October 1, 1978 - March 31, 1979
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

Animal and Plant Health Inspection Service

Charles P. Schwalbe
Edward C. Paszek
Victor C. Mastro
Robert G. Reeves
Joyce A. Finney
Winfred H. McLane
Othel T. Forrester
Jessie J. Baker
Leonard F. Kennedy
John A. Tanner
Joseph G. R. Tardif
Robert N. Wassell
Rebecca Upton
Madeleine E. Perry

Center Director
Agriculturist
Entomologist
Chemist
Biological Technician
Biological Technician
Entomologist
Biological Aid
Supervisory Biological Technician
Entomologist
Equipment Specialist
Maintenance Worker
Clerk-Stenographer
Clerk-Typist
<table>
<thead>
<tr>
<th>Project Number</th>
<th>Project Title</th>
<th>Report Type</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM 6.1.5</td>
<td>Regulatory Treatments</td>
<td>McLane</td>
<td>4</td>
</tr>
<tr>
<td>GM 8.1.1</td>
<td>Evaluation of Ultraviolet Screening Agents for Microbials</td>
<td>McLane</td>
<td>4</td>
</tr>
<tr>
<td>GM 8.1.2</td>
<td>Field Studies of Insecticides and Microbials Applied by Aircraft</td>
<td>McLane</td>
<td>4</td>
</tr>
<tr>
<td>GM 8.1.3</td>
<td>Laboratory Screening of Candidate Pesticides Against the Gypsy Moth</td>
<td>McLane</td>
<td>4</td>
</tr>
<tr>
<td>GM 8.1.5</td>
<td>Field Studies of Insecticides and Microbials Applied by Ground Equipment</td>
<td>McLane</td>
<td>4</td>
</tr>
<tr>
<td>GM 9.1.1</td>
<td>Preseason Evaluation of Formulation of Carbaryl</td>
<td>McLane</td>
<td>4</td>
</tr>
<tr>
<td>GM 9.1.2</td>
<td>1979 Field Tests of Bay Sir 1845, Dimilin and Bacillus thuringiensis</td>
<td>Reeves</td>
<td>5</td>
</tr>
<tr>
<td>JB 9.1.1</td>
<td>Management of Japanese Beetle Populations in Airports</td>
<td>McLane</td>
<td>4</td>
</tr>
<tr>
<td>GM 9.1.4</td>
<td>The Effects of Encapsulated Disparlure Formulations, and Individual Components on Automotive Paints</td>
<td>Reeves, Walthall</td>
<td>6</td>
</tr>
<tr>
<td>Project Number</td>
<td>Project Title</td>
<td>Report Type</td>
<td>Page</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>GM 6.2.3</td>
<td>Periodicity of Pheromone Release from Female Gypsy Moths</td>
<td>Reeves</td>
<td>14</td>
</tr>
<tr>
<td>GM 7.2.1</td>
<td>Efficiency of (+) Disparlure-Baited Traps in Arrays for Estimating Male Moth Density and Delimitation of Infestations</td>
<td>Schwalbe</td>
<td>15</td>
</tr>
<tr>
<td>GM 7.2.4</td>
<td>Development of Laboratory Evaluation Techniques for Gypsy Moth Male Competitiveness Comparisons</td>
<td>Mastro</td>
<td>16</td>
</tr>
<tr>
<td>GM 8.2.2</td>
<td>Radiological Sterilization of Male Gypsy Moths</td>
<td>Schwalbe</td>
<td>17</td>
</tr>
<tr>
<td>GM 8.2.3</td>
<td>Gypsy Moth Mating Behavior</td>
<td>Schwalbe</td>
<td>17</td>
</tr>
<tr>
<td>GM 8.2.4</td>
<td>Assessing Male Competitiveness Traits of Various Strains of Gypsy Moths</td>
<td>Mastro</td>
<td>39</td>
</tr>
<tr>
<td>GM 8.2.5</td>
<td>Preliminary Evaluation of Large Capacity Traps</td>
<td>Schwalbe</td>
<td>40</td>
</tr>
<tr>
<td>GM 8.2.6</td>
<td>Large-Capacity Milk Carton Traps - Design Improvements</td>
<td>Paszek</td>
<td>41</td>
</tr>
<tr>
<td>GM 8.2.7</td>
<td>1978 Field Evaluation of Microcapsular Formulations of Disparlure</td>
<td>Schwalbe</td>
<td>42</td>
</tr>
<tr>
<td>GM 9.2.1</td>
<td>Effects of Cold Treatment on Competitiveness</td>
<td>Mastro</td>
<td>43</td>
</tr>
<tr>
<td>GM 9.2.2</td>
<td>Efficiency of Overflooding ratios</td>
<td>Mastro</td>
<td>43</td>
</tr>
<tr>
<td>GM 9.2.3</td>
<td>SMT Trial in Simulated Infestation</td>
<td>Mastro</td>
<td>43</td>
</tr>
<tr>
<td>GM 9.2.4</td>
<td>1979 Preseason Evaluation of Competitiveness</td>
<td>Mastro</td>
<td>44</td>
</tr>
<tr>
<td>Project Number</td>
<td>Project Title</td>
<td>Report Type</td>
<td>Page</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>GM 6.3.6</td>
<td>Establishing Standards</td>
<td>Tanner</td>
<td>48</td>
</tr>
<tr>
<td>GM 7.3.4</td>
<td>Insect Production and Distribution</td>
<td>Kennedy</td>
<td>54</td>
</tr>
<tr>
<td>GM 8.3.2</td>
<td>Rearing Techniques and Production Costs</td>
<td>Forrester</td>
<td>55</td>
</tr>
<tr>
<td>GM 8.3.4</td>
<td>Evaluating the Sampling Techniques Used to Establish Standards I. Egg Sampling Techniques</td>
<td>Tanner</td>
<td>56</td>
</tr>
<tr>
<td>GM 8.3.5</td>
<td>Incidence and Duration of Egg Hatch in Relation to Chilling Duration for Various Laboratory Strains of Gypsy Moth</td>
<td>Tanner</td>
<td>63</td>
</tr>
<tr>
<td>GM 9.3.1</td>
<td>Laboratory Evaluation of Eggs Collected from Oconomowoc Infestation</td>
<td>Forrester</td>
<td>65</td>
</tr>
<tr>
<td>GM 9.3.2</td>
<td>Silk Removal from Gypsy Moth Pupae</td>
<td>Forrester</td>
<td>65</td>
</tr>
<tr>
<td>GM 9.3.3</td>
<td>The Effect of Temperature on Vitamin Pre-mix Relative to Development and Egg Hatch</td>
<td>Forrester</td>
<td>65</td>
</tr>
<tr>
<td>GM 9.3.4</td>
<td>Alternatives to Wheat Germ in Artificial Gypsy Moth Diet</td>
<td>Forrester</td>
<td>65</td>
</tr>
<tr>
<td>GM 9.3.5</td>
<td>Evaluation of No Transfer Rearing Technique</td>
<td>Forrester</td>
<td>65</td>
</tr>
</tbody>
</table>
Because of increased workload associated with the expanded Japanese Beetle and Gypsy Moth Retardation Projects, preparation of the following reports will be delayed until October, 1979.

- GM 6.1.5 Regulatory Treatments
- GM 8.1.1 Evaluation of Ultraviolet Screening Agents for Microbials
- GM 8.1.2 Field Studies of Insecticides and Microbials Applied by Aircraft - 1978
- GM 8.1.3 Laboratory Screening of Candidate Pesticides for Gypsy Moth
- GM 8.1.5 Field Studies of Insecticides and Microbials Applied by Ground Equipment
- GM 9.1.1 Preseason Evaluation of a New Formulation of Carbaryl
- JB 9.1.1 Management of Japanese Beetle Populations in Airports
Tests detailed in the subject work plan have been completed and results will be presented in the next Laboratory Report.
The objective of this study was to observe the effect of 2% encapsulated disparlure formulations, and the individual components of these formulations on automotive paint.

Through telephone conversations with factory representatives from the "big three" automobile manufacturers, we learned that virtually all of the automobiles made in the past several years were painted with acrylic enamel or acrylic lacquer. General Motors (GM) and Chrysler Corporation furnished paint samples which were sprayed on small metal plates. According to company spokesmen, these plates were exposed to the same curing process as assembly line automobiles. The GM plates were painted with various colors of acrylic lacquer, and the Chrysler Corporation samples were all acrylic enamel. The disparlure used in this study was 2% encapsulated formulations; one formulated in 1976, the other in 1978. Samples from each formulation were sprayed on separate sections of each painted plate in a laboratory spray tower at a rate equivalent to 3 lb/acre. The spots were allowed to dry for 24 hours, and each plate was then evaluated for permanent paint damage after washing, waxing and compounding. Also, a 2 cm diameter droplet was put on each plate and similarly evaluated.

Another aspect of the test involved placing droplets of the individual components of the disparlure formulation directly on the panels. The compounds were neat disparlure, Rhoplex B-15, RA 1645, xylene and amyl acetate. After these spots dried, they were cleaned off the plates with hexane. Visual evaluations of the damage caused by these chemicals were made before and after waxing the plates. The 2% encapsulated disparlure was also sprayed aerially onto the painted plates at a rate of 3 lb/acre at altitudes of 25', 50', 100' and 150'. The plates were evaluated for damage after washing with water and waxing.
Table 1. Effects of spray tower application of 2% microencapsulated disparlure formulation on auto paint samples.

<table>
<thead>
<tr>
<th>Paint Type</th>
<th>Paint Color</th>
<th>After water wash</th>
<th>After Compound</th>
<th>After Wax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylic lacquer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-metallic</td>
<td>Conestoga tan</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ranch Green</td>
<td>2</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Ruby red</td>
<td>2</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Classic white</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Regal black</td>
<td>1</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td>Acrylic lacquer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metallic</td>
<td>Andes copper</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Mediterranian blue</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Cranberry firemist</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Acrylic enamel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-metallic</td>
<td>Chianti red</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>School bus yellow</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Formal black</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Spitfire orange</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Eggshell white</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Designers cream</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Acrylic enamel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metallic</td>
<td>Light chestnut</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Astral blue</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Bittersweet</td>
<td>1</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

N  No spot
1  Spot faint (barely visible)
2  Spot definite
3  Deep spot (almost to metal)
4  Spot to the metal
Table 2. Effects of drop application of 2% microencapsulated disparlure formulation on auto paints.

<table>
<thead>
<tr>
<th>Paint Type</th>
<th>Paint Color</th>
<th>Reaction to 2.0 cm drop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylic lacquer</td>
<td>Conestoga tan</td>
<td>3</td>
</tr>
<tr>
<td>non-metallic</td>
<td>Ranch green</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ruby red</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Classic white</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Regal black</td>
<td>3</td>
</tr>
<tr>
<td>Acrylic lacquer</td>
<td>Andes copper</td>
<td>1</td>
</tr>
<tr>
<td>metallic</td>
<td>Mediterranean blue</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cranberry firemist</td>
<td>1</td>
</tr>
<tr>
<td>Acrylic enamel</td>
<td>Chianti red</td>
<td>1</td>
</tr>
<tr>
<td>non-metallic</td>
<td>School bus yellow</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Formal black</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Spitfire orange</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Eggshell white</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Designers cream</td>
<td>1</td>
</tr>
<tr>
<td>Acrylic enamel</td>
<td>Light Chestnut</td>
<td>1</td>
</tr>
<tr>
<td>metallic</td>
<td>Astral blue</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bittersweet</td>
<td>2</td>
</tr>
</tbody>
</table>

N  No spot
1  Spot faint (barely visible)
2  Spot definite
3  Deep spot (almost to metal)
4  Spot to the metal
Table 3. Results of spotting of individual ingredients of disparlure formulation on paint panels prior to waxing.

<table>
<thead>
<tr>
<th>Paint type</th>
<th>Paint color</th>
<th>Xylene</th>
<th>Disparlure</th>
<th>Rhoplex B-15</th>
<th>RA-1645</th>
<th>Amyl Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylic lacquer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-metallic</td>
<td>Conestoga tan</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ranch green</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ruby red</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Classic white</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Regal black</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td>Acrylic lacquer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metallic</td>
<td>Andes copper</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Med. blue</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cran. Firemist</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td>Acrylic enamel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-metallic</td>
<td>Chianti red</td>
<td>2</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>School bus yellow</td>
<td>2</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Formal black</td>
<td>2</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Spitfire oran.</td>
<td>2</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Eggshell white</td>
<td>2</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Designers cream</td>
<td>2</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td>Acrylic enamel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metallic</td>
<td>Lt. chestnut</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Astral blue</td>
<td>2</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bittersweet</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
</tbody>
</table>

N  No spot
1  Spot faint (barely visible)
2  Spot definite
3  Spot deep (almost to metal)
4  Spot to the metal
Table 4. Results of spotting of individual ingredients of dispersalure formulation to paint panels after waxing.

