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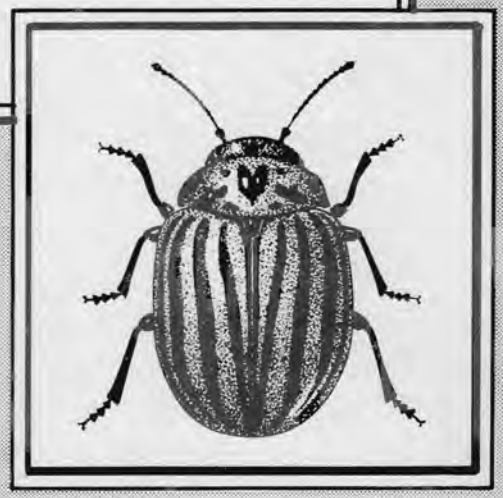

October 1, 1987 - September 30, 1988

Laboratory Report
Otis Methods Development Center
Animal and Plant Health Inspection Service
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Project Number: ABC 86.1.1
Project Title: Artificial Diets for Predatory Coccinellids
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leader: O. T. Forrester

Work is continuing on the development of artificial diets for the predatory coccinellids Coccinella septempunctata (C7), Hippodamia variegata (HV) and Propylea quatuodecempunctata (P14). Major emphasis has been placed on pre- and post-storage feeding. A post-storage artificial diet has been developed for C7 that compares favorably with pea aphid in terms of weight gain and survival after 15 days. Results of these tests are shown in Table 1.

Table 1. Effects of diet on survival, weight gain and fecundity of C7 from storage at Niles.

Diet	N	% female survival 16 days	% weight gain	Mean number eggs/ ovipositing female	Number to oviposit
1	7	85.7	39.0 a	13.0 a	4
6	7	85.7	38.9 a	50.2 a	5
5	7	100	36.7 a	39.3 a	3
7	7	100	35.8 a	68.0 a	4
8	7	85.7	31.1 a	23.0 a	4

Means within the columns followed by the same letter are not significantly different (P> 0.05 Duncan's multiple range test).

Diets 6, 7 and a new formulation 9 were selected for further testing. Results are shown in Table 2. The formulations for diets tested are shown in Table 3.

Table 2. Effect of diet on survival, weight gain and fecundity of C7 from storage at Niles.

Diet	N	% female survival at 15 days	% weight gain	Mean number eggs/ ovipositing female	Number to oviposit
7	15	93.3	53.2 a	29.33 b	6
Pea Aphid	15	73.3	50.3 a	206.6 a	8
9	15	86.6	46.6 a	46.9 b	9
6	15	73.3	41.6 a	18.2 b	5

Means within columns followed by the same letter are not significantly different (P> 0.05 Duncan's multiple range test).

No significant differences were observed in weight gain between the artificial diets and pea aphid. Oviposition of C7 fed pea aphid was significantly higher than the artificial diets, however, this is not the most important criterion in a post-storage diet. The important criteria are survival and evidence of ovarian development. If the stored C7 are for field release to establish, then any eggs produced in the laboratory are wasted.

If the stored C7 are to be used to introduce and establish populations in areas where they do not occur, then any eggs produced in the laboratory prior to release are wasted.

Table 3. Post storage feeding diets used for C7, HV and P14.

Ingredient (gm)	Diet type 1	5	6	7	8	9
Raw pork liver 1/	60	60	60	84	60	72
Sucrose	40	30	40	15	40	27.5
Inositol	-	10	-	10	1.0	5
Invert sugar	-	10	0	10	-	5
Wheast	5	2	5	-	5	2.5
Pollen substitute	5	-	5	-	5	2.5
Glycerol	5ml	-	5	-	5	2.5
Olive oil	5ml	-	5	-	5	2.5
Vanderzant vitamin mix	-	.5	-	-	-	-
Water	5ml	-	5ml	8ml	5	6.5
Diethylstilbesterol	-	-	-	50mg	-	25mg
Methyl testosterone	-	7.5mg	50mg	-	-	25mg
Royal jelly	-	.1	-	-	-	-
Lipid mix 2/		5ml				

1/ Homogenized
2/ Mixture of 30ml cod liver oil, 60ml olive oil, 60ml wheat germ oil, and 2ml tween 80.

Results from this test suggest that diet types 6, 7 and 9 would be good post-storage diets for C7 if diethylstilbesterol and methyl testosterone are acceptable ingredients with respect to worker safety.

The post-storage diets tested with C7 were also tested using HV and P14. Results are shown in Tables 4 and 5.

Table 4. Effect of diet on survival, weight gain and fecundity on HV from storage at Niles.

