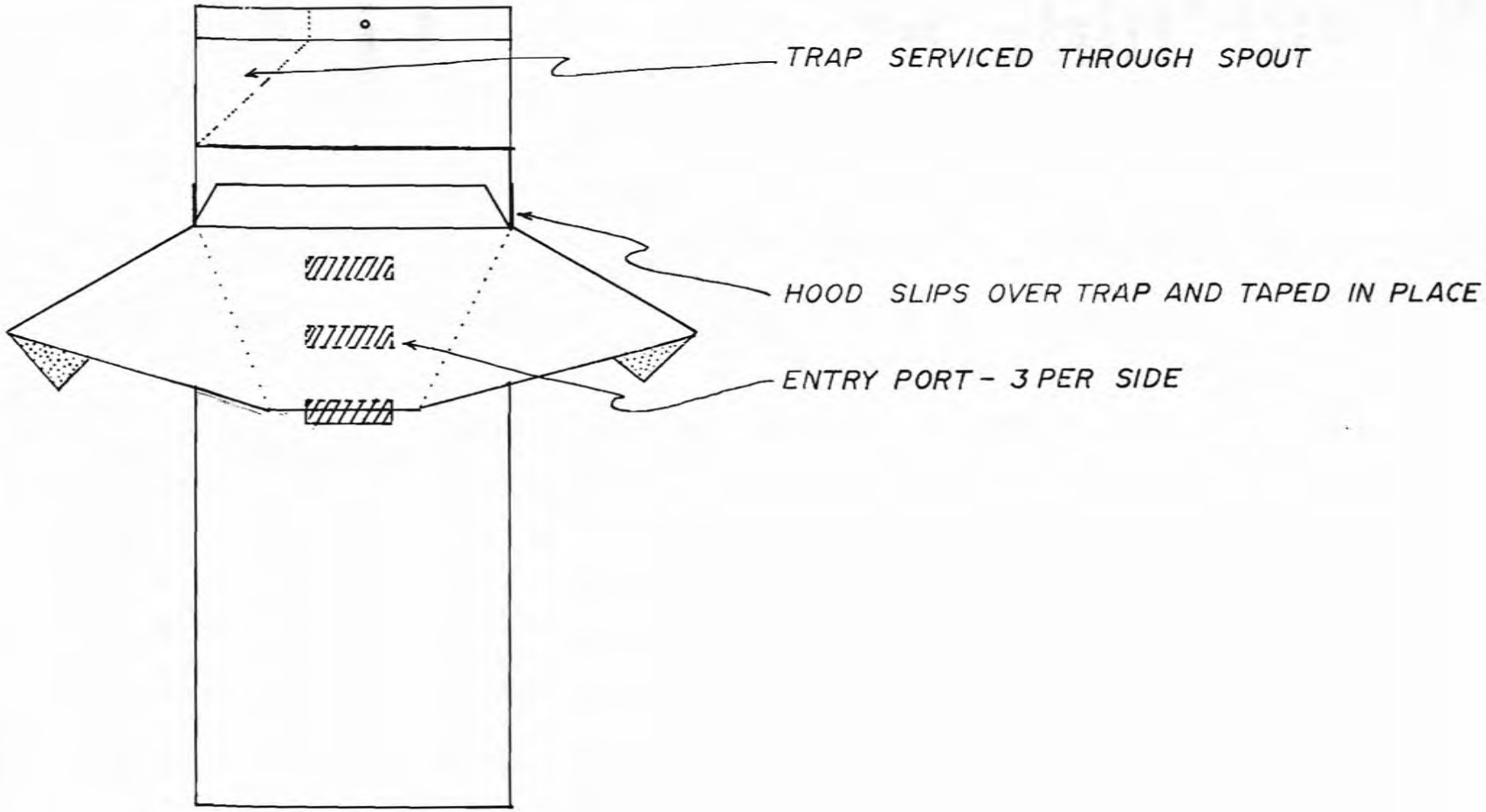
Results:

Table 4. Percent of moths escaped from 4 designs of MC trap.

| Trap Type and Color | Percent Escaped |
|---|---|
| 12 ports - black hood | 4 |
| 12 ports - orange hood | 30 |
| 4 ports - black hood | 12 |
| 4 ports - orange hood | 12 |

Four percent of the moths escaped in 8 days from 1/2 gal MC with 12 slotted entrance ports with the hoods dyed black. Thirty percent of the moths escaped in similar design traps with the hoods orange in color. The escape rate was similar, 12% from both the black and orange hood samples of the 1/2 gal modified MC with the 4 slotted entrance ports. The test indicates that untreated traps hold moths without the use of insecticides. These trap modifications with the hoods over the entrance ports make the traps much more efficient when insecticides are used in them as killing agents.

From these tests, the following is a description of a trap recommended for capturing large numbers of moths in dense populations: 1/2 gal milk carton trap with twelve 1" x 5/16" entrance ports per trap. Three of these openings are centrally placed on the upper portion of each of the four 7-1/2" x 3-3/4" trap panels. They are spaced 1/2" apart with top opening 1" from the top of each panel. A 3-3/4" x 3-3/4" hood slips over the trap with its 1" crown fitting tightly to the milk carton. The bottom 2-3/4" section of the hood projects away from the milk carton like an awning and at its lowest point is 1-3/4" away from it. The trap is serviced through the pouring spout which may be secured in the closed position with a staple or with a small spring type binder clip. (See Figure 1).