<table>
<thead>
<tr>
<th>Paint type</th>
<th>Paint color</th>
<th>Xylene</th>
<th>Disparlure</th>
<th>Rhoplex B-15</th>
<th>RA-1645</th>
<th>Amyl Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylic lacquer</td>
<td>Conestoga tan</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ranch green</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ruby red</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Classic white</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Regal black</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td>Acrylic lacquer</td>
<td>Andes copper</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td>metallic</td>
<td>Med. blue</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cran. firemist</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td>Acrylic enamel</td>
<td>Chianti red</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td>non-metallic</td>
<td>School bus</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>yellow</td>
<td>2</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Formal black</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Spitfire</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>orange</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Eggshell white</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Designers cream</td>
<td>2</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td>Acrylic enamel</td>
<td>Light chestnut</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>metallic</td>
<td>Astral blue</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bittersweet</td>
<td>2</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>1</td>
</tr>
</tbody>
</table>

N  No spot
1  Spot faint (barely visible)
2  Spot definite
3  Spot deep (almost to metal)
4  Spot to the metal
Table 5. Reaction to spraying at various altitudes prior to waxing.

<table>
<thead>
<tr>
<th>Paint type</th>
<th>Paint Color</th>
<th>REACTION TO SPRAYING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25'</td>
</tr>
<tr>
<td>Acrylic</td>
<td>Red</td>
<td>1</td>
</tr>
<tr>
<td>lacquer</td>
<td>White</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Lt. blue metallic</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dk brown metallic</td>
<td>N</td>
</tr>
<tr>
<td>Acrylic</td>
<td>Ok Brown metallic</td>
<td>N</td>
</tr>
<tr>
<td>enamel</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

- N: No spots
- 1: Spots barely visible
- 2: Spots definite

Table 6. Reaction to spraying at various altitudes post waxing.

<table>
<thead>
<tr>
<th>Paint type</th>
<th>Paint Color</th>
<th>REACTION TO SPRAYING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25'</td>
</tr>
<tr>
<td>Acrylic</td>
<td>Red</td>
<td>N</td>
</tr>
<tr>
<td>lacquer</td>
<td>White</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Lt. blue metallic</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Dk brown metallic</td>
<td>N</td>
</tr>
<tr>
<td>Acrylic</td>
<td>Ok Brown metallic</td>
<td>N</td>
</tr>
<tr>
<td>enamel</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

- N: No spot
- 1: Spot barely visible
- 2: Spot definite
Results:

Placing the plates in a spray tower and spraying with 2% disparlure determined whether or not the formulation caused any damage to auto paint under laboratory conditions.

The acrylic lacquer non-metallic plates were all spotted after washing with water (Table 1), but the acrylic lacquer metallic plates were not spotted after washing. Waxing the plates (Table 1) removed some of the spots, and compounding the plates (Table 1) removed most of the spots. The acrylic enamel non-metallic plates and metallic plates were slightly spotted after washing. Compounding and waxing removed these spots.

Placing a 2.0 cm. spot (Table 2) on acrylic non-metallic plates resulted in severe damage to the paint. The paint was removed almost to bare metal and was wrinkled. The spot on the acrylic lacquer metallic plates produced a faint spot, and there was only a slight wrinkling of the paint. The acrylic enamel non-metallic plates had a moderate to faint spot, and very little paint wrinkling. The acrylic enamel metallics sustained slight to moderate damage which was mostly discoloration but no wrinkling was evident.

Testing with the individual components of the 2% disparlure formulation revealed which component was causing the most damage. The acrylic lacquer plates, before waxing (Table 2), revealed two components that caused damage. Xylene which caused dulling of the paint and moderate spotting, and amyl acetate which caused severe damage. In 2 out of 5 plates this chemical removed the paint to the metal. Disparlure, Rhoplex B-15 and RA-1645 had no effect on the acrylic lacquer paint. Metallic paints vs. non-metallic showed no significant difference except that the metallic paint was damaged less by amyl acetate.

The acrylic enamel paint, before waxing (Table 2), had three components that damaged the paint. Xylene which dulled and spotted the plates; disparlure which produced varying results in as much as non-metallic colors sustained slight to moderate damage, and metallics showed very little or no damage; amyl acetate which caused minimal damage.

Spraying at various altitudes determined which height produced the least damage. The acrylic lacquer plates prior to waxing, (Table 5) had slight damage at 25', 50' and 100'. The damage at 150' was more evident. There was no damage to the acrylic enamels at any height. After waxing, (Table 6), spotting on the acrylic lacquers at 25' and 50' was eliminated. However, waxing the acrylic lacquer plates that had been sprayed at 100' and 150' did not have any effect. There was no apparent difference between metallic and non-metallic plates before or after waxing.
Conclusions:

The 2% encapsulated disparlure formulations damage automotive paints under laboratory and aerial spraying conditions. The acrylic lacquers are more susceptible to damage from these materials than acrylic enamels. Xylene and amyl acetate are the two most damaging components of the formulations. We conclude that these formulations should not be used in areas where the spray might contact automobiles.
Conclusions:

The 2% encapsulated disparlure formulations damage automotive paints under laboratory and aerial spraying conditions. The acrylic lacquers are more susceptible to damage from these materials than acrylic enamels. Xylene and amyl acetate are the two most damaging components of the formulations. We conclude that these formulations should not be used in areas where the spray might contact automobiles.
Project Number: GM 6.2.3
Project Title: Periodicity of Pheromone Release from Female Gypsy Moth
Report Type: Interim
Project Leader: R. G. Reeves

No progress to report this period.
Results of 1978 field tests were summarized in the last Laboratory Report. Since then, no additional work has been conducted and we do not presently plan to continue the project.

Appendix 1 is a manuscript for a USDA Handbook presently in galley proof form. It is a general publication intended to serve as a field guide for gypsy moth survey operations.
Two techniques are currently being considered for laboratory evaluation of strain competitiveness: an actograph system for monitoring periodicity of male activity, and a flight tunnel for comparing male behavioral response to pheromone cues. Testing of both techniques is still in the developmental stages. M. Waldvogel began a cooperative study with the flight tunnel during the 1978 field season. Preliminary results of these studies have been reported before. A final summary will be presented in the next semi-annual report with the completion of Mike's Masters Thesis. Because of personnel and time constraint, further work on development of procedures for use of the flight tunnel may have to be delayed until September 1979.

Development of the actograph system is continuing. Recent changes in the insect holding cage design and sensor placement have made the system much more sensitive to insect activity. We are currently building insect cages of the new design and plan to be ready to compare feral and laboratory strains during the 1979 field season. Because this system is unique, much time has been spent calibrating, changing circuitry and generally familiarizing ourselves with the system. Once the equipment problems have been resolved, the biological problems should go easily.
This project was initiated to determine the age:dosage formula best suited for inducing acceptable (90-95%) degree of sterility in male pupae. Effects on the P₁ and F₁ generations were summarized in the last Laboratory Report. Based on those results, we have adopted irradiation of 8-11 day old pupae with 15 krad as the standard treatment. Because of logistical considerations, however, irradiation in the adult stage seemed desirable and additional tests with adult males are underway.

The following pages summarize results of fertility tests performed on progeny of treated P₁ male pupae. In all treatments other than the 2 krad treatments a high degree of inherited sterility was observed in the F₁ and F₂ generations. F₃ progeny are presently being reared. These results suggest that carry-over effects may be observed in a population the years following release, thus increasing the efficacy of the SMT for gypsy moth control.
$P_1$ males irradiated with 2 krad at 46 days

$n = 56$
- 846 eggs/mass
- 95% embryo
- 47% hatch
- 81% $\delta$ surv
- 79% $\Phi$ surv

$n = 17$
- 869 eggs/mass
- 99% embryo
- 58% hatch
- 80% $\delta$ surv
- 81% $\Phi$ surv

$n = 73$
- 651 eggs/mass
- 88% embryo
- 27% hatch
- 81% $\delta$ surv
- 79% $\Phi$ surv

$n = 48$
- 775 eggs/mass
- 85% embryo
- 30% hatch
- 81% $\delta$ surv
- 79% $\Phi$ surv

$n = 62$
- 848 eggs/mass
- 90% embryo
- 42% hatch
- 81% $\delta$ surv
- 79% $\Phi$ surv

$n = 25$
- 528 eggs/mass
- 88% embryo
- 41% hatch
- 81% $\delta$ surv
- 79% $\Phi$ surv
$P_1$ males irradiated with 2 krad at 6-7 days

- $n = 33$
  - 660 eggs/mass
  - 94% embry
  - 21% hatch
  - 71% $\sigma$ surv
  - 95% $\varphi$ surv

- $n = 35$
  - 808 eggs/mass
  - 87% embry
  - 55% hatch
  - 77% $\sigma$ surv
  - 99% $\varphi$ surv

- $n = 43$
  - 779 eggs/mass
  - 67% embry
  - 33% hatch
  - % $\sigma$ surv
  - % $\varphi$ surv

- $n = 41$
  - 644 eggs/mass
  - 75% embry
  - 25% hatch
  - % $\sigma$ surv
  - % $\varphi$ surv

- $n = 54$
  - 685 eggs/mass
  - 89% embry
  - 30% hatch
  - % $\sigma$ surv
  - % $\varphi$ surv

- $n = 40$
  - 943 eggs/mass
  - 94% embry
  - 33% hatch
  - % $\sigma$ surv
  - % $\varphi$ surv
P1 males irradiated with 2 krad at 8-9 days

- P1
  - F1
    - F2
      - F3

n = 46
814 eggs/mass
97% embryo
52% hatch
78% ♂ surv
78% ♀ surv

n = 40
1133 eggs/mass
69% embryo
13% hatch
38% ♂ surv
32% ♀ surv

n = 50
1279 eggs/mass
79% embryo
15% hatch
54% ♂ surv
54% ♀ surv

n = 23
785 eggs/mass
95% embryo
58% hatch
85% ♂ surv
81% ♀ surv

n = 28
1207 eggs/mass
91% embryo
21% hatch
98% ♂ surv
51% ♀ surv

n = 50
1279 eggs/mass
79% embryo
15% hatch
54% ♂ surv
54% ♀ surv

n = 28
1207 eggs/mass
91% embryo
21% hatch
98% ♂ surv
51% ♀ surv

-20-
$P_1$ males irradiated with 2 krad at 10-11 days

$P_1$

$n = 71$
- 838 eggs/mass
- 99% embry
- 40% hatch
- 55% $\delta$ surv
- 113% $\varphi$ surv

$F_1$

$n = 29$
- 763 eggs/mass
- 99% embry
- 61% hatch
- 64% $\delta$ surv
- 110% $\varphi$ surv

$F_2$

$n = 38$
- 668 eggs/mass
- 79% embry
- 18% hatch
- $\% \delta$ surv
- $\% \varphi$ surv

$n = 29$
- 759 eggs/mass
- 74% embry
- 13% hatch
- $\% \delta$ surv
- $\% \varphi$ surv

$n = 48$
- 61 eggs/mass
- 90% embry
- 26% hatch
- $\% \delta$ surv
- $\% \varphi$ surv

$n = 31$
- 814 eggs/mass
- 94% embry
- 42% hatch
- $\% \delta$ surv
- $\% \varphi$ surv

$F_3$
P_1 males irradiated with 8 krad at 4-5 days

- P_1
  - n = 47
  - 765 eggs/mass
  - 92% embryo
  - 25% hatch
  - 87% σ surv
  - 63% Φ surv

- F_1
  - n = 40
  - 349 eggs/mass
  - 27% embryo
  - 9% hatch
  - 65% σ surv
  - 35% Φ surv

- F_2
  - n = 38
  - 419 eggs/mass
  - 5% embryo
  - 0.27% hatch
  - 64% σ surv
  - 36% Φ surv

- F_3
  - n = 35
  - 491 eggs/mass
  - 64% embryo
  - 3% hatch
  - 64% σ surv
  - 36% Φ surv

- n = 17
  - 869 eggs/mass
  - 99% embryo
  - 58% hatch
  - 80% σ surv
  - 81% Φ surv

- n = 25
  - 528 eggs/mass
  - 88% embryo
  - 41% hatch
  - 88% σ surv
  - 12% Φ surv
$P_1$ males irradiated with 8 krad at 6-7 days