Diet	N	% female survival 17 days	% weight gain	Mean number eggs/ ovipositing female	Number to oviposit
7	7	85.7	32.1 a	88.2 a	6
8	7	71.4	30.8 a	34.8 b	4
1	7	71.4	28.3 a	28.0 b	3
5	7	57.1	26.5 a	28.0 b	2
6	7	85.7	26.0 a	39.0 ab	4

Means within columns followed by the same letter are not significantly different (P>0.05 Duncan's multiple range test).

While no significant differences were indicated in percent weight gain, beetles fed diet 7 laid significantly more eggs. If the criteria of survival and evidence of ovarian development are used, diet 7 and 6 would be good candidate for post-storage feeding diets for HV.

Table 5. Effect of diet on survival, weight gain and fecundity of P14 from storage at Niles.

Diet	N	% female survival	% weight gain	Mean number of eggs/ ovipositing female	Number to oviposit
8	7	71.4	45.1 a	12.0 a	2
6	7	71.4	35.4 a	9.0 a	1
5	7	100	34.4 a	22.6 a	3
1	7	57.1	33.9 a	0.0	0
7	7	57.1	24.4 a	0.0	0

Means within columns followed by the same letter are not significantly different (P> 0.05 Duncan's multiple range test).

If the criteria of survival and evidence of ovarian development are used, diet 5 is a good candidate for further testing.

Five artificial diets were tested for pre-storage conditioning (Table 8.) Results are given in Tables 6 and 7.

Table 6. Effect of diet on survival and weight gain of teneral adult C7.

Diet	N	Percent female survival 36 days	Percent female weight gain
3	9	55.6	40.9 a
1	9	66.6	33.1 a
Pea aphid	9	77.8	32.9 a
7	9	100	30.1 a
2	9	55.6	21.7 a
6	9	55.6	19.9 a

Table 7. Effect of diet on survival and weight gain of teneral adult C7.

Diet	N	Percent female survival 22 days	Percent female weight gain
1	18	83.3	61.0 a
2	18	94.4	56.4 ab
6	18	94.4	55.0 ab
7	18	83.3	51.3 ab
5	18	94.4	49.1 b
3	18	94.4	49.0 b
Frozen pea aphid	18	88.9	48.56 b
4	18	94.4	47.9 b

Means within columns followed by the same letter are not significantly different ($P > 0.05$ Duncan's multiple range test).

Table 8. Diet ingredients for pre-storage feeding C7.

Ingredient	Diet type 1	2	3	4	5	6	7
Pork liver	70	70	70	70	70	70	70
Sucrose	23	23	23	23	23	23	23
Glycerol	4	4	4	4	4	4	4
Olive oil	4						
Wheat germ oil	-	2.0	2.0	-	-	-	-
Soy bean oil	-	2.0	2.0	-	-	-	-
Fornesol	-	-	.3ml	-	-	.3ml	-
Lipid mix 1/	-	-	-	4.0ml	4ml	-	4.0
Vanderzant vitamin mix	-	1.0	-	1.0	.5	.5	1.0
Fortification mix	-	-	-	-	.5	.5	1.0

1/ Homogenized
2/ Mixture of 30ml cod liver oil, 60ml olive oil, 60ml wheat germ oil, and 2ml tween 80.

Results of this series of tests suggest that artificial diet can be used for pre-storage feeding. Survival and percent weight gain of C7 fed artificial diets are not significantly different from those fed pea aphid and the simplest diet formulation is as good as the complex. Survival after storage was not evaluated.

Project Number: BT 87.1.1
Project Title: Development of Browntail Moth Rearing Techniques
Report Period: October 1, 1987- September 30, 1988
Report Type: Interim
Project Leaders: Alida Pellegrini-Toole, V. C. Mastro, L. Robbins

No change in rearing techniques were made this year. Twenty-five groups of insects were reared. Adult females were used for pheromone extraction; adult males were used in the flight tunnel for bioassay of extractions and candidate synthetic pheromones.

Project Number: ABC 87.1.1
Project Subtitle: Laboratory Study of Aphid Suitability for Coccinella septempunctata Larvae and Adults
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leader: William C. Kauffman, assisted by P. Iorio and C. Paris

Objective: To determine, through life table analyses, the nutritional suitability of four aphids for immature development and reproduction of C. septempunctata (C7).

Rational: Environmental heterogeneity influences both the quantity and quality of aphids available for predation. Spatial and temporal differences in aphid abundance and species diversity are due to a variety of biotic and abiotic factors including:

1) differential susceptibility of plants, both crops and weeds, to aphid infestation,
2) crop phenology relative to aphid invasion,
3) cropping practices including crop diversity, proximity of fields, and amount of land in cultivation,
4) influence of weather on aphid movements and population growth, and
5) role of indigenous and exotic species of beneficial insects.