TRAP SERVICED THROUGH SPOUT
HOOD SLIPS OVER TRAP AND TAPED IN PLACE
ENTRY PORT - 3 PER SIDE

SCALE, 1.0" = 0.5"

Project Number: GM 8.2.7
Project Title: 1978 Field Evaluation of Microcapsular Formulations of Disparlure
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: C. P. Schwalbe, E. C. Paszek

In 1976 a series of field tests were performed to evaluate the effectiveness of seven candidate controlled release formulations of disparlure in disrupting gypsy moth mating. In those tests, application rate was 8 g/A. The incidence of mating throughout the entire season in treated plots was reduced as much as 83% with the most effective formulation (2% NCR microcapsules). However, 27% mating still occurred during peak male flight and it was felt that the formulation performance was still inadequate for pilot testing. Therefore, additional formulation research was conducted by SEA-AR. This report details field evaluations of a newly developed candidate formulation. The 1978 formulation was similar to that used in 1976, but capsule size was reduced to ca. 50 μ. In addition to testing the new formulation, dosage rates were increased 2.5 fold to 20 g/A in an effort to improve performance.

All tests were conducted in the Fall River State Forest. Each formulation was sprayed onto 40 A plots on July 16. After treatment, 10 female moths were placed in each plot. They were replaced every other day. Also, 10 traps were placed in each plot and they were examined every other day. These evaluations were carried out from 7/27 to 8/24 when male activity terminated.

Table 1. Experimental design of mating disruption tests. Fall River, 1978

| Formulation 1/ | No. Plots | Application Volume | Application Rate (AI) |
|---|---|---|---|
| Check | 3 | - | - |
| 1976 2% NCR | 3 | 10 pt/A | 20 g/A |
| 1978 2% NCR | 3 | 10 pt/A | 20 g/A |
| 1978 2% NCR | 3 | 1 pt/A | 2 g/A |

1/ All formulations contained 1.1% RA-1645 as a sticker.

Male moth activity in the treated and control plots is tabulated in Table 2.

Table 2. Male moth activity in disparlure-treated and control plots. Fall River, 1978.

| Formulation | Dosage | Total No. ♂♂ trapped 1/ | No. ♀♀ Determined | % ♀♀ Mated |
|---|---|---|---|---|
| Control | - | 1237 | 294 | 65 |
| 1976 2% NCR | 20 g/A | 147 | 290 | 3 |
| 1978 2% NCR | 20 g/A | 170 | 201 | 2 |
| 1978 2% NCR | 2 g/A | 812 | 276 | 16 |

1/ Total males captured in 30 traps in the 3 replicate plots.

Total trap catch and the incidence of female mating was significantly reduced in all treated plots. A number of observations warrant special comment: trap catch and incidence of mating were lower in plots treated with 20 g than in those where 2 g/A were applied. This indicates that the degree of mating disruption is dependent upon the amount of material applied and, therefore, upon the performance of the slow release formulation. There did not seem to be any differences in the data between the 1976 and 1978 20 g/A formulation plots. Either the reduced capsule size in the 1978 formulation did not result in increased effectiveness or population levels or application rates were such that resolution between the two efficient formulations was not possible. It is possible that results in the 1976 tests would have been more favorable if the increased dosage of 20 g/A would have been used. However, differences between the two formulations may be more obvious at lower application rates or higher insect population densities. It also appears that the incidence of mating is correlated with the number of males captured in (+) disparlure-baited traps. If this pattern is confirmed with other studies, it may be possible to use traps as monitors of efficacy in larger scale trials of mating disruption.

It is felt that the results of this study are sufficiently encouraging to conduct a pilot scale test in a natural low-level, isolated infestation in 1979. Plans for this trial are presently being prepared.

Project Number: GM 6.3.6
Project Title: Establishing Standards
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: John Allen Tanner, Donna Houle

## Introduction:

The purpose of this project is to explain the current insect quality monitoring techniques used in the rearing facility. Data is collected for each strain/generation and will be used to establish standards, develop life tables, and detect changes from normal development so that corrective action can be taken.

## Developmental Biology:

The ensuing procedure is followed for each laboratory strain. Sampling is random and commences with neonates infested on B-4 diet (high wheat germ) the Tuesday of each week. On the first post-infestation day, ten cups are selected and the mean number of larvae/cup determined. On the 7th, 14th and 21st post-infestation day, five cups are selected and the mean larval weight, the percentage of larvae/developmental stage, and the percent survival determined.

Percent survival is estimated by the following formula:

$$\frac{\bar{x} \text{ larvae/cup - weighing day}}{\bar{x} \text{ larvae/cup - day one post-infestation}}$$

The average of the three percentages is used to express larval survival from infestation to the 21 post-infestation day. Larvae are sexed on the 21st post-infestation day and fed modified hormworm diet. Males are consolidated 15/cup and females 5/cup. Three cups of males and 8 cups of females are selected and specified for quality control use. Each cup is examined daily to determine the pupation rate. The pupae are harvested and segregated by sex. The mean pupal weight, the accuracy of larval sexing, the percent pupal survival, and the percent pupal deformity are determined for each sex.

The adults are allowed to emerge. Ten newly emerged males are mated to determine male longevity and fertility. The remaining adults are examined each week day for deformity then discarded. Percent emergence, percent deformity, and percent survival from neonate to adult are determined.

Fecundity and egg hatch data is taken directly from the colony. The females were reared at approximately the same time as the males used to determine longevity and fertility. A mating container (20-25 matings) is selected each week on the first post-mating day. The masses are allowed to embryonate for 28 days. The percentage of females laying egg masses is determined. Ten masses are selected and weighed. Each weight is multiplied by 1.33 to estimate the number of eggs/mass. The masses are returned to the colony for chilling.