<table>
<thead>
<tr>
<th>Generation</th>
<th>Eggs/mass</th>
<th>Embryos</th>
<th>Hatch</th>
<th>Sex Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_1$</td>
<td>841</td>
<td>96%</td>
<td>30%</td>
<td>65% male, 78% female</td>
</tr>
<tr>
<td>$F_1$</td>
<td>328</td>
<td>13%</td>
<td>0.36%</td>
<td>56% male, 116% female</td>
</tr>
<tr>
<td>$F_2$</td>
<td>338</td>
<td>34%</td>
<td>0.59%</td>
<td>78% female</td>
</tr>
<tr>
<td>$F_3$</td>
<td>463</td>
<td>80%</td>
<td>0.76%</td>
<td>74% male, 56% female</td>
</tr>
<tr>
<td>$F_4$</td>
<td>907</td>
<td>99%</td>
<td>74%</td>
<td>56% male, 116% female</td>
</tr>
<tr>
<td>$F_5$</td>
<td>802</td>
<td>97%</td>
<td>62%</td>
<td>74% male, 56% female</td>
</tr>
</tbody>
</table>
P₁ males irradiated with 8 krad at 8-9 days

-24-
P₁ males irradiated with 8 krad at 10-11 days

- P₁
  - n = 71
    - 670 eggs/mass
    - 96% embry
    - 17% hatch
    - 71% ♂ surv
    - 71% ♀ surv

- F₁
  - n = 22
    - 674 eggs/mass
    - 99% embry
    - 53% hatch
    - 64% ♂ surv
    - 110% ♀ surv

- F₂
  - n = 35
    - 379 eggs/mass
    - 28% embry
    - 2% hatch
    - 28% ♂ surv
    - 28% ♀ surv

  - n = 29
    - 378 eggs/mass
    - 2% embry
    - 0% hatch
    - 2% ♂ surv
    - 2% ♀ surv

  - n = 33
    - 473 eggs/mass
    - 74% embry
    - 2% hatch
    - 74% ♂ surv
    - 74% ♀ surv

- F₃
  - n = 31
    - 814 eggs/mass
    - 94% embry
    - 42% hatch
    - 94% ♂ surv
    - 94% ♀ surv
F1 males irradiated with 10 krad at 4-5 days

F1

n= 39
731 eggs/mass
91 % embry
28 % hatch
79 % ♂ surv
36 % ♀ surv

F2

n= 44
525 eggs/mass
5% embry
0% hatch
% ♂ surv
% ♀ surv

n= 12
379 eggs/mass
83 % embry
0 % hatch
% ♂ surv
% ♀ surv

n= 16
392 eggs/mass
53 % embry
19 % hatch
% ♂ surv
% ♀ surv

F3

n= 18
773 eggs/mass
99 % embry
83 % hatch
56 % ♂ surv
116 % ♀ surv

n= 24
730 eggs/mass
97 % embry
13 % hatch
% ♂ surv
% ♀ surv
P₁ males irradiated with 10 krad at 6-7 days

- P₁
- F₁
- F₂
- F₃

n= 80
852 eggs/mass
96 % embry
26 % hatch
69 % ♂ surv
62 % ♀ surv

n= 34
820 eggs/mass
95 % embry
84 % hatch
64 % ♂ surv
132 % ♀ surv

n= 36
370 eggs/mass
18 % embry
19 % hatch
 % ♂ surv
 % ♀ surv

n= 25
328 eggs/mass
3 % embry
0 % hatch
 % ♂ surv
 % ♀ surv

n= 43
497 eggs/mass
62 % embry
64 % hatch
 % ♂ surv
 % ♀ surv

n= 25
802 eggs/mass
97 % embry
62 % hatch
 % ♂ surv
 % ♀ surv
$P_1$ males irradiated with 10 krads at 8-9 days

$P_1$

$F_1$

$F_2$

$F_3$

- $n = 44$
- 705 eggs/mass
- 95% embry
- 5% hatch
- 74% $\delta$ surv
- 24% $\varphi$ surv

- $n = 35$
- 808 eggs/mass
- 87% embry
- 55% hatch
- 77% $\delta$ surv
- 99% $\varphi$ surv

- $n = 40$
- 414 eggs/mass
- 2% embry
- 0% hatch
- 37% $\delta$ surv
- 63% $\varphi$ surv

- $n = 5$
- 271 eggs/mass
- 0% embry
- 0% hatch
- 33% $\delta$ surv
- 67% $\varphi$ surv

- $n = 13$
- 447 eggs/mass
- 56% embry
- 6% hatch
- 67% $\delta$ surv
- 93% $\varphi$ surv

- $n = 40$
- 943 eggs/mass
- 94% embry
- 33% hatch
- 73% $\delta$ surv
- 84% $\varphi$ surv
Pl males irradiated with 10 krad at 10-11 days

F1

n= 65
700 eggs/mass
97% embryo
18% hatch
71% ♂ surv
27% ♀ surv

F2

n= 20
395 eggs/mass
5% embryo
0% hatch
71% ♂ surv
0% ♀ surv

n= 1
59 eggs/mass
0% embryo
0% hatch
71% ♂ surv
0% ♀ surv

n= 6
344 eggs/mass
60% embryo
33% hatch
71% ♂ surv
71% ♀ surv

F3

n= 24
730 eggs/mass
97% embryo
13% hatch
71% ♂ surv
71% ♀ surv
$P_1$ males irradiated with 15 krad at 4-5 days

- $P_1$
  - $n = 23$
  - 320 eggs/mass
  - 9% embry
  - 17% hatch
  - 25% $\delta$ surv
  - 0% $\varphi$ surv

- $F_1$
  - $n = 35$
  - 808 eggs/mass
  - 87% embry
  - 55% hatch
  - 77% $\delta$ surv
  - 99% $\varphi$ surv

- $F_2$
  - $n = 1$
  - 331 eggs/mass
  - 0% embry
  - 0% hatch
  - 0% $\delta$ surv
  - 0% $\varphi$ surv

- $F_3$
  - $n = 0$
  - eggs/mass
  - % embry
  - % hatch
  - % $\delta$ surv
  - % $\varphi$ surv
$P_1$ males irradiated with 15 krad at 6-7 days

- $P_1$
  - $n = 57$
  - 831 eggs/mass
  - 92 % embry
  - 8 % hatch
  - 59 % $\delta$ surv
  - 16 % $\varphi$ surv

- $F_1$
  - $n = 24$
  - 861 eggs/mass
  - 95 % embry
  - 61 % hatch
  - 64 % $\delta$ surv
  - 110 % $\varphi$ surv

- $F_2$
  - $n = 36$
  - 373 eggs/mass
  - 8 % embry
  - 2 % hatch
  - % $\delta$ surv
  - % $\varphi$ surv

- $F_3$
  - $n = 3$
  - 405 eggs/mass
  - 1 % embry
  - 0 % hatch
  - % $\delta$ surv
  - % $\varphi$ surv

- $F_4$
  - $n = 8$
  - 390 eggs/mass
  - 44 % embry
  - 8 % hatch
  - % $\delta$ surv
  - % $\varphi$ surv

- $F_5$
  - $n = 28$
  - 647 eggs/mass
  - 96 % embry
  - 58 % hatch
  - % $\delta$ surv
  - % $\varphi$ surv
$P_1$ males irradiated with 15 krad at 8-9 days

<table>
<thead>
<tr>
<th>Generation</th>
<th>Sample Size</th>
<th>Eggs/Mass</th>
<th>Embryo Survival</th>
<th>Hatch Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_1$</td>
<td>n=86</td>
<td>750</td>
<td>94%</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>62% surv</td>
<td>32% surv</td>
</tr>
<tr>
<td>$F_2$</td>
<td>n=35</td>
<td>330</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200% surv</td>
<td>0% surv</td>
</tr>
<tr>
<td></td>
<td>n=10</td>
<td>407</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0% surv</td>
<td>0% surv</td>
</tr>
<tr>
<td></td>
<td>n=11</td>
<td>321</td>
<td>48%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6% surv</td>
<td>32% surv</td>
</tr>
<tr>
<td></td>
<td>n=31</td>
<td>755</td>
<td>88%</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>88% surv</td>
<td>38% surv</td>
</tr>
</tbody>
</table>

$P_2$ males irradiated with 15 krad at 8-9 days

<table>
<thead>
<tr>
<th>Generation</th>
<th>Sample Size</th>
<th>Eggs/Mass</th>
<th>Embryo Survival</th>
<th>Hatch Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_1$</td>
<td>n=51</td>
<td>820</td>
<td>95%</td>
<td>56%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>116% surv</td>
<td>6%</td>
</tr>
<tr>
<td>$F_2$</td>
<td>n=10</td>
<td>407</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0% surv</td>
<td>0% surv</td>
</tr>
<tr>
<td></td>
<td>n=11</td>
<td>321</td>
<td>48%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0% hatch</td>
<td>6% surv</td>
</tr>
<tr>
<td></td>
<td>n=31</td>
<td>755</td>
<td>88%</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>88% surv</td>
<td>38% surv</td>
</tr>
</tbody>
</table>
P₁ males irradiated with 15 krad at 10-11 days

P₁

F₁

F₂

F₃

n = 50
903 eggs/mass
90 % embry
4 % hatch
114 % ♂ surv
17 % ♀ surv

n = 0
802 eggs/mass
98 % embry
81 % hatch
51 % ♂ surv
110 % ♀ surv

n = 11
660 eggs/mass
0 % embry
0 % hatch
% ♂ surv
% ♀ surv

n = 0
eggs/mass
% embry
% hatch
% ♂ surv
% ♀ surv

n = 1
338 eggs/mass
19 % embry
0 % hatch
% ♂ surv
% ♀ surv

n = 28
1207 eggs/mass
91 % embry
21 % hatch
% ♂ surv
% ♀ surv
P₁ males irradiated with 20 krad at 4–5 days.

- F₁
- P₁
- n = 21
- 808 eggs/mass
- 97% embry.
- 87% hatch
- 99% surv.

- F₂
- n = 35
- 808 eggs/mass
- 55% embry.
- 77% hatch
- 99% surv.

- F₃
- n = 0
- 0% embry.
- 0% hatch
- 0% surv.

- eggs/mass
- % embry
- % hatch
- % surv.
$P_1$ males irradiated with 20 krad at 6-7 days

- **$P_1$**
  - n = 65
  - 859 eggs/mass
  - 87% embryo
  - 1% hatch
  - 35% $\sigma$ surv
  - 14% $\phi$ surv

- **$F_1$**
  - n = 9
  - 336 eggs/mass
  - 56% embryo
  - 0% hatch
  - % $\sigma$ surv
  - % $\phi$ surv

- **$F_2$**
  - n = 1
  - 785 eggs/mass
  - 9% embryo
  - 0% hatch
  - % $\sigma$ surv
  - % $\phi$ surv

- **$F_3$**
  - n = 5
  - 699 eggs/mass
  - 50% embryo
  - 33% hatch
  - % $\sigma$ surv
  - % $\phi$ surv

- **$F_4$**
  - n = 24
  - 861 eggs/mass
  - 95% embryo
  - 61% hatch
  - 64% $\sigma$ surv
  - 110% $\phi$ surv
$P_1$ males irradiated with 20 krad at 8-9 days

- $P_1$
  - $n = 50$
  - 751 eggs/mass
  - 92% embry
  - 1% hatch
  - 200% ♂ surv
  - 0% ♀ surv

- $F_1$
  - $n = 23$
  - 847 eggs/mass
  - 94% embry
  - 58% hatch
  - 51% ♂ surv
  - 110% ♀ surv

- $F_2$
  - $n = 3$
  - 782 eggs/mass
  - 0% embry
  - 0% hatch
  - 200% ♂ surv
  - 0% ♀ surv

- $F_3$
  - $n = 0$
  - eggs/mass
  - % embry
  - % hatch
  - % ♂ surv
  - % ♀ surv

- $F_3$
  - $n = 0$
  - eggs/mass
  - % embry
  - % hatch
  - % ♂ surv
  - % ♀ surv

- $F_3$
  - $n = 28$
  - 1207 eggs/mass
  - 91% embry
  - 21% hatch
  - 98% ♂ surv
  - 51% ♀ surv
$P_1$ males irradiated with 20 krad at 10-11 days

- $F_1$
  - $n=23$
  - 649 eggs/mass
  - 93% embry
  - 22% hatch
  - 0% $\sigma$ surv
  - 0% $\varphi$ surv

- $F_2$
  - $n=0$
  - eggs/mass
  - % embry
  - % hatch
  - % $\sigma$ surv
  - % $\varphi$ surv

- $F_3$
  - $n=0$
  - eggs/mass
  - % embry
  - % hatch
  - % $\sigma$ surv
  - % $\varphi$ surv

- $F_4$
  - $n=13$
  - 802 eggs/mass
  - 98% embry
  - 81% hatch
  - 51% $\sigma$ surv
  - 110% $\varphi$ surv

- $F_5$
  - $n=0$
  - eggs/mass
  - % embry
  - % hatch
  - % $\sigma$ surv
  - % $\varphi$ surv
No additional work has been accomplished on this project since the last Laboratory Report. We are in the process of planning a series of experiments focusing on the phenomenon of multiple mating and how it relates to sperm translocation and egg embryonation. Results will be summarized in the next report.
All planned studies associated with this project have been completed and are reported in the following manuscripts.