This generalist predator of aphids is, therefore, often exposed to several species of aphids co-existing within a single crop (i.e., greenbug and oat bird cherry aphid on sorghum or wheat) or two or more species feeding on different crops in adjacent fields (i.e., greenbug on wheat and pea aphid on alfalfa). Thus, consistent feeding by C7 on a single aphid species throughout an entire life stage or through both larval and adult generations is highly unlikely.

A further impetus for "spreading the risk" (also called "bet-hedging") by predation on more than one aphid species is the typically ephemeral nature of aphid outbreaks. The ability of aphids to rapidly invade a crop and expand their population size exponentially (primarily by parthenogenetic reproduction) may result in extensive plant damage, particularly in young plants, leading to crop deterioration and a crash in the aphid population. A critical factor which determines the level of aphid suppression by coccinellids is, therefore, the ability of the predator to invade infested fields sufficiently early to prevent this aphid-induced plant collapse. In the event of an aphid crash due to excessive herbivory or, alternatively, to high predation levels, C7 adults and large larvae will emigrate from the field in search of other local outbreaks of aphids. This ephemeral nature of aphid pressures, aphid diversity in agriculture, and the mobility of C7 within and between fields virtually ensures that C7 individuals will not feed exclusively on one aphid species for extended periods. Consequently, the past history of predation and the present status of prey species consumed may determine the fitness of C7 populations (field or laboratory), and research data elucidating the relative nutritional value of potential aphid prey is thus warranted. Such laboratory life table data, if available, would enable one to suggest the likelihood of suppressing known field populations of aphids by C7 predation based on the population growth parameters of C7 determined for a particular aphid species. This knowledge would also greatly improve our present mass-

rearing potentials for C7.

Materials and Methods: The laboratory colony of C7 was maintained on a mixture of cereal aphids and pea aphid so that experimental results would not reflect a bias in prey acceptance and suitability. In this experiment, four species of aphids (pea aphid, Acyrthosiphum pisum; greenbug, Schizaphis graminum; oat bird cherry aphid, Rhopalosiphum padi; and corn leaf aphid, R. maidis) were offered first to C7 larvae then to next-generation adults to determine relative nutritional quality of these prey for C7 development and reproduction. Aphids were provided in excess throughout the experiment. Replicated treatments (that is, species of aphids) were repeated over time and were conducted under constant laboratory conditions of 27°C (± 1), 20-50% RH, and 16:8 (L:D) photoperiod.

The experimental arena used for both the larval and adult studies consisted of a clear plastic cage, 18 cm high X 11 cm dia, with removable lid of white netting material. This cage was placed over a pot containing one fava bean plant (for pea aphid) or 15-20 barley plants (for the three cereal aphids). A foam closure surrounded the plant stems at soil level. Aphids and predators were thus maintained on live, vigorous plants which, by incorporating plant effects on predator and prey behavior, more closely resembled the natural field situations.

Larval C7 tests were initiated with a single neonate placed into an arena containing aphid-infested plants. Four larvae were randomly selected from an egg mass, and a single larva was placed into each of the four arenas enclosing a prey species. The survival and stadium of each individual were recorded daily until adult emergence; pupal weights were also recorded.

Newly eclosed adults were sexed and weighed. Male and female beetles from each treatment were randomly paired and introduced into new arenas and presented the aphid species upon which the larvae had fed. An oviposition substrate consisting of a single cell-pack (6 cm high x 3-4.5 cm dia, cut from black plastic Pro-Tray) was placed onto the foam bottom of the cage to facilitate collection of C7 eggs. Adult survival and oviposition were recorded daily through day 25. Cell-packs containing eggs were held to determine egg viability and time to ≥50% hatch for each egg clutch.

Results: C7 development from egg hatch to adult eclosion was longer when larvae had fed on GB than when other prey were consumed (Table 1). This difference was primarily due to the extended duration of the fourth stadium of GB-fed larvae. Mean mortality of C7 immatures (excluding the egg stage) ranged from 80.6-88.7% and was not significantly different among species of aphids eaten. Pupal weight, which is generally considered a good indicator of nutritional status of larvae, was highest with PA prey and lowest with GB and OBC aphids.

In the ensuing adult generation, the only cohort with all females fecund was the adults which consumed PA (Table 2). When fecundity was measured both as total oviposition and as daily egg production, C7 adults which consumed PA belonged to the group with the highest fecundity, whereas C7 fed GB was in the lowest fecundity group (Table 2 and Fig.1). A diet of CL or OBC resulted in intermediate levels of eggs oviposited per day. In addition, GB-fed adults experienced a significant delay in first oviposition as opposed to other aphid

diets. Data recorded from these egg clutches indicated that there were no differences in mean egg hatch (i.e., range = 32-42%) nor mean duration of egg stage (range = 3.2-3.4 days) among the four aphid treatments.