After 90 days of chilling, a sample of five masses is taken and a hatch test conducted. Twenty eggs are excavated from the core of each mass and incubated in petri dishes.

Sex ratio information is precluded by the sampling technique used after larval sexing. To obtain this information, a group of larvae (10 cups, 15/cup) are infested the same day as the quality control larvae. The larvae are allowed to develop to adults without sampling. These larvae are also sexed on the 21 post-infestation day but all are retained. One group is started each week for the NJ strain and each month for the minor strains.

Results:

The developmental biologies for the New Jersey, Ludlow and Pennsylvania strains are presented in tables 1-3. Each table is segregated into four columns. Column one data was presented in the last report (Oct. 1, 1977 - March 31, 1978). Column two data concerns larvae infested onto modified hornworm diet and transferred to fresh modified hornworm diet on the 14 and 28 post-infestation day. Column three and four data concerns larvae infested onto B-4 diet and transferred to fresh hornworm diet on the 21st post-infestation day. Column four insects were handled as described under the developmental biology section.

The B-4-hornworm diet combination and feeding schedule resulted in larger pupae and increase female fecundity compared to the hornworm diet-feeding-schedule. The former diet-feeding schedule also increased male longevity in the Pennsylvania and Ludlow strain and slanted the sex ratio towards a predominance of males.

Table 4 presents the developmental data for the wild strain collected at Yards Creek, New Jersey. A major rearing "bottleneck" occurred during the larvae stage. Only 53% of the male larvae and 58% of the female larvae reached the adult stage. However, 92% of the male larvae and 94% of the female larvae, that pupated, emerged as adults.

Table 5 describes the accuracy in sexing larvae on the 21 post-infestation day. In all cases the mean accuracy was above 90% for both males and females.

Colony Hatch Test:

Hatch tests are conducted with colony egg masses after they have been chilled 90 days and at the time they are utilized in the rearing program (colony hatch test). The 90 day hatch test estimates the number of eggs that must be incubated to provide neonates for program maintenance. The 90 day hatch test also provides advance warning of low hatch periods and allows long term planning and adjustment. The colony hatch tests estimate the accuracy of the 90 day hatch test and examine the effects of treating eggs with formalin

Individual egg masses are sampled as described under the developmental biology section. Ninety day hatch tests are established weekly for the New Jersey and Yards Creek strain, and biweekly for the remaining strains. Fifteen masses are used in each test. Five masses are drawn from egg masses initially chilled at the beginning of a week, five from masses chilled in the middle of a week, and five from masses chilled at the end of a week

The colony hatch test is set up weekly for all the strains. New Jersey masses are sampled before and after the formalin treatment. The other strains are sampled only after the formalin treatment.

Results:

Fig. 1 presents the hatch rates for New Jersey eggs chilled 90, 120 and 180 days. The ninety day hatch tests showed two periods of low hatch, one in mid March and the other in May. The hatch in May was less than 10%. Lately the hatch has increased.

There is an indication that the 90 day hatch test does not adequately estimate the true hatch. Egg masses with low, 90 day hatch are producing considerably higher hatch after 120 days of chilling. Further testing will be necessary before a final hatch test technique is established.

Air Quality Test:

Air quality tests are performed weekly. Each test consists of an air particle count and a microbial plate count. Air particle counts (>0.5 ul) are taken with a Coulter Particle Counter (Model #550). Two or three different locations are chosen within a room. Two counts are taken in the morning and two counts are taken in the afternoon at each location.

Microbial contamination is measured with Trypticase Soy Agar (TSA) plates. Two or three TSA plates are placed into each room. The plates are exposed to the air for 6 hours (10 a.m. to 4 p.m.), incubated (32$^{o}$C) for 48 hours, then refrigerated until counted. The numbers of bacteria and fungal colonies are determined but not identified.

Results:

Particle counts have been persistently below the maximum acceptable level of 10000/$ft^3$ of air/min (Owens, Personal Communication). The major microbial contaminate has been bacteria. The major source of contamination seems to be the workers. However, little bacteria or fungi has been found growing on our diet.

The techniques described above may be changed over the next several months. In depth description of these changes will appear in future reports as they occur.

Table 1. Performance of the New Jersey Laboratory Strain Gypsy Moth Under Mass Production.

| | $NJ_{F16}$ 1/ | $NJ_{F16}$ 2/ | $NJ_{F16}$ 3/ | $NJ_{F16}$ 4/ |
|---|---|---|---|---|
| Eggs laid/female | 755±88 | 616±25 | 733±29 | 757±89 |
| % Hatch 5/ | 33 | - | - | - |
| $HT_{50}$(days) | - | - | - | - |
| Pupal weights | | | | |
| Males (gms) | .60±.02 | .52±.06 | .59±.04 | .60±.05 |
| Females (gms) | 1.98±.10 | 1.72±.17 | 2.01±.11 | 2.06±.08 |
| Developmental Time | | | | |
| To Adults ($DT_{50}$- days) | | | | |
| Males | 44±2.0 | 41±0.7 | 42±1.0 | 40±0.5 |
| Females | 46±0.3 | 42±1.0 | 43±2.0 | 41±0.9 |
| % Adult Deformative | | | | |
| Males | 3 | 3 | 3 | 0.3 |
| Females | 21 | 10 | 15 | 15 |
| % Adult Recovery | | | | |
| Males | 95 | 93 | 96 | 96 |
| Females | 93 | 96 | 90 | 90 |
| % Survival | | | | |
| (Larvae to Adults) | | | | |
| Males | - | - | - | 82±10 |
| Females | - | - | - | 81±09 |
| Sex Ratio (Male/Female) | 1.0 | .9 | 1.6 | - |
| % Mating Pairs | 95 | 98 | 93 | 98 |
| Male Longevity | | | | |
| ($Lt_{50}$-days) | 6.3±0.8 | 6.1±0.4 | 6.7±0.3 | 6.2±0.6 |

1/ Neonates infested onto modified hornworm diet and refed modified hornworm diet on the 14 and 28 post-infestation day - data from the Oct. 1, 1977 - March 31, 1978 Report.