Crepuscular Activity of Gypsy Moth Adults. ODell and Mastro. Submitted to Environmental Entomology - Appendix 2.

A Comparison of Laboratory-Reared and Wild Type Gypsy Moth Males. Mastro and ODell. In final draft form soon to be submitted to Environmental Entomology. The manuscript will be included in the next Laboratory Report.

Periodicity of Activity of the Adult Gypsy Moth. In preparation and to be included in the next Laboratory Report.

Male Gypsy Moth Response to Various Trap Types. In preparation and to be included in the next Laboratory Report.
Preliminary screening of large capacity traps was completed in 1978 and results were summarized in the last Laboratory Report. Further design refinements will be reported under Project No. 8.2.6. Therefore, this represents the Final Report for this project.
No progress to report.
Results of 1978 field trials were summarized in the last Laboratory Report. A manuscript is in preparation and will be the Final Report.
Project Numbers: GM 9.2.1, GM 9.2.2 and GM 9.2.3
Project Titles: Effects of Cold Treatment on Competitiveness/Efficiency of Overflooding Ratios/SMT Trial in Simulated Infestation
Report Type: Interim
Project Leaders: V. C. Mastro, T. O'Dell

All three projects are field tests slated for the 1979 field season. Presently, plots are being established for all tests and equipment and materials are being prepared. Insect rearing for these tests began on May 3 and adults should be ready for testing by June 18. Field tests will be run essentially in the order of work plans numerical order.
The objectives of the trials described below were to compare the competitiveness of males reared, harvested and surface-disinfected by various techniques. The plan of work outlined in Work Plan 9.2.5 was amended to offset difficulties encountered with synchrony of eclosion from the various treatment groups. The actual method and materials used in conducting the work are detailed below.

Five treatment groups were compared in these tests. They were reared and handled as follows:

1) 1978 technique - larvae were reared, 15/cup, on B-4 diet. At 21 days they were sexed and reared at 15° per cup to pupation on hornworm diet. Pupae were harvested by hand from rearing cups (1978-TT).

2) Males reared with the same technique as in treatment (1), but irradiated as pupae (10-11 days old) with 15 k - CO (37.3 min exposure).

3) With 1979 technique - larvae were reared, 15/cup, on B-4 diet to pupation and harvested by hand from rearing cups (1979-NTT).

4) Males reared using the technique described in treatment 3 but harvested by placing lids with attached pupae and loose pupae in a chlorox (1.31% sodium hypochlorite) bath for 6 minutes then rinsing twice with fresh water.

5) Males reared using the technique described in treatment 3 and harvested in the manner described in treatment 4. Adults were chilled when less than 6 hours old, irradiated with 15 krad C060 and held for 20-24 hours at 50° F until release.

Treatment groups were distinguished by day-glo fluorescent dust. In all treatment groups, males were eclosed in the laboratory and cooled for handling and marking (ca 1/2 hour at 50° F) prior to release. However, the 1979-NTT group which was irradiated as adults was held for ca. 24 hours at 50° F.

To monitor male activity, traps were placed on two concentric circles (70 and 140 m radii) around the center release point. In all, 66 traps were placed in each plot; 22 and 44 traps on the inner and outer rings respectively. Five types of traps were placed in each plot to compare trap design efficiency. These designs were:
1) Delta trap
2) MC-A - 12 entry ports 5/16" x 1".
3) MC-B - 4 entry ports 1/2" x 1" and 8 ports 5/16" x 1".
4) MC-C - 12 triangular entry ports 1¼" x 7/8" x 7/8".
5) MC-D - 16 entry ports 5/16" x 1".

All traps were baited with Hercon wicks (+ disparlure) and hung from the branches of trees. Each milk carton trap design was represented 4 times, on the inner circle and twice on the outer circle (for a total 6 milk carton traps of each design in each plot). Six delta traps were placed on the inner circle and 36 on the outer circle. For randomization, roughly each quadrant (90°) of the trap circle was assigned one of the 5 trap treatments. Within each quadrant trap positions were randomized with each replicate (male release). Plots were separated by at least 1 mile.

In all, 3 replicates of the test described above were run with releases on March 20, 22 and 23.

Males irradiated as adults in replicate 1 were tattered and had lost most wing scales during irradiation. This condition occurred even though freezer packs had been placed on the top and bottom of the irradiation canister. In subsequent treatments small freeze packs were placed directly into the treatment cylinder containing the males. This procedure reduced male activity and damage but did not completely eliminate it. These observations emphasized the critical need to keep males cool when irradiating, shipping and handling. Shipping techniques and procedures will have to be worked out carefully because only a short time at higher temperature (ca 1/2 hour) will release male activity and cause loss of wing scales and wing damage. Tests have been initiated to determine how low insulated shipping cartons can be held at inactivating temperatures using prefrozen packs.

Allowing males to eclose in the laboratory and expand and harden their wings before eclosing appears to be an acceptable technique. Males usually remained quiescent after eclosion and the eclosion box was easily transported to a chilling room (50°F). After ca. 1/2 hour males were sufficiently inactive to mark, count and drop them through a metal funnel into a 16 oz. squat cup. Fifty males were placed in each cup. Cups for field release were loaded in insulated shipping cartons and transported to the field. The temperature was held at ca. 50°F until release by placing frozen freezer packs in the carton. A few (ca. 5) minutes were required after removal of males from insulated containers for them to regain mobility.
Result and Discussion:

Results of the strain comparison are not clear. Males reared according to 1979 – NTT and harvested by hand (no chlorox) performed well in all three replicates (Table 1). Males reared in the same manner but harvested with chlorox were not recovered with the same frequency as the former group and were significantly different from the standard (1978 - TT) male in 2 of 3 replicates. It appears that chlorox treatment in some way affects male competitiveness. However, because of the conditions when the chlorox treatment were carried out (i.e. excessive and rough handling and cold temperatures) competitiveness may have been affected by other factors. The technique, if developed more completely may save considerable harvesting expense. Males irradiated as adults and held for ca. 24 hours at 50°F performed as well as the NTT treatment which were not irradiated. Surprisingly, in trials 1 and 3, males irradiated as pupae performed poorly. In 1978 field trials, males reared and irradiated in a similar manner performed as well as untreated adults. Perhaps the inconsistency between the results of these two tests can be explained. Male pupae used in the Brownsville trials were shipped in insulated cartons using freezer packs for cooling. Because of airline delays these pupae remained at 50°F for ca. 36 hours. Normally, chilling slows or delays eclosion because developmental processes are slowed down. Even though the pupae in this group were irradiated 10-11 days after pupation, the development of the insects may have been delayed and developmentally they were, in fact, younger. 1978 trials demonstrated that pupae irradiated as 8-9 days old were competitively lacking and, possibly, the Brownsville insects were irradiated in much the same developmental stage.

Generally, no transfer rearing appears to be an acceptable method. Harvesting and separation of pupae using a chlorox bath requires further investigation. Irradiation of adults did not significantly decrease the competitiveness of adults except in replicate 3 and males from this treatment performed as well as males eclosing from irradiated pupae. Again, it should be emphasized that procedures need to be refined to prevent the warming of adults in radiation treatment and shipping.

Of the four milk carton trap designs tested, analysis did not reveal any significant difference between entry port design (Table 2). However, trap type MC-A caught a larger proportion of the male population than any other type. Interestingly, all four milk carton traps averaged higher male catches than the delta trap (Table 3). Even though significant differences could not be found between the four milk carton traps, the larger average catch per trap by MC-A indicates it is more efficient in capturing males.
Table 1. Brownsville Test - Strain comparison - pairwise $X^2$ at 5% level of significance.

<table>
<thead>
<tr>
<th>STRAIN (Treatment)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Recovered</td>
<td>N</td>
<td>% Recovered</td>
</tr>
<tr>
<td>1</td>
<td>15.83 c</td>
<td>259</td>
<td>50.00 a</td>
</tr>
<tr>
<td>2</td>
<td>7.07 d</td>
<td>283</td>
<td>34.83 b</td>
</tr>
<tr>
<td>3</td>
<td>37.15 a</td>
<td>506</td>
<td>32.11 b</td>
</tr>
<tr>
<td>4</td>
<td>19.96 bc</td>
<td>531</td>
<td>28.47 b</td>
</tr>
<tr>
<td>5</td>
<td>24.34 b</td>
<td>189</td>
<td>25.24 b</td>
</tr>
</tbody>
</table>

Table 2. Comparison of 1/2 gallon milk carton traps with four entry port designs.

<table>
<thead>
<tr>
<th>TRAP CODE</th>
<th>REPlicate</th>
<th>No. Males Captured</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>42 74 62 41</td>
<td>219 54.75</td>
</tr>
<tr>
<td>B</td>
<td>31 56 46 22</td>
<td>155 38.75</td>
</tr>
<tr>
<td>C</td>
<td>37 63 41 17</td>
<td>158 39.50</td>
</tr>
<tr>
<td>D</td>
<td>50 56 52 21</td>
<td>179 44.75</td>
</tr>
</tbody>
</table>

F=0.79

Table 3. Comparison of capture in different trap designs on the 70 m ring of circular plots.

<table>
<thead>
<tr>
<th>Trap Type</th>
<th>$\bar{x}$ No. of Males captured/trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>6.25</td>
</tr>
<tr>
<td>MC-A</td>
<td>13.69</td>
</tr>
<tr>
<td>MC-B</td>
<td>9.69</td>
</tr>
<tr>
<td>MC-C</td>
<td>9.88</td>
</tr>
<tr>
<td>MC-D</td>
<td>11.19</td>
</tr>
</tbody>
</table>
Introduction:

The purpose of this project is to explain the current insect quality monitoring techniques used in the rearing facility. Data are 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.

Production Monitoring:

Developmental and reproductive data were collected by the procedure described by Tanner and Houle (1978). This procedure was found to be time consuming (5-7 man hours/day) making it impractical for use in the daily monitoring of production. A modified version of this procedure is used for the daily monitoring of production.

The number of man hours needed to collect the data was reduced three ways:

1. Only the major production strain (New Jersey) was monitored.
2. Unnecessary measurements were eliminated.
3. The sample size was reduced wherever possible.

The modified procedure is described below. The number of man hours required to collect data was reduced to 1-1/2 to 2 man hours/day.

Daily Monitoring of Production:

An internal standard (Chambers 1977) was developed with the data collected by Tanner and Houle (1978). Daily production is compared to this standard with significant variation indicating a possible change in colony development. Sampling is random and commences with neonates infested onto diet each weekday. On the 7th, 14th and 21st post-infest day (PID), five cups are selected and the percentage of larvae in various developmental stages is determined. The results are compared to the standard.

On the 28th PID (Calculated male pupae DT 50 ) and the 30 PID (Calculated female pupae DT 50 ), five cups are selected and the percentage of male or female pupae determined. The percentages are compared with that of the standard.
The 35th PIO, all pupae are harvested and the percent survival from neonate to pupa determined by the following formula:

$$\frac{\# \text{ pupae harvested}}{\# \text{ neonates infested}} \times 100$$

Fifty male and fifty female pupae are weighed and the percent deformity determined. Percent survival, percent deformity and pupal weights are compared with the standard.

The weighed pupae are used for adult emergence data. Newly emerged adults are examined for deformity then discarded or used for colony maintenance. Percent emergence, percent deformity, and calculated male and female DT$_{50}$ are determined and compared with the standard. The sex ratio is determined by the following formula:

$$\frac{\# \text{ male pupae harvested} \times \% \text{ adult male emergence}}{\# \text{ female pupae harvested} \times \% \text{ adult female emergence}}$$

Mating, fecundity and egg hatch data are taken directly from the colony. Males are mated with females from the same larval infest date. A mating container (25 matings) is selected for each larval infest date. A pre-weighed oviposition paper is inserted into each container. Seven days after mating, the paper is removed and the percentage of females laying egg masses and the mean egg mass weight are determined. The mean number of eggs/mass is determined by multiplying the mean egg mass weight by 1.33. The masses are returned to the colony.

After 120 days of chilling, a sample of five masses is taken and a hatch test conducted. Approximately 20-40 eggs are taken from the "core" of each mass. Care is taken not to remove the hairs as tests by Tanner and Buck (1979) have shown dehairing to be detrimental to the embryo. The eggs are placed into petri dishes and incubated in an environmental room (25°C, 50-55% RH).

Results:

The mass rearing procedure has been changed since the Tanner-Houle data were accumulated, thus, those data are no longer applicable as a standard. However, the data can be used to evaluate the new rearing procedure. The basic differences between the two rearing procedures are the number of neonates infested/cup and the transferring of larvae to new diet on the 21st post infest date (PID). Under the old system (Transfer system), 15 neonates were infested onto high wheat germ diet (B-4 diet) and then transferred to modified hornworm diet after being sexed on the 21st PID (Tanner and Houle, 1978, Forrester 1978). The new system (No Transfer System) consists of infesting 8 neonates onto B-4 diet then allowing them to develop to pupae with no transferring or sexing (Forrester, personal communication).

Table 1 presents the developmental and reproductive data for gypsy moth (New Jersey strain) reared under the two systems. The significant developmental and reproductive differences are listed in Table 2.
Table 1. Performance of the New Jersey Laboratory strain of Gypsy Moth Under Mass Production.

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pupal Weights</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males (gms)</td>
<td>.57 ± .03 B</td>
<td>.72 ± .04 A</td>
</tr>
<tr>
<td>Females (gms)</td>
<td>2.02 ± .17 B</td>
<td>2.41 ± .14 A</td>
</tr>
<tr>
<td><strong>% of Male Pupae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 PID (Cal DT&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>47.1 ± 10.9 A</td>
<td>11.8 ± 10.7 B</td>
</tr>
<tr>
<td><strong>% of Female Pupae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 PID (Cal DT&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>52.6 ± 18.0 A</td>
<td>19.8 ± 11.1 B</td>
</tr>
<tr>
<td><strong>% Pupal Deformity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>&gt;1 B</td>
<td>7.5 ± 3.7 A</td>
</tr>
<tr>
<td>Females</td>
<td>49.0 ± 14.4 B</td>
<td>93.0 ± 5.3 A</td>
</tr>
<tr>
<td><strong>% Survival</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Neonate to Pupae)</td>
<td>88.1 ± 6.6 A</td>
<td>79.0 ± 7.7 B</td>
</tr>
<tr>
<td><strong>Developmental Time</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to Adults (DT&lt;sub&gt;50&lt;/sub&gt; - Days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>40.5 ± .5 B</td>
<td>44.5 ± .7 A</td>
</tr>
<tr>
<td>Females</td>
<td>40.8 ± 1.0 B</td>
<td>43.1 ± .8 A</td>
</tr>
<tr>
<td><strong>% Adult Deformity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0.6 ± 1.4 A</td>
<td>1.6 ± 4.0 A</td>
</tr>
<tr>
<td>Females</td>
<td>13.9 ± 7.5 A</td>
<td>6.0 ± 4.5 B</td>
</tr>
<tr>
<td><strong>% Adult Recovery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>94.3 ± 6.4 B</td>
<td>99.0 ± 1.6 A</td>
</tr>
<tr>
<td>Females</td>
<td>91.3 ± 8.7 A</td>
<td>95.9 ± 4.0 A</td>
</tr>
<tr>
<td><strong>% Survival</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Neonated to adults)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>83.5 ± 10.9 A</td>
<td>78.2 ± 8.0 B</td>
</tr>
<tr>
<td>Females</td>
<td>80.1 ± 9.7 A</td>
<td>75.7 ± 8.2 A</td>
</tr>
<tr>
<td><strong>Sex Ratio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td># Males/Females</td>
<td>1.48 ± .53 A</td>
<td>1.12 ± .26 B</td>
</tr>
<tr>
<td>% Mating Pairs</td>
<td>93.8 ± 6.5 A</td>
<td>91.2 ± 9.6 A</td>
</tr>
<tr>
<td>Eggs laid/female</td>
<td>887.8 ±101.5 B</td>
<td>1042.9 ±160.5 A</td>
</tr>
</tbody>
</table>

1/ Means within a row not followed by the same letter are significantly different (P=0.05)

2/ Data collected by Tanner-Houle (1978). Neonates infested (15/cup) onto high wheat germ diet and the larvae sexed and refed modified hornworm diet on the 21st PID

3/ Neonates infested (8/cup) onto high wheat germ diet with no transfer on the 21st PID
The major developmental and reproductive differences between New Jersey laboratory gypsy moth strains reared under the no transfer rearing procedures and the standard rearing procedures are as follows:

Adoption of the no transfer system resulted in:

I. Increased larval developmental period
II. Increased pupal weights
III. Increased pupal deformity
IV. Reduced survival from neonate to pupae
V. Delayed adult emergence
VI. Increased adult male emergence
VII. A change in the adult emergence pattern from males and females with the same DT$_{50}$ to males with the DT$_{50}$ one day later than the females
VIII. Decreased adult female deformity
IX. Reduced number of adult males/female (sex ratio)
X. Increased number of eggs laid/female

The transfer and no transfer rearing systems produced different developmental and reproductive data (Table 1). The differences probably reflect different larval densities. The lower larval density (8/cup), no transfer rearing system, produces the longest larval and pupal developmental period. It also produces the heaviest pupae and resulted in females with a higher fecundity. Larval development is directly related to larval density (Leonard 1968, Campbell 1978). Lower larval densities result in longer developmental periods. Lower larval densities also result in heavier pupae (Leonard 1968) and heavier female pupae produce adult females with a higher fecundity (Maksimovic 1958, Campbell 1978).

In our colony, heavier pupae have more banding (deformity) but there is no relationship between banding and adult deformity (Bell, personal communication). This accounts for the greater percentage of pupal banding obtained with the heavier pupae produced by the no transfer rearing system. The lack of a relationship between banding and adult deformity also explains why there was less adult female deformity even though there was more female pupal banding in the no transfer system.

A lower percentage of pupae was harvested with the no transfer method. This was not due to increased larval mortality but indirectly the result of the longer larval developmental period. Pupal harvest was terminated before many of the last larvae pupated. These larvae were discarded (Kennedy, personal communication).
The no transfer system changed the adult emergence pattern. With the transfer system, the adult males and females had the same DT<sub>50</sub> (Developmental Time to 50% adult emergence). With the no transfer system, the males DT<sub>50</sub> was one day later than the females. This change can be traced back to the larval developmental period. The no transfer system extended the male larval developmental period by 3 days the females by only 2 days.

The no transfer system had a sex ratio that was closer to 1:1 than the transfer system. This was the result of a new neonate infestation method and not due to the rearing system (Kennedy, personal communication). Bell (personal communication) observed a predominance of male larvae in the first 3 or 4 days of hatch. During the next several days of hatch, the sex ratio was more 1 to 1. The last few days of hatch were predominantly females. When the transfer method was used, neonates were selected from masses that have been hatching only 3 or 4 days. Neonates are not selected from masses that have been hatching for at least 6 days.

Colony Hatch Test:

The colony hatch test procedure described by Tanner and Houle (1978) was found to be unreliable in predicting the true colony hatch (Tanner and Buck 1979). Other procedures are being tested but none have shown promise. Until this problem is resolved, colony hatch tests will not be conducted.

Air Quality Tests:

Air particle counts have been temporarily discontinued. The procedure used by Tanner and Houle (1978) does not give an accurate picture of the number of particles in the air during a work day. Adapting the machine with a paper record and allowing the machine to run 24 hours in one room should give a good profile of the number of particles in the air during an average work day. Microbial plates are still taken weekly. No serious contamination has been found.
References Cited


Project Number: GM 7.3.4
Project Title: Insect Production and Distribution
Report Type: Interim
Project Leader: L. F. Kennedy

No progress to report.
No progress to report.

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 selected for use in our quality control program will be evaluated for their accuracy in expressing true population mean. Unreliable or inaccurate techniques will be modified or discarded.

The first technique to 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 number hatch of an egg mass.

Methods:

New Jersey (F₁) egg masses were sampled after 90, 120, 150 and 180 days of chilling. Each sample consisted of five masses. Twenty eggs were removed from the core of each mass and placed into petri dishes (50 x 9 mm). The remaining portion of each mass was also placed into petri dishes. All the eggs were incubated in an environmental room (25°C, 60-65% RH). Mortality was removed daily and counted. Upon completion of hatch, the percent survival, the percent hatch and the eclosion rate were determined. Data were analyzed by a t-test for paired observation. A total of ten replicates were conducted over a period of time.
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 to 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 number hatch of an egg mass.

Methods:

New Jersey (F_1) egg masses were sampled after 90, 120, 150 and 180 days of chilling. Each sample consisted of five masses. Twenty eggs were removed from the core of each mass and placed into petri dishes (50 x 9 mm). The remaining portion of each mass was also placed into petri dishes. All the eggs were incubated in an environmental room (25°C, 50-55%RH). Hatch was removed daily and counted. Upon completion of hatch, the percent embryonation, the percent hatch and the eclosion rate were determined. The data were analyzed by a t-test for paired observation. A total of ten replicates were conducted over a period of time.
Results:

The percent hatch of a 20 egg core sample was not very reliable in predicting the percent hatch of the whole egg mass. Only 20 to 27 percent of the core samples had a percent hatch that varied by 10 or less percentage points from the percent hatch of the corresponding whole egg mass (Fig 1).

A reliable hatch test may be possible if the hatch samples were selected from a homogenous mixture of several masses. The following test was developed to examine this possibility.

Methods and Materials:

Egg masses were selected for colony maintenance two weeks prior to their expected date of incubation. The masses were treated with a 10% formalin solution (60 min) then rinsed and dried. The masses were weighed and the total number of eggs determined by multiplying the weight by 1.33 (Bell, personal communication). Ten percent of the eggs were selected by weight. The eggs were dehaired and five - 20 egg samples were drawn from the homogenous mixture of eggs. The samples were placed into individual petri dishes and incubated directly in an environmental room (25°C, 50-55% RH). The remaining egg masses and dehaired eggs were returned to chilling for two weeks before they were used for colony maintenance. The additional chilling did not effect the hatch of the dehaired eggs (unpublished data).

When the egg masses and the dehaired samples were to be incubated, a predicted hatch was available from the hatch samples. This predicted hatch was compared to the actual hatch.

Results:

Figure 2 presents the predicted and the actual hatch of egg masses incubated for colony maintenance. The sample (predicted) hatch followed a similar trend as observed by Tanner and Houle (1978) with their sample hatch. There was a period of high hatch followed by a period of low hatch. The actual colony hatch varied between 80 to 90 percent. The low sample hatch was unique to the samples and may be due to our egg selecting and/or egg handling technique. The factor(s) which caused the discrepancy between the predicted and the actual percent hatch must be identified before a reliable hatch test can be developed. Presented below are tests which have been conducted in hopes of identifying the factor(s).
Effects of dehairing on egg hatch:

A hatch test consists of 20 eggs excavated from the core of an egg mass (Tanner and Houle, 1978). To obtain exactly 20 eggs, a small core section must be removed from the egg mass and the setae mechanically removed (dehaired). This allows for a visual count of exactly 20 eggs. Mechanical dehairing may damage the eggs. Dehairing may also expose the eggs to desiccation. The following test was developed to determine the effects of mechanical dehairing on percent hatch.

Methods and Materials:

A sample of eggs was removed from the core of an egg mass and mechanically dehaired by rubbing the sample between two pieces of paper. Twenty eggs were randomly selected and incubated in a petri dish. A second sample was removed from the core of the mass and placed directly into a petri dish without being dehaired. The mass was then sectioned in half. One half of the mass was placed into a petri dish without being dehaired. The other half was dehaired. A 20 egg sample was randomly selected from the dehaired half mass and placed into a petri dish. The remaining dehaired eggs were also placed into a petri dish. All the eggs were incubated in an environmental room (25.5°C, 50-55% RH). Neonates were removed daily. Five, 120 day chilled egg masses were used/replication. Four replications were conducted. The data were analyzed by AOV and the treatments separated by Duncan's Multiple-Range test.

Table 1. The effects of dehairing on the percent hatch of gypsy moth eggs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Percent Hatch a/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non dehaired - half mass</td>
<td>97.3 ± 3.1 A</td>
</tr>
<tr>
<td>Non dehaired - sample</td>
<td>96.8 ± 6.9 A</td>
</tr>
<tr>
<td>Dehaired - half mass</td>
<td>61.3 ± 27.0 B</td>
</tr>
<tr>
<td>Dehaired - sample</td>
<td>51.8 ± 28.4 BC</td>
</tr>
<tr>
<td>Dehaired - half mass - sample</td>
<td>33.7 ± 29.1 C</td>
</tr>
</tbody>
</table>

a/ Means within a column not followed by the same letter are significantly different (P=0.05) (Duncan's multiple range test)
b/ Mean of two replicates only
Results:

Dehaired eggs produced a significantly lower percent hatch than non-dehaired eggs (Table 1). Also the wide standard deviation (dehaired samples) indicates that egg masses varied considerably in the response to dehairing. Dehairing the eggs may mechanically damage the eggs and/or expose the eggs to some adverse environmental condition.