2/ Neonates infested onto modified hornworm diet and refed modified hornworm diet on the 14 and 28 post-infestation day - data obtained after March 31, 1978.

3/ Neonates infested onto high wheat germ diet and refed modified hornworm diet on the 21st post-infestation day.

4/ Neonates infested onto high wheat germ diet and the larvae sexed and refed hornworm diet on the 21st post-infestation day.

5/ Eggs chilled 90 days.

Table 2. Performance of the Ludlow Laboratory Strain Gypsy Moth Under Mass Production.

| | $Lud_{F15}$ 1/ | $Lud_{F16}$ 2/ | $Lud_{F16}$ 3/ | $Lud_{F16}$ 4/ |
|---|---|---|---|---|
| Eggs laid/female | 732±90 | 576±75 | 769±77 | 830±44 |
| % Hatch 5/ | 37 | - | - | - |
| $HT_{50}$(days) | - | - | - | - |
| Pupal weights | | | | |
| Males (gms) | .59±.02 | .48±.02 | .58±.03 | .54±.04 |
| Females(gms) | 1.98±0.2 | 1.61±.03 | 1.89±.09 | 2.07±.17 |
| Developmental Time to Adults ($DT_{50}$-days) | | | | |
| Males | 44±1.0 | 41±1.0 | 43±0.5 | 41±1.0 |
| Females | 45±2.0 | 43±2.0 | 43±1.5 | 42±1.0 |
| % Adult Deformative | | | | |
| Males | 6 | 4 | 3 | 2 |
| Females | 17 | 7 | 13 | 6 |
| % Adult Recovery | | | | |
| Males | 98 | 93 | 96 | 93 |
| Females | 90 | 99 | 96 | 90 |
| % Survival (Larvae to Adults) | | | | |
| Males | - | - | - | 84±07 |
| Females | - | - | - | 77±11 |

Table 2. (Cont'd)

| | $Lud_{F15}$ 1/ | $Lud_{F16}$ 2/ | $Lud_{F16}$ 3/ | $Lud_{F16}$ 4/ |
|---|---|---|---|---|
| Sex Ratio | | | | |
| (Male/Female) | 1.3 | 1.0 | 1.3 | - |
| % Mating Pairs | 93 | 95 | 96 | 94 |
| Males Longevity | | | | |
| ($LT_{50}$- days) | $6.2 \pm 0.7$ | $5.0 \pm 0.5$ | $6.6 \pm 0.5$ | $5.8 \pm 1.0$ |

1/ Neonates infested onto modified hornworm diet and refed modified hornworm diet on the 14 and 28 post-infestation day - data from the October 1, 1977-March 31, 1978 Report.

2/ Neonates infested onto modified hornworm diet and refed modified hornworm diet on the 14 and 28 post-infestation day - data obtained after March 31, 1978.

3/ Neonates infested onto high wheat germ diet and refed modified hornworm diet on the 21st post-infestation day.

4/ Neonates infested onto high wheat germ diet and the larvae sexed and refed modified hornworm diet on the 21st post-infestation day.

5/ Eggs chilled 90 days.

Table 3. Performance of the Pennsylvania Laboratory Strain Gypsy Moth Under Mass Production.

| | $PA_{F7}$ 1/ | $PA_{F8}$ 2/ | $PA_{F8}$ 3/ | $PA_{F8}$ 4/ |
|---|---|---|---|---|
| Eggs laid/female | 738±95 | 634±80 | 842±75 | 791±58 |
| % Hatch 5/ | 29 | - | - | - |
| $HT_{50}$ (days) | - | - | - | - |
| Pupal weights | | | | |
| Males (gms) | .60±.02 | .51±.02 | .63±.03 | .56±.02 |
| Females (gms) | 1.99±.10 | 1.73±.09 | 2.15±.04 | 2.07±.18 |
| Developmental Time to Adults ($DT_{50}$-days) | | | | |
| Males | 45±3.0 | 41±1.0 | 44±1.0 | 42±0.7 |
| Females | 46±3.0 | 43±0.5 | 45±1.0 | 43±0.7 |
| % Adult Deformative | | | | |
| Males | 4 | 2 | 2 | 0 |
| Females | 19 | 13 | 17 | 10 |
| % Adult Recovery | | | | |
| Males | 96 | 95 | 96 | 96 |
| Females | 97 | 99 | 90 | 96 |
| % Survival (Larvae to Adults) | | | | |
| Males | - | - | - | 85±06 |
| Females | - | - | - | 82±12 |
| Sex Ratio | | | | |
| (Male/Female) | 1.2 | 1.0 | 1.3 | - |
| % Mating Pairs | 94 | 95 | 100 | 100 |
| Male Longevity | | | | |
| ($LT_{50}$- days) | 6.1±0.6 | 5.6±0.7 | 6.5±0.4 | 6.5±0.5 |

1/ Neonates infested onto modified hornworm diet and refed modified hormworm diet on the 14 and 28 post-infestation day - data from the Oct. 1, 1977 - March 31, 1978 Report.