The percent hatch of gypsy moth eggs as affected by their incubation in a saturated atmosphere:

When observed under a microscope, many of the unhatched, dehaired eggs contained desicated embryos. Removal of the hydrophobic setae may expose the embryo to excessive drying and result in increased embryo mortality through desication. The following test was developed to examine the effect of relative humidity on hatch.

Methods and Materials:

This test was conducted within an environmental room programmed for a 25°C temperature. Four samples (ca. 20 eggs/sample) were taken from an individual egg mass and placed into individual petri dishes. One half of the samples were dehaired between two pieces of paper toweling. The remaining samples were not dehaired. One dehaired and one non-dehaired egg sample was incubated directly within the room (50-55% RH). The two remaining samples were incubated in a saturated atmosphere (100% RH) maintained within a desicator. Newly hatched neonates were removed daily. Percent hatch was computed based on the total number of embryonated eggs. Five masses were used per replication. Five replications were conducted over a period of time. The treatments were analyzed by an AOV test and compared by Duncan's multiple range test.

Table 2. The percent hatch of gypsy moth eggs as affected by their incubation in a saturated atmosphere.

<table>
<thead>
<tr>
<th>Percent Humidity</th>
<th>Percent Hatch /</th>
<th>Non dehaired</th>
<th>Dehaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>92.8 ± 4.5 A</td>
<td>92.3 ± 4.1 A</td>
<td></td>
</tr>
<tr>
<td>50 - 55</td>
<td>89.2 ± 4.7 A</td>
<td>40.5 ± 17.2 B</td>
<td></td>
</tr>
</tbody>
</table>

/ Means not followed by the same letter are significantly different (P=0.05) (Duncan's multiple range test).
Results:

Table 2. summarizes the results of the test. Hatch percentages were similar when dehaired and non dehaired eggs were incubated in a saturated atmosphere. Incubating dehaired eggs directly in the environmental room (55% RH) resulted in lower hatch percentages compared to dehaired and non dehaired eggs incubated in the saturated atmosphere and non dehaired eggs incubated directly in the environmental room. Non dehaired eggs incubated directly in the environmental room had a slightly lower mean percent hatch than non dehaired eggs incubated in the saturated atmosphere.

Incubating dehaired eggs directly in the environmental room may have caused the percent hatch discrepancy observed between the dehaired core sample and the whole egg mass (Figure 1). The environmental room may be programmed for a too low humidity and/or a too high airflow. Dehairing the eggs exposes the embryo to excessive drying and results in death through desication.

A reliable hatch test may be developed with dehaired samples if the samples are incubated in a saturated or near saturated atmosphere. A dehaired sample is preferred because the number of eggs incubated can be kept constant. With a non dehaired sample, the hairs (seta) obscure the eggs. The actual number of eggs incubated can never be accurately determined until after the hatch test is completed. The actual number of eggs incubated was found to vary considerably between non dehaired egg sample (unpublished data).

Testing will continue until a reliable hatch test is developed.
Figure 1. The distribution in the percentage points difference between the percent hatch of a whole egg mass and its corresponding 20 egg sample (New Jersey F17).

1. Percentage Points Difference = The number of percentage points between the percent hatch of the whole egg mass and the percent hatch of the 20 egg sample.
2. The percentage of samples within a percentage points difference range.
Figure 2. The predicated and actual eclosion rates of colony (New Jersey) egg masses chilled 120 days.
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 Type: Final
Project Leaders: John Allen Tanner and Terry Buck

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_{9}, Abington F_{16}). 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 compares the New Jersey (F_{17}), Ludlow (F_{17}), Pennsylvania (F_{8} + F_{2}) and Abington (F_{1}) strains. All the masses came from insects reared at the same time. Each mass was embryonated 28 days and chilled at 6-7°C. Hatch tests commenced with 90 day chilled eggs and continued at 30 intervals, until the masses have been chilled for 180 days. A hatch test consisted of 10 masses/strain. Twenty eggs were excavated from the core of each mass and placed into individual petri dishes. The eggs were incubated in an environmental room programmed for 25°C and 50-55% RH. Differences in percent hatch and hatch rates were tested by AOV tests and linear regression.

Results:

All the hatch tests have been completed. Each test was conducted within the same environmental room used by Tanner and Buck (1979). Their data indicated that within this environmental chamber, a dehaired 20 egg core sample gave an inadequate estimate of the percent hatch of an egg mass. For this reason our data will not be presented. However, the test will be repeated when an adequate hatch test is developed.
References Cited:


There is no progress to be reported on the following projects:

- **GM 9.3.1** Laboratory Evaluation of Eggs Collected from Oconomowoc, WI
- **GM 9.3.2** Silk Removal from Gypsy Moth Pupae
- **GM 9.3.3** The Effect of Temperature on Vitamin Pre-mix Relative to Development and Egg Hatch
- **GM 9.3.4** Alternatives to Wheat Germ in Artificial Gypsy Moth Diet
- **GM 9.3.5** Evaluation of No Transfer Rearing Technique
Appendix 1

Using Pheromone-Baited Traps to Detect and Evaluate Populations of the Gypsy Moth

Introduction

The gypsy moth, *Lymantria dispar*, is a destructive insect pest of hardwood forests and shade trees in the United States. The larva feeds on leaves of many types of deciduous trees, and severe or successive defoliations result in decreased tree growth and vigor. Mortality may occur because these trees are predisposed to attack by secondary insect pests and microbial pathogens. Large caterpillars will feed on coniferous trees that are not preferred by smaller larvae, and a single defoliation of these species usually results in death.

The range of this insect now extends over most of the Eastern United States. Although the gradual enlargement of this generally infested region is due to natural spread, infestations are caused by artificial spread also. Natural spread occurs when newly hatched larvae spin down on long strands of silk and are buoyed by air currents. Such dispersal is highly seasonal, occurring during the time of egg hatch, and is dependent upon wind speed and direction. Artificial spread occurs when gypsy moth life stages are inadvertently carried to uninfested areas by man. Logs, firewood, mobile homes, and recreational vehicles are commonly found to be infested and have been implicated with the establishment of a number of small infestations far from the generally infested areas. Quarantine and inspection activities are conducted to minimize the incidence of such movement.
In spite of the efforts to retard artificial spread, new isolated infestations are frequently established. During the mid-1970's, three to four infestations were discovered each year. Early detection of these infestations is necessary if they are to be prevented from building to damaging population levels. The boundaries of the infestation must also be clearly delineated so that appropriate control measures can be accurately applied. Traps baited with attractants are effective devices for determining the occurrence and distribution of pest insects.

The Attractant

Female gypsy moths produce a chemical sex pheromone that attracts mates to them and evokes male mating behavior. The chemical, (+)-cis-7, 8-epoxy-2-methyloctadecane, has been isolated, identified, and synthesized. The common name of the pheromone, disparlure, is derived from the scientific name of the gypsy moth, Lymantria dispar. This optically active form of disparlure is being phased into the USDA survey program to replace the less effective racemic mixture previously used.

Disparlure is commercially formulated for use by incorporation into three-layer plastic laminated dispensers. Each dispenser measures 1x1/8 in. and contains approximately 1 mg disparlure that is released into the air at a constant rate throughout the field season. Because research is continuing in an effort to utilize more efficiently this costly attractant, these specifications may change.
The Trap

The trap currently in use for gypsy moth survey is called the delta trap, because of its triangular shape (fig. 1). It is fabricated of bleached sulfate, medium-density, solid paperboard, .022 in. nominal thickness. Polyplastic coating .75 mil thick prevents water absorption and improves the field weathering characteristics of the trap. The trap is die cut, creased, and perforated, as shown in fig. 2. Certain creases are perforated to facilitate folding of the baffled ends inward (fig. 3). Approximately 42 in$^2$ of surface area is covered with 0.3 oz. of adhesive, Tack-Trap, which is sufficient to ensnare 15-20 moths.

The first step in preparing the trap for field use is to install the pheromone dispenser. The technique used should allow for ample air circulation around the dispenser to facilitate pheromone release. At no time should it come in contact with the sticky adhesive. An acceptable method is to staple one end of the dispenser to panel A (fig. 2) in such a way that it hangs freely within the assembled trap. Folding the trap is facilitated by first "breaking" all creases and perforations. If the trap will be checked frequently, it can be stapled to a tree through panel D, folded into place, and held closed with two paper clips. Traps that do not require frequent inspection can be assembled and stapled several times through panels D, E, and F. The completed trap is then stapled to a tree or suspended with string or wire threaded through the holes in panels D, E, and F.
Traps should be placed as low as possible on the boles of trees since most moth flight activity is near the ground. The standard height for placement is between 3 and 5 ft. To discourage vandalism, traps may be placed higher. However, increasing the height of trap placement decreases the probability of male capture. Also, fixing the trap to a vertical silhouette such as a tree, instead of free hanging the trap, will increase the probability of male capture. When placing traps, care should be taken that entrance ports are not obstructed by leaves or branches. Also, if the trap is in a location exposed to direct sunlight, it should be hung on the north side of the structure. High temperatures within the trap caused by direct sunlight may cause the adhesive to dry out and become ineffective.

Detection Survey Methods

One of the most important features of an effective survey is consistency. Following a standard plan allows comparison of results from location to location and from time to time. The probability of detecting an infestation is dependent upon the size and density of the pest population and the distance between traps (trap density). For routine detection surveys, trap density should be 1 trap per 3 mi². If resources are limited, it is advisable to trap smaller areas of a region on a rotating basis. This way trap density is not compromised in the interest of surveying large areas, and each area will be surveyed on a regular basis. In planning the survey, a uniform grid is overlayed on a map, and trap locations are approximated. In many situations traps can thus be identified according to State, county, township, and section locations. Actual placement of the traps should be as close to the grid point as possible.
A general rule of thumb is not to deviate from a trap site by more than 25 percent of the distance between traps (1/4 mi in a detection survey). This flexibility in locating traps permits placement in accessible areas most likely to harbor infestation. When locating trap positions, fence rows and wood lots should not be disregarded. When host material is present, the area should be regarded as a possible trap location. Since artificial movement of gypsy moth is associated with the movement of people and their commodities, it is often worthwhile to concentrate detection survey efforts in populated areas or on commercial or recreational sites. Examples are roadside rest stops, campgrounds, mobile-home parks, logging mills, nurseries, and forested urban and suburban communities that generally favor the introduction and establishment of the pest. In small, high-risk sites such as campgrounds, parks, and sawmills, trap density may be increased up to several traps per square mile, thus improving the probability of detecting very small, isolated infestations.

Monitoring

Detection traps should be placed in the field by mid-June, before male flight normally occurs. Frequent checking of these traps is not necessary as they are designed to remain effective throughout the season. It is good practice, however, to check them at least once. Traps are often lost to vandalism, and those near dusty roads may become ineffective as the Tack Trap® becomes covered with dust. Trap replacement may even occasionally be necessary. Also, if a midseason check reveals evidence of infestation, it is possible to begin a delimitation survey to determine the extent of infestation.
Delimitation Survey

When male moths are captured in a detection survey it is necessary to confirm the existence of a reproducing population and to determine the area of infestation. This is done through a delimitation survey. Under most circumstances, male moths are not likely to disperse far (more than 1 mile) from their point of origin. Therefore, it is recommended that delimitation be conducted by placing 25 traps per \( \text{mi}^2 \) over 9 \( \text{mi}^2 \) centered on the positive detection trap(s). Such an array will contain 225 traps on a grid and traps will be about 1,000 ft apart (fig. 4). A system similar to that used for planning and laying out the detection survey can be used. If, in areas of broken woodland, a trap site cannot be located on or near potential host material by deviating from the site by 25 percent of the distance between traps (250 feet), that site should be left untrapped.

In areas subdivided into 640-acre sections, placing 25 traps per square mile is not practical, because very few trap locations will fall on natural boundaries such as roads and fence rows. In such settings, uniform trap distribution can be accomplished by placing 32 traps per square mile, as shown in fig. 5. This plan provides for uniform distances between traps, and most trap sites will be on natural boundaries where host plants are most likely to occur. Trap placement and recovery will also be facilitated by following these boundaries.
Timing

The timing of the delimitation survey should be the same as that used in detection survey. Traps should be in place by June 15 and can be removed by September 15. As mentioned earlier, the delta trap has capacity for about only 15 moths. If an infestation does in fact exist within the area under delimitation, traps may quickly fill with moths and become ineffective. Therefore, they should be examined as frequently as practical. Merely removing insects from the trap is generally not adequate because wing scales and other debris will coat the Tack-Trap®. Traps which have captured 15 or more moths should be replaced. It is not necessary, however, to use a new lure dispenser; simply transfer it into the new trap.