2/ Neonates infested onto modified hormworm diet and refed modified hornworm diet on the 14 and 28 post-infestation day - data obtained after March 31, 1978.

3/ Noenates infested onto high wheat germ diet and refed modified hornworm diet on the 21st post-infestation day.

4/ Neonates infested onto high wheat germ diet and the larvae sexed and refed hornworm diet on the 21st post-infestation day.

5/ Eggs chilled 90 days.

Table 4. Performance of the Yards Creek wild Strain Gypsy Moth Under Mass Production.

| | Y.C. (Wild)[1/] |
|---|---|
| Eggs laid/female | 840±45 |
| % Hatch [2/] | - |
| $HT_{50}$ (days) | - |
| Pupal Weights | |
| Males (gms) | .55±.03 |
| Females (gms) | 2.13±.23 |
| Developmental Time to Adults ($DT_{50}$ - days) | |
| Males | 44±0.3 |
| Females | 44±1.0 |
| % Adult Deformative | |
| Males | 0 |
| Females | 6 |
| % Adult Recovery | |
| Males | 92 |
| Females | 94 |
| % Survival (Larvae to Adults) | |
| Males | 53±07 |
| Females | 58±17 |
| Sex Ratio | |
| (Male/Female) | - |
| % Mating Pairs | 96 |
| Males Longevity | |
| ($LT_{50}$ -days) | 6.1±0.6 |

1/ Neonates infested onto high wheat germ diet and the larvae sexed and refed modified hornworm diet on the 21st post-infestation day.

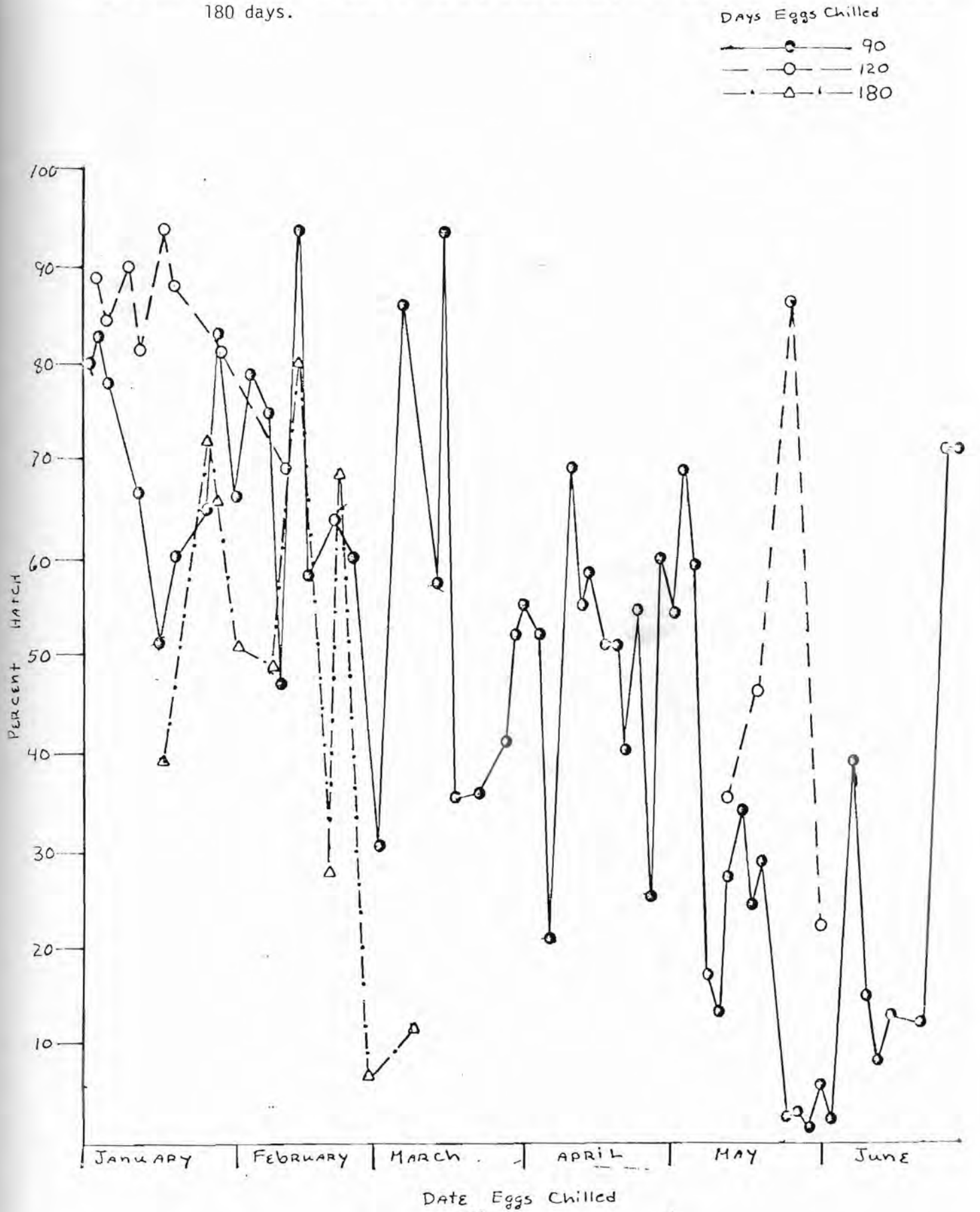
2/ Eggs chilled 90 days.

Table 5. The Accuracy of Sexing Larvae on the 21st Post-infestation day.

| | STRAIN 1/ | | | |
|---|---|---|---|---|
| | $NJ_{F16}$ | $Lud_{F16}$ | $PA_{F8}$ | Y.C.(wild) |
| Males | 97 | 100 | 99 | 95 |
| Females | 94 | 94 | 96 | 91 |

1/ NJ = New Jersey
Lud = Ludlow, Massachusetts
PA = Pennsylvania
Y.C. = Yards Creek, New Jersey

Figure 1. - The Eclosion Rates of Colony (New Jersey) Egg Masses Chilled 90, 120 and 180 days.



Project Number: GM 7.3.4
Project Title: Insect Production and Distribution
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: L. F. Kennedy, J. J. Baker, O. T. Forrester

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

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

A. Production - Infested 879,180 Neonates
Refrigerated 19,169 Masses

B. Distribution

1. Methods Development and A.R.S.

| | | |
|---|---|---|
| Insecticide screening | - | 63,710 larvae |
| Sterile Male | - | 30,960 male pupae |
| | - | 17,180 female pupae |
| Pheromone Studies | - | 5,120 Adult males |
| | - | 135 Adult females |
| Virus project | - | 156,255 larvae |

2. Cooperators

Michigan State University - R. Carde

36,925 Male pupae
20,925 Female pupae

Michigan State University - J. Miller

900 Male pupae

Massachusetts University - P. Barbosa

2,400 - Female pupae
2,400 - Larvae

Pennsylvania State University - M. Waldvogel

2,800 Male Pupae
600 Female pupae

Connecticut Agricultural Exp. Station - J. Anderson

4,400 Female pupae

Agricultural Res. Center, Beltsville, MD - R. Webb

10,000 Male pupae
15,000 Female pupae

Forest Service - Hamden, CT - H. Smith

23,285 Larvae

Syracuse University - Wasserman

Eggs - 8 Masses

Beltsville - Dr. Hayes

Eggs - 20 Masses

Penn State University

Eggs - 70 Masses

Project Number: GM 8.3.2
Project Title: Rearing Techniques and Production Costs
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leaders: O. T. Forrester, L. F. Kennedy, J. J. Baker

The following rearing techniques are those in effect May 31, 1978. The major changes are: the elimination of the 16 oz. 2186 SE Dixie cup as a rearing container, sexing and segregating larvae at the 21 day transfer and the use of high wheat germ diet for infest and modified hornworm diet for refeed. Costs of each operation are expressed per 1000 insects. With these new procedures current rearing costs have been reduced to $28.79 per 1000 insects.

Diet Preparation:

Diet preparation and dispensing procedures have not changed. Diet preparation cost for neonate infestation is $1.17. This requires 67 cups with 70 ml of diet each. Labor cost for transfer and sexing diet is $2.39 and it requires 134 cups with 70 ml of each. Total labor cost for diet preparation is $3.56.

Neonate Infestation:

Neonate larvae are infested at 15 per 6 oz. ME-6R Sweetheart plastic cup and closed with an AS 306 paper lid. Cost for this operation is; labor $2.65, diet $1.69 and containers $2.12 for a total of $6.46.

Transfer and Sexing:

Under the previous system 15, 21 day old larvae were swept from the 6 oz. infest cup into a 16 oz. 2186 SE Dixie cup with 90 ml of diet in a 3 oz. L0311 plastic cup. The cost of this operation was $10.84. The new procedure is to separate the sexes at 21 days and put 15 male larvae in a 6 oz. cup with 70 ml of diet. Female larvae are transferred at 5 per 6 oz. cup on 70 ml of diet. The cost for this operation is; labor $2.99, diet $4.67 and containers $4.12 for a total of $11.78.

Pupal Harvest:

The previous pupal harvest system was quite time consuming because of the silking in the paper cup, labor cost was $4.50 and container cost was $1.62 for a cost of $6.12.

The present system of removing the pupae, pre sexed as larvae, reduced labor cost to $1.00 for male pupa and $1.20 for female pupa (the increased cost of female pupae harvest is because 3 times as many cups have to be handled). Container cost $1.60, cost for new pupal harvest is $3.80.

Mating and Egg Harvest:

This procedure has not changed, estimated cost is $2.65 per 250 mating and egg mass harvest.

Preparing Egg Masses for Hatch:

This procedure has not changed - cost $0.54.

Further cost reductions will occur with "no transfer" rearing. Good quality insects have been produced without transfer. Eight neonates were infested on 80 ml of modified hornworm diet in ME-6R and XE-6 cups, and allowed to develop to pupa. Pupal recovery was good, adult eclosion was excellent, 100% of the adult males and 93.5% of the adult females were normal. The sex ratio in this test was 35.4% male and 64.6% female. The mean egg mass weight was 752.5 mg. Use of this system of rearing would reduce rearing cost to $19.00/1000 or less.

Project Number: GM 8.3.4.
Project Title: Evaluating the Sampling Techniques Used to Establish Standards I. Egg Sampling Techniques.
Report Period: April 1, 1978 - September 30, 1978
Report Type: Preliminary
Project Leaders: John Allen Tanner and D. Houle

Introduction:

A quality control program must have sampling techniques which accurately estimate the true population mean. This requires careful evaluation of each sampling technique before it is accepted for use in a quality control program.