Careful and accurate recordkeeping is fundamental to effective survey. All moth recoveries should be immediately recorded in the field in an appropriate record book. The entry should include the date, exact trap location, number of specimens, and the name of the observer. Observations on weather and trap and insect condition may also be noted. If it is necessary to submit the specimen for positive identification, replace the trap with a new one and forward the insect within the trap to the identifier. If laboratory identification is not necessary, the moth should be removed from the trap so it is not counted again the next time the trap is checked. At the end of each day, field records should be transcribed to a master log. It is important to stress that data regarding empty traps should also be reported; this will serve to determine specifically when each trap was examined.
Survey Interpretation

About all that can be said about the capture of a gypsy moth in a
detection survey is that it probably originated within a mile of the trap
in which it was captured. As mentioned earlier, if it is discovered early
in the season it may be possible to place additional traps in the area. It
is not unusual, however, to capture isolated moths in an area where no
further evidence of infestation is ever discovered. Those incidents are
likely the result of "hitchhiking" insects on recreational vehicles. If a
delimitation survey the year following detection is negative -- that is,
no moths are captured - it may be concluded that a reproducing population
does not exist in the area surveyed.

If, however, insects are captured in the delimitation survey, it is
possible to characterize the distribution of the infestation. As a general
rule, it can be stated that moths are most likely to be captured in those
delimitation traps closest to their point of origin. The probability of
capture decreases dramatically as this distance increases. Thus, mapping
the percentage of captured insects found at each trap location gives an
image of relative population density. Establishing the absolute boundaries
of the infestation is somewhat more difficult, and it should be kept in mind
that a small proportion (less than 1 percent of the captured population) is
expected to be found in traps up to 1 mi from where they originated. Delimi-
tation is not necessarily done by encircling all traps containing moths.
The recommended procedure to follow in mapping insect distribution is to total the number of moths caught in all traps in the delimitation survey, then to divide the number of moths found in each trap by this total. Each figure will represent the relative population density near that trap. Those traps containing the most insects represent the densest area of the infestation, and it is in this area that egg-mass scouting should be concentrated. The boundaries of the infestation can be defined by connecting those traps that contain more than 1 percent of the total captured population.

Because adult male behavior may be affected by influences of temperature, humidity, wind, topography, abundance of female moths and openness of terrain, it is difficult to apply standard survey procedures and interpretation methods universally. Each survey plan must consider factors such as distribution of host-plant material, probable time and location of introduction, and potential for spread of the infestation. Factors such as these may warrant modification of the guidelines for survey described in this handbook. Also, it should be stressed that research and development are continuing in an effort to improve effectiveness of survey techniques, and these guidelines may be changed as new information becomes available.
Figures

Figure 1. Assembled delta trap. The orange color is readily visible to facilitate recovery. Green and brown traps are used in areas where high visibility is not desired.

Figure 2. Schematic layout of the delta trap. Dimensions are in centimeters. Shaded areas indicate where adhesive is applied. Creases are indicated by solid lines and perforations by dotted lines.

Figure 3. Perspective drawing showing partially assembled and completed trap.

Figure 4. Delimitation survey grid with 25 traps per square mile. In practice, this trap arrangement would be deployed over 9 mi².

Figure 5. Delimitation survey grid with 32 traps per square mile. Note that maximum utilization is made of roads and fence rows. This design is recommended for use in areas subdivided into quarter sections.
Appendix 2

Intended for publication in:
Environmental Entomology

Crepuscular Activity of Gypsy Moth Adults

by

Thomas M. ODell 1/ and Victor C. Mastro 2/

1/ U.S. Department of Agriculture, Forest Service, Forest Insect and Disease Laboratory, 151 Sanford Street, Hamden, CT 06514.

INTRODUCTION

The twilight (evening and morning) migrations (= crepuscular activity) of gypsy moth larvae to and from their feeding sites is an interesting behavioral trait that has promulgated speculation as to its survival strategy (Leonard 1970, Campbell and Sloan 1976, Campbell 1978). Of seemingly lesser interest has been the evening activity of adult gypsy moths reported by Carde and Doane (1974) for males and by ODell (1978) for males and females. Recently, while investigating the competitive behavior of laboratory-reared and wild type gypsy moth adults we were able to closely examine adult crepuscular activity and observed an activity periodicity quite similar to that of the immature stages. Here we describe the sequential behavior and consistency of the adult crepuscular activity, and discuss the survival strategy for its maintenance in gypsy moth populations.
METHODS AND MATERIALS

The studies were conducted in wooded sites on or near Otis Air Force Base, Cape Cod, Ma., during July and August of 1977 and 1978.

Wild adults (WT) were collected as pupae from Massachusetts, New York, and Pennsylvania. Laboratory-reared (LR) pupae had been reared for 16 (1977) and 17 (1978) generations at the APHIS Gypsy Moth Methods Development Laboratory, Otis Air Force Base, Ma.

Male activity was observed in field plots and in 12 x 12 x 8 ft screen cages placed in a wooded area adjacent to the APHIS laboratory. Female activity was monitored in a similar field cage.

For field studies, male pupae were held by strain (LR or WT) in open wood boxes with 1/4-in hardware cloth bottoms. These were placed in field plots at least 24 h before eclosion was initiated. Eclosion and dispersal were recorded hourly from 0500 h until moth activity stopped at ca. 2000 h.
Since the age of moths could not be readily determined during field observations, flight cages were used to determine activity periodicity of moths of different ages. Recently, eclosed males were placed in cages according to sex and strain. A grid was drawn on the inner surface of each cage side in order to locate and record moth position. Before and after sunrise (0300 and 0600 h) and sunset (1800 and 2100 h), the position of resting males was recorded; activity during each of these periods was recorded on July 28, 29, and 30 (all times are Eastern Standard Time). The proportion of moths dispersing was calculated by comparing moth positions before and after each respective observation period.

Females were placed in the cage as pupae (in 16 oz cups).

Shortly after eclosion they were removed from the cups and marked sequentially, according to time of eclosion, with a number, applied with a blue felt tip marker, to the left wing; this allowed measurement of motor activity for any particular time period. After marking, the moth was placed in the grid drawn on the cage side; the female readily clung to the screen.
RESULTS

1977 Field Observations

Fig. 1 summarizes the first-flight periodicity of LR and WT males during July 1977. First-flight dispersal was recorded on 12 days during this period. Of the 9,285 first-flights, 6,221 (67%) occurred before 1500 h, 714 (8%) occurred between 1500 and 1900 h, and 2,350 (25%) occurred between 1900 and 2000 h (crepuscular flight).

Generally, evening flight began shortly after sunset and continued for ca 45 min; LR moths consistently began activity 3-5 min before WT moths. Table 1 summarizes the evening activity of LR and WT male gypsy moths. As the season progressed, and the diel period shortened, the change in timing of initial activity paralleled the change in timing (earlier) of sunset. We also noted that moth activity consistently began at about the same time as lightening bug flashing and the occurrence of whippoorwill calls.

Male moth crepuscular activity began with crawling and/or wing-fanning, with movement mostly in a sideways or backwards direction rather than straight ahead. This lasted 10 to 40 sec depending, apparently, on the ambient temperature; i.e. at relatively low temperatures moths took longer to initiate flight. Often, if other males were encountered during the pre-flight activity, the active male displayed a typical lepidopteran courtship and attempted
to mate with the other male. This usually continued until the other male moved out of contact, or until flight was initiated.

Wing-fanning and walking culminated in hovering and then dispersal from the box, or, if flight did not occur before 2000 h, the moths ceased activity. Although most moths displayed some crepuscular activity many did not fly. This was particularly evident on cooler evenings; e.g. Table 1, 25% dispersal on July 26, 27, and 28 at $17^\circ \pm 1^\circ C$ vs 71% dispersal on July 18, 19, and 20 at $23^\circ \pm 1^\circ C$.

Once in flight moths flew up into the crowns of nearby trees and settled on the underside of leaves and branches. Although we were not able to quantitate the duration of these first flights, warm evenings (21-24° C), and slight breezes appeared to prolong flight; moths hovered above shrubs and around the eclosion boxes before ascending into the crown.

After 2100 h, using flashlights, we were able to observe moths resting on foliage 5 to 10 m from the ground. Usually these moths had disappeared by 0700 h the next day. On 2 evenings, between 2100 and 2200 h, voles, mice, and chipmunks were observed taking moths resting on boles of trees. On several mornings (0600-0700 h) blue jays, chickadees, and a worm-eating warbler (single sighting) were observed taking and eating moths from the tree foliage. In addition, evening grosbeaks (two sightings) and blue jays took moths from low shrubs; jays flushed resting moths by flying at, and
landing on shrubs. We presumed, at that time, that predator activity was largely responsible for the overnight disappearance of moths. However, the 1978 cage observations indicated that an early morning flight period resulted in a redistribution of moths, and thus, predators were only partially responsible for moth disappearance.

1978 Field Cage Observations - Males

On July 24 and 25 newly eclosed male moths, 25 WT and 48 LR, were released by strain in two adjacent flight cages to determine if cage confinement influenced first-flight crepuscular activity, as compared to 1977 field observations. No significant difference in activity was observed. Males of both strains began wing-fanning and walking at ca. 1925 h; peak activity occurred at ca. 1945 h. Moth to moth interference due to confinement, and the presence of the observer appeared to increase flight activity, thus peak activity occurred ca. 10 min later than in the 1977 field observations. Nevertheless, by 2010 h all moths were resting.

Subsequently, on July 28 at 1500 h, newly eclosed male moths (93 LR and 75 WT, Bridgewater, Ma.) were released by strain in respective flight cages. During the following 53-h observation period the number of live moths declined so that by 2100 h on July 30 only 18 (19%) LR moths and 27 (35%) WT moths were active. Nevertheless, crepuscular activity occurred regularly between 1900 and 2000 h, and between 0330 and 0445 h, respectively (Fig. 2).
Less than 2% of all moths changed position between 0000 and 0330 h. Crepuscular behavior of 1 and 2-day old males were similar to pre-flight evening activity described previously. On each day the morning crepuscular period began at 0330 h and ended by 0445 h. General diel activity began around 0900 h (19°C); initial diel flight was preceded by wing-fanning but not necessarily walking. Diel peak flight (50%) of moths in flight) occurred between 1200 and 1400 h (ca. 25°C). Flight generally declined after 1400 h so that only three (July 29) and two moths (July 30) were flying between 1600 and 1900 h. Evening activity began at 1923 h and ended by 2000 h.

Measurable differences between evening and morning crepuscular activity were, (1) the proportion of moths dispersing between 1923 and 2000 h was 70% (LR) and 80% (WT), while only 48% (LR) and 50% (WT) dispersed between 0330 and 0445 h, and (2) following evening dispersal 44% of all moths were resting on the top of the cage, while only 14% were on the top after morning dispersal. Temperature at the time of activity probably influenced the degree of activity; i.e. evening temperature ranged from 15.5°C to 25°C while morning temperatures ranged from 7°C to 16°C.
Less than 2% of all moths changed position between 2000 and 0330 h. Crepuscular behavior of 1 and 2-day old males were similar to pre-flight evening activity described previously. On each day the morning crepuscular period began at 0330 h and ended by 0445 h. General diel activity began around 0900 h (19° C); initial diel flight was preceded by wing-fanning but not necessarily walking. Diel peak flight (50%) of moths in flight occurred between 1200 and 1400 h (ca. 25° C). Flight generally declined after 1400 h so that only three (July 29) and two moths (July 30) were flying between 1600 and 1900 h. Evening activity began at 1923 h and ended by 2000 h.

Measurable differences between evening and morning crepuscular activity were, (1) the proportion of moths dispersing between 1923 and 2000 h was 70% (LR) and 80% (WT), while only 48% (LR) and 50% (WT) dispersed between 0330 and 0445 h, and (2) following evening dispersal 44% of all moths were resting on the top of the cage, while only 14% were on the top after morning dispersal. Temperature at the time of activity probably influenced the degree of activity; i.e. evening temperature ranged from 15.5°C to 25°C while morning temperatures ranged from 7°C to 16°C.
1978 Field Cage Observations - Females

On July 24, 213 newly eclosed female gypsy moths were placed on the screen grid and their positions recorded. Their movement over the next 60 h is summarized in Fig. 3 and Table 2.