In this and future reports, sampling techniques proposed for use in our quality control program will be evaluated for their accuracy in expressing the true population mean. Unreliable or inaccurate techniques will be modified or discarded.

The first technique that will be evaluated is the 20 egg hatch sample. A preliminary test will determine the accuracy in which a 20 egg sample expresses the hatch of an egg mass. The results of this experiment will determine if further testing is necessary.

Objective:

To determine the accuracy of a 20 egg sample in expressing the true hatch of an egg mass.

Methods:

New Jersey ($F_{17}$) egg masses will be sampled after 90, 120, 150 and 180 days of chilling. Each sample will consist of five masses. Twenty eggs will be removed from the core of each mass and incubated in petri dishes (50 x 9 mm). The remaining portion of each mass will also be incubated in petri dishes. Hatch will be removed daily and counted. Upon completion of hatch, the percent embryonation, the percent hatch and the eclosion rate will be determined. The data will be analyzed by a t-test for paired observation, an AOV test, and regression test. A total of ten replicates will be conducted over a period of time.

Project Number: GM 8.3.5
Project Title: Incidence and Duration of Egg Hatch in Relation to Chilling Duration for Various Laboratory Strains of Gypsy Moth.
Report Period: April 1, 1978 - September 30, 1978
Report Type: Preliminary
Project Leaders: John Allen Tanner, Donna Houle

## Introduction:

Personal observations seem to indicate less chilling is necessary to break diapause in our older gypsy moth strains (New Jersey $F_{16}$, Ludlow $F_{16}$, and Pennsylvania $F_8$) than our newer gypsy moth strains (Pennsylvania $F_2$, Abington $F_1$). Hoy (1977) reported breeding a non-diapausing gypsy moth strain after only eight generations. Our rearing techniques may be selecting for a less intense diapausing strain. If this is the case, a general 90 day hatch test and a common chilling period may not be the optimum technique to use. This would be true if the difference between the newer and older strains are extreme.

## Objective:

1) To determine the response of each laboratory strain to a constant chilling temperature and 2) to determine the optimum time to sample masses for hatch estimates.

## Methods:

*This chilling test will compare the New Jersey ($F_{17}$), Ludlow ($F_{17}$), Pennsylvania* ($F_8$ + $F_2$) and Abington ($F_1$) strains. All the masses will come from insects reared at the same time. Each mass will be embryonated 28 days and chilled at 6-7° C. Hatch test will commence with 90 day chilled eggs and continue, at 30 intervals, until the masses have been chilled for 180 or 210 days. A hatch test will consist of 10 masses/strain. Twenty eggs will be excavated from the core of each mass and incubated in individual petri dishes. Differences in percent hatch and hatch rates will be tested by AOV tests and linear regression.

## References Cited:

Hoy, M. A. 1977. Rapid response to selection for a nondiapausing gypsy moth. Science. 196. 1462-1463.

Project Number: GM 9.3.2
Project Title: Silk Removal from Gypsy Moth Pupae
Report Period: April 1, 1978 - September 30, 1978
Report Type: Preliminary
Project Leader: O. T. Forrester

Sodium hyporchlorite has been used to remove silk from several different lepidopterous insects. It is also effective in removing silk from gypsy moth pupae. Mechanical sexing of gypsy moth pupae may be facilitated with a silk removal process. Collected pupae normally aggregate into clumps when their cremasters become entangled with silk.

Pupae were emersed in various concentrations of sodium hyporchlorite and the time required for 100% separation was recorded. The results are in Table 1.

Table 1. Length of treatment with sodium hypochlorite required to dissolve silk connecting pupae.

| | % Sodium hyporchlorite | | | | |
|---|---|---|---|---|---|
| Replicate | 5.25% | 2.63% | 1.32% | 1.05% | 0.52% |
| 1 | 81 sec. | 75 sec. | 320 sec. | 206 sec. | 325 sec. |
| 2 | 35 sec. | 67 sec. | 142 sec. | 233 sec. | 201 sec. |
| 3 | 25 sec. | 45 sec. | 80 sec. | 316 sec. | 544 sec. |
| 4 | 23 sec. | 30 sec. | 134 sec. | 181 sec. | 740 sec. |
| $\bar{x}$ | 41.0±27.1 | 54.3±20.5 | 170±103 | 234±58.6 | 452±238 |

A complete treatment schedule, adult eclosion data and operational silk removal procedure will be developed and presented during the next reporting period.

Project Number: GM 9.3.3
Project Title: The Effect of Temperature on Vitamin Pre-mix Relative to Development and Egg Hatch.
Report Period: April 1, 1978 - September 30, 1978
Report Type: Interim
Project Leader: O. T. Forrester

There has been considerable discussion about the effect of temperature on the vitamin pre-mix used in the gypsy moth artificial diet. Diet preparation procedures have included: 1) cooling the agar-water mixture to 70°C before adding the complete dry mix containing the vitamins, 2) adding the complete dry mix at 90°C and 3) adding non-vitamin dry ingredients at 90°C with the vitamin mix added when diet cools to 70°C.

In this test vitamin pre-mix (Hoffman LaRoche) was added to 1600 ml of boiling water and returned to a boil for 1 minute. This mixture was added to a 1 gallon Waring blender with agar and 1 ml raw linseed oil. The remaining dry ingredients were added at 90°C. In the second treatment the dry ingredients including the vitamin pre-mix were added to the agar-water-raw linseed oil mixture at 90°C. In the third treatment the dry mix including vitamin pre-mix was added to the agar-water-raw linseed after cooling to 70°C. Treatment four was the same as treatment 1 without raw linseed oil. All diets were blended for 1 minute at 1500 RPM. The diet was dispensed into 6 oz. ME-6R Sweetheart plastic cups at 70 ml/cup. The insects were transferred to fresh diets at 21 days. The test was replicated 3 times at 21 day intervals.