The crepuscular periodicity of the female approximated that of the male. In the evening wing-fluttering (not as rapid as wing fanning) and walking began at ca. 1930 h with peak activity occurring between 1940 and 1950 h. Morning activity began at ca. 0345 h and continued until 0420 h; peak activity occurred shortly after 0400 h. During the first 24 h the moths moved an average of 1.6 m (N=213); crepuscular wing-fanning and walking accounted for 92% of this movement. Single moths moved up to 3.4 m; rapid wing-fanning and walking characterized these sudden bursts of activity. The direction of female movement was usually down and to the right so that after 60 h most moths were clustered in a bottom corner of the cage (Fig. 3). As with males, most of the females displayed some crepuscular activity. When wing-fluttering and/or walking ceased all females folded their wings, tent-like, and the sex gland was everted. This position was maintained until at least 2400 h, when observations ceased. Since males were not flying during this period we were not able to evaluate whether or not pheromone was being released. In the morning (0330 h) the gland was not visible, and was not everted immediately following morning crepuscular activity; the sex gland was next everted at ca. 0900 h at which time males flying in the vicinity were attracted to the cage.
DISCUSSION

The most fascinating aspect of adult crepuscular activity is its similarity with the crepuscular migrations of larvae, particularly since the major resting period has changed from day (larva) to night (adult). Larval migrations, particularly stages IV, V, and VI, are easily observed; by ca 0345 h larvae are moving down the tree from their feeding sites in the crown to resting sites on the bole or in the litter (ODell and Godwin 1979). Robert Neely (1977, personal communication) recorded the evening migration of gypsy moth larvae in Branford, Ct., as beginning at 1915 h, with peak activity at 1930 h. If one is able to observe the activity of instars II and III in the upper crown of trees, similar timing of crepuscular migrations to and from cracks and crevices of branches are evident (ODell and Godwin, unpublished). Allowing for subtle seasonal and geographic differences in the timing of sunset and sunrise, crepuscular redistribution of larvae and adults appears to be remarkably synchronous.

The retention of this energy-intensive behavior, from molt to molt, and through the adult metamorphosis period, would seem to signify its importance in the survival strategy of the gypsy moth.

Predator and parasite avoidance (Campbell 1978), and the maintenance of heat budgets (Leonard 1970) have been suggested as selective factors effecting the maintenance of crepuscular migrations of the larvae, while predation appears to be the major selective pressure effecting retention of crepuscular activity in the adult stage.
The tendency for gypsy moth larvae to aggregate in particular niches during resting periods and subsequently to pupate there, provides a predictable site for predators and parasites to search and, thus, decreases their chance of survival (Campbell 1978). The crepuscular first flight of males, and the first crepuscular movement of females provides a mechanism for escape from these relatively hazardous sites. Since males are most sexually active 24 h after eclosion, and females tend to mate and lay eggs within 24 h of eclosion (Richerson 1976) the first crepuscular movement appears to be of primary importance.

The extent of movement by a female also determines where her eggs will be laid; e.g. the female aggregation formed at the bottom of the cage (Fig. 2) suggests that the clusters of egg masses often found at the base of trees, might result from female crepuscular activity. Thus, parameters affecting egg survival must also be considered as selective factors in the maintenance of female crepuscular activity.
Crepuscular flight of males appears to result in a vertical redistribution of moths within the immediate vicinity of their afternoon resting site; e.g. evening dispersal is predominantly upward toward the crown, and in field cages moths tended to rest at the top of the cage. Conversely, moths were not readily apparent in tree crowns and were not as likely to be resting on the top of the field cage following morning crepuscular flight. We propose that the function of crepuscular activity is to remove the insect from the dominant place and time of activity of different groups of predators; i.e. small vertebrates on or near the ground at night, and birds in the trees early in the morning.

Recently, Miller and Cardé (1978) demonstrated the ability of male gypsy moths to monitor ultrasonic stimuli, and proposed that the moth used this mechanism to escape bat predation during nocturnal flight periods. The maintenance of crepuscular flight, as a predation avoidance behavior, would seem to require just such a back-up system.
Bats have long been recognized as important predators of nocturnal and crepuscular insects (Constantine 1970), but Lymantriids have not been reported among their prey (lists by Poulton 1929, Buckhurst 1930, and others). However, since records of moth predation are usually based on the recovery of wings near bat roosts, or as particles in their stomach, the relatively small, soft-bodied gypsy moth adult would probably go undetected.

Crepuscular activity of bats closely corresponds with the timing of sunset and sunrise; with light intensity (via "light sampling behavior") the most important factor influencing the timing of flight from the usually darkened roost (Erkert 1978, Kunz 1974, DeCoursey and DeCoursey 1964). Gould (1955) reported that the most active feeding period occurred in the first hour after bat emergence, with another relatively active feeding period during morning twilight. Most of Gould's data were from bats collected near Mashpee, Ma., ca 10 mi from the site of our studies. His calculations indicate that the typical hourly catch of the little brown bat, *Myotis lucifugus*, one of the most common bats in his study area, weighed ca 1 g. By our calculations if *M. lucifugus* fed solely on male gypsy moths (each ca 15 mg), a single bat could consume ca 600 moths during the evening redistribution period of *L. dispar*. 
Baker and Cardé (1978) were unable to elicit behavioral reaction to ultrasound by L. dispar females, but their studies concentrated on reactions during the diel, particularly on the withdrawal of the ovipositor. Females may well respond to ultrasonic emissions during their crepuscular walking/fluttering forays.

Although most bats catch moths on the wing, at least one, the European long-eared bat, Plecotus auritus, also picks insects off the foliage of trees and bushes (Gouw 1974). This, plus reports of L. dispar female flight in the Soviet Union (Leonard 1974) and in Pennsylvania (Sandquist 1970), should stimulate further investigation of female reaction to ultrasonics during the crepuscular activity period.

The onset of crepuscular activity of L. dispar adults closely paralleled the time of sunset and sunrise, even with shortening days during July; on a heavily overcast day evening flight occurred somewhat earlier (Table 1). These results are similar to those obtained for bats and implicate light intensity as the chief exogenous factor effecting crepuscular activity. Whether or not such exogenous cues interact with an endogenous rhythm, as proposed for bats (Erkert and Kracht 1978, DeCoursey and DeCoursey 1964) is a question that can only be answered by careful laboratory experiments with controlled lighting.
Table 1. The timing and extent of adult male gypsy moth crepuscular (evening) first flights in 1977

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature (°C)</th>
<th>Sky Condition</th>
<th>Sunset</th>
<th>Species</th>
<th>Time of Activity</th>
<th>Total Eclosion for Day</th>
<th>Number adults remaining at 1900 h</th>
<th>Total dispersed 1900-2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 6</td>
<td>19.4</td>
<td>PCL</td>
<td>1924</td>
<td>NJ16 PA</td>
<td>1935 1943 1955</td>
<td>375</td>
<td>192</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1935 1945 1955</td>
<td>321</td>
<td>93</td>
<td>63</td>
</tr>
<tr>
<td>July 7</td>
<td>21.1</td>
<td>CLR</td>
<td>1924</td>
<td>NJ16 PA</td>
<td>1930 1946 2010</td>
<td>492</td>
<td>291</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1942 1955 2010</td>
<td>764</td>
<td>256</td>
<td>127</td>
</tr>
<tr>
<td>July 8</td>
<td>18.3</td>
<td>CLR</td>
<td>1924</td>
<td>NJ16 PA</td>
<td>1932 1942 2005</td>
<td>286</td>
<td>217</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1940 1945 2005</td>
<td>427</td>
<td>305</td>
<td>123</td>
</tr>
<tr>
<td>July 11</td>
<td>17.2</td>
<td>PCL</td>
<td>1922</td>
<td>NJ16 DI</td>
<td>1930 1940 2000</td>
<td>131</td>
<td>93</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1934 1943 2000</td>
<td>152</td>
<td>68</td>
<td>29</td>
</tr>
<tr>
<td>July 13</td>
<td>20.6</td>
<td>PCL</td>
<td>1921</td>
<td>NJ16 DI</td>
<td>1930 1940 2000</td>
<td>289</td>
<td>97</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1932 1940 2000</td>
<td>233</td>
<td>47</td>
<td>34</td>
</tr>
<tr>
<td>July 14</td>
<td>20.0</td>
<td>PCL</td>
<td>1921</td>
<td>NJ16 DI</td>
<td>1925 1943 2000</td>
<td>310</td>
<td>68</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1928 1945 2005</td>
<td>221</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>July 18</td>
<td>26.7</td>
<td>CLD rain</td>
<td>1918</td>
<td>NJ16 AB</td>
<td>1906 1935 1955</td>
<td>698</td>
<td>286</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1915 1940 1955</td>
<td>309</td>
<td>112</td>
<td>50</td>
</tr>
<tr>
<td>July 19</td>
<td>24.4</td>
<td>CLR</td>
<td>1917</td>
<td>NJ16 AB</td>
<td>1920 1940 2000</td>
<td>563</td>
<td>152</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1929 1945 2000</td>
<td>352</td>
<td>124</td>
<td>103</td>
</tr>
<tr>
<td>July 20</td>
<td>24.4</td>
<td>CLR</td>
<td>1916</td>
<td>NJ16 AB</td>
<td>1924 1930 1950</td>
<td>581</td>
<td>102</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1930 1935 1950</td>
<td>442</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>July 26</td>
<td>17.8</td>
<td>CLR</td>
<td>1911</td>
<td>NJ16 PL</td>
<td>1921 1931 1955</td>
<td>110</td>
<td>61</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1924 1935 1955</td>
<td>127</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>Date</td>
<td>Temperature (°C)</td>
<td>Sky Condition¹/</td>
<td>Sunset²/</td>
<td>Species³/</td>
<td>Time of Activity</td>
<td>Total Ecolson for Day</td>
<td>Number adults remaining at 1900 h</td>
<td>Total dispersed 1900-2000</td>
</tr>
<tr>
<td>----------</td>
<td>------------------</td>
<td>-----------------</td>
<td>----------</td>
<td>------------</td>
<td>------------------</td>
<td>-----------------------</td>
<td>----------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>July 27</td>
<td>16.8</td>
<td>CLR</td>
<td>1910</td>
<td>NJ¹⁶</td>
<td>1923 1931 1955</td>
<td>182</td>
<td>121</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FL¹⁶</td>
<td></td>
<td>1927 1935 1955</td>
<td>71</td>
<td>45</td>
<td>3</td>
</tr>
<tr>
<td>July 28</td>
<td>17.8</td>
<td>CLR</td>
<td>1909</td>
<td>NJ¹⁶</td>
<td>1921 1930 1955</td>
<td>282</td>
<td>115</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FL¹⁶</td>
<td></td>
<td>1921 1935 1958</td>
<td>80</td>
<td>39</td>
<td>24</td>
</tr>
</tbody>
</table>

¹/ CLR=c1ear, CLD=c10udy, PCL=p1artly c10udy
²/ The Old Farmers' Almanac 185th Anniversary Edition
³/ NJ¹⁶=lab reared, PA=collected in Centre Co., Pa., AB=collected in Abington, Ma., FL=collected in Fern Lake, N.Y., DI=collected in Dighton, Ma.
Table 2. The timing and extent of adult female gypsy moth crepuscular activity in 1978

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature (°C)</th>
<th>Sunrise-SR</th>
<th>Sunset-SS</th>
<th>Time of Activity</th>
<th>Distance moved (mm)</th>
<th>( \bar{x} )</th>
<th>( s^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 24</td>
<td>18</td>
<td>1912 SS</td>
<td></td>
<td>1930-2001</td>
<td>507</td>
<td>14008</td>
<td></td>
</tr>
<tr>
<td>July 25</td>
<td>16</td>
<td>0430 SR</td>
<td></td>
<td>0400-0430</td>
<td>447</td>
<td>12982</td>
<td></td>
</tr>
<tr>
<td>July 25</td>
<td>--</td>
<td>--</td>
<td></td>
<td>0630-1530</td>
<td>124</td>
<td>7211</td>
<td></td>
</tr>
<tr>
<td>July 25</td>
<td>17</td>
<td>1910 SS</td>
<td></td>
<td>1930-1958</td>
<td>535</td>
<td>17795</td>
<td></td>
</tr>
<tr>
<td>July 26</td>
<td>12</td>
<td>0431 SR</td>
<td></td>
<td>0400-0430</td>
<td>103</td>
<td>1659</td>
<td></td>
</tr>
<tr>
<td>July 26</td>
<td>18</td>
<td>1909 SS</td>
<td></td>
<td>1930-2002</td>
<td>269</td>
<td>10091</td>
<td></td>
</tr>
</tbody>
</table>

\(4/\) The Old Farmers' Almanac, 186th Anniversary Edition

\(=U.S. \text{GOVERNMENT PRINTING OFFICE: 1979-601-784/151}\)