Results of this test are summarized in Table 1. Developmental rates, pupal weights and incidence of adult abnormalities were comparable. Egg hatch was higher with boiled vitamin mix and 90°C diet (Treatment 1). Boiling vitamin mix did not adversely effect developmental rate, pupal weights, or adult normality. However, when linseed oil was deleted from the diet, hatchability of eggs declined.

Table 1. The effect of diet preparation temperature on vitamin pre-mix in relation to developmental time and hatch.

| Treatment | Pupal Wt (mg) ♂ | Pupal Wt (mg) ♀ | Time to Adult (days) ♂ | Time to Adult (days) ♀ | % Normal | | 90% Egg Hatch |
|---|---|---|---|---|---|---|---|
| Boiled vitamin mix | .58±.05 | 2.36±.1 | 41.5±2.6 | 43.1±2.9 | 98.7 | 89.2 | 83.1±11.8 |
| Vitamins added at 90°C | .54±.06 | 2.22±.17 | 40.8±1.4 | 42.6±2.1 | 96.4 | 81.8 | 82.7±4.6 |
| Vitamins added at 70°C | .60±.07 | 2.35±.6 | 40.7±1.8 | 42.5±1.9 | 97.9 | 82.6 | 69.2±14.4 |
| Boiled vitamin mix no oil | .59±.01 | 2.35±.16 | 40.7±2.1 | 42.3±2.9 | 98.1 | 78.9 | 52.7±24.6 |

Project Number: GM 9.3.4
Project Title: Alternatives to Wheat Germ in Artificial Gypsy Moth Diet.
Report Period: April 1, 1978 - September 30, 1978
Report Type: Preliminary
Project Leader: Othel T. Forrester

Unreliable quality of wheat germ in the past has increased interest in alternative sources of protein in the artificial diet of the gypsy moth. Bran, middlings, oatmeal, barley flakes and corn germ are being tested as substitutes for wheat germ in the gypsy moth artificial diet.

Oatmeal used as an alternative for wheat germ produced normal adult females and 100% abnormal adult males; the wings and abdomen were severely deformed. The wheat germ control produced normal adult males and females. Furthermore, the insects produced with the oatmeal diet were slightly smaller than the control. The mean adult eclosion time was 2 days earlier for oatmeal males and 3 days later for the oatmeal females than in the control.

Egg hatch was also determined from insects reared on alternate diets. The 90 day hatch for oatmeal reared insects was 17.1% and 26.8% for wheat germ; the 180 day egg hatch was 42.2% and 50% respectively.

In a second series of tests wheat germ was replaced with bran, middlings, and oatmeal. Two of the replacements, bran and middlings, produced adults and egg masses. Neonate larvae reared on the oatmeal diet did not develop beyond second instar; cholesterol was omitted from the diet. In continuing tests of 1:1 bran-wheat germ, bran, middlings and oatmeal, three of these diets produced adults and egg masses. Larvae infested onto oatmeal again failed to develop beyond second instar larvae, presumeably because of the omission of cholesterol. The 180 day egg hatch data was: bran=40%, 1:1 bran-wheat germ=50.4%, wheat germ=52.4%, bran=36.8%, middlings=26% and wheat germ=34% . Using egg hatch as the criterion all of these are possible replacement protein sources. On all diets, mean adult eclosion took two days longer than with wheat germ. Male pupal weights and physical normalcy were equivalent to wheat germ diets. Female weights for corn germ and barley flakes were slightly higher than wheat germ.

Cholesterol and B-sitosterol were tested to determine the level required for the gypsy moth to develop normally on oatmeal diet. Fifty, 100, 150 and 200 mg of cholesterol/liter and 200 mg B-sitosterol/liter of diet were tested. The sterol levels were too low or the diets were not properly blended. The insects did not develop beyond second instar after 22 days. Normal development has occurred at 500 mg of cholesterol/liter. The threshold for cholesterol will be determined in subsequent tests.

One hundred percent of males reared on oatmeal diets without added lipids had deformed wings and abdomens. These data were used to determine the critical period for wing development. Neonate larvae were infested onto modified hornworm diet with 1 ml raw linseed oil/liter. Groups of larvae were transferred to oatmeal diet without added lipids after 14, 17, 19, 21, 24 and 26 days of development. Adult males eclosing from these treatments ranged from 100% abnormal wings and abdomen from the 14 day transfer to 6.5% abnormal from the 26 day transfer. A transitional area for normal and abnormal occurred between 18 and 21 days. Insects transferred at 21, 24 and 26 days produced 21.2, 10.7 and 6.5% abnormal adult male respectively. Adult females from this test were normal. The critical time for normal adult male wing formation was between 19 and 21 days. This test indicates a difference in the dietary requirements for normal male and female development. Insects from this test were not mated.

More testing is needed to determine lipid and sterol requirements for the gypsy moth. Oatmeal diets may be useful in this work.

In the final test of the preliminary series corn germ, barley flakes and oatmeal were tested. Mean adult eclosion time was two days later than wheat germ diets. Male and female weights were comparable to wheat germ. The 120 day hatch was 45.5% with oatmeal, 42.0% with corn germ, and 38.8% with barley. Corn germ and barley are also possible replacements for wheat germ.

With further testing all of these products can be developed into low cost gypsy moth diets.