The following life table parameters were calculated from the combined data of immature and adult generations so to quantify the effect of aphid diet on the potential population growth of C7: net reproductive rate (R_o), generation time (t), intrinsic rate of increase (r_m), finite capacity for increase ( ), and generation doubling time (DT). These calculations establish that the most nutritionally suitable prey for C7 was PA, followed by CL and OBC, and lastly, GB. Fitness of C7 is clearly dependent upon the species of aphid consumed. These results, therefore, reject the null (H_o) hypothesis which states that C7 diet is inconsequential to the development, survival and fecundity of this introduced aphid predator. Thus, past history and current status of aphid prey quality must be considered in laboratory studies of C7 even when prey supply is unlimited. Based on these data, PA is the preferred aphid for mass-rearing of C7. These results, although not directly applicable to field populations, infer the following: 1) C7 feeding exclusively on a nutritionally inferior aphid, such as GB, will experience lower fecundity and thus diminished aphid reduction potential by its progeny; 2) the significance of species of prey encountered may, therefore, depend on whether aphid suppression can be achieved solely by adult predation or must be augmented with predation by C7 progeny; 3) however, frequent movement by C7 between foci of different species of aphids may serve to dilute or ameliorate any nutritional limitations experienced theretofore by larvae or adults. There is a conspicuous need to model factors influencing the dynamics of C7 and aphid populations (i.e., nutrition, inter- and intra-field movements, relative growth potentials, influences of abiotic conditions, etc.) so to achieve a more complete understanding of this predator-prey-crop relationship.

Table 1. Mean development of Coccinella septempunctata immature stadia when larvae consumed four species of aphids[a].

Aphid prey	No. of neonates	Mean duration ± SE (days)						Mean ± SE (mg)
		Instar I	Instar II	Instar III	Instar IV	Pre-pupa/ pupa	Neonate to adult	Pupal wt.
Pea Aphid	62	2.3 ± 0.06	2.1 ± 0.06	2.1 ± 0.06	3.6 ± 0.13bc	5.5 ± 0.08	15.4 ± 0.10b	40.8 ± 0.79a
Corn Leaf Aphid	52	2.5 ± 0.10	2.0 ± 0.09	2.0 ± 0.08	3.3 ± 0.10c	5.3 ± 0.12	14.9 ± 0.24b	37.8 ± 0.84b
		NS	NS	NS		NS		
Oat Bird-Cherry Aphid	57	2.5 ± 0.08	2.0 ± 0.07	2.1 ± 0.06	3.7 ± 0.14b	5.4 ± 0.10	15.4 ± 0.15b	34.0 ± 0.78c
Greenbug	62	2.4 ± 0.07	2.1 ± 0.07	2.0 ± 0.08	4.1 ± 0.12a	5.4 ± 0.09	16.0 ± 0.17a	32.3 ± 1.03c

[a] ANOVA (SAS GLM, PS ≤ 0.05) and vertical separation of means by Ryan-Einot-Gabriel-Welsch (SAS REGWF) multiple F test; NS indicates no significant difference among aphid diets.

Table 2. Mean reproductive potentials of Coccinella septempunctata adults fed four aphid species.

Aphid prey	No. of adult pairs	% fecund	Mean ± SE[a]		
			Preovipositional period (days)	Eggs/ female	Eggs/ female/ day
Pea Aphid	15	100.0	7.8 ± 0.44b	596.8 ± 116.62a	24.8 ± 1.94a
Corn Leaf Aphid	16	81.2	7.2 ± 0.92b	325.7 ± 88.56ab	13.9 ± 1.38b
Oat Bird-Cherry Aphid	10	60.0	7.8 ± 1.01b	267.5 ± 94.01b	12.4 ± 1.69b
Greenbug	15	60.0	10.9 ± 0.59a	94.9 ± 26.66b	4.1 ± 0.65c

[a] ANOVA (SAS GLM, $P \leq 0.05$) and vertical separation of means (SAS REGWF).

Table 3. Net reproductive rate (R_o), generation time (t), intrinsic rate of increase (r_m), finite capacity for increase ( ) and generation doubling time for Coccinella septempunctata due to diet of aphids[a].

Aphid prey	R_o	t	r_m		DT
Pea Aphid	114.3	35.3	0.134	1.144	5.17
Corn Leaf Aphid	58.8	33.2	0.123	1.130	5.64
Oat Bird-Cherry Aphid	39.4	33.3	0.110	1.117	6.30
Greenbug	13.7	36.9	0.071	1.074	9.76

[a] R_o, females/female/generation; t and DT days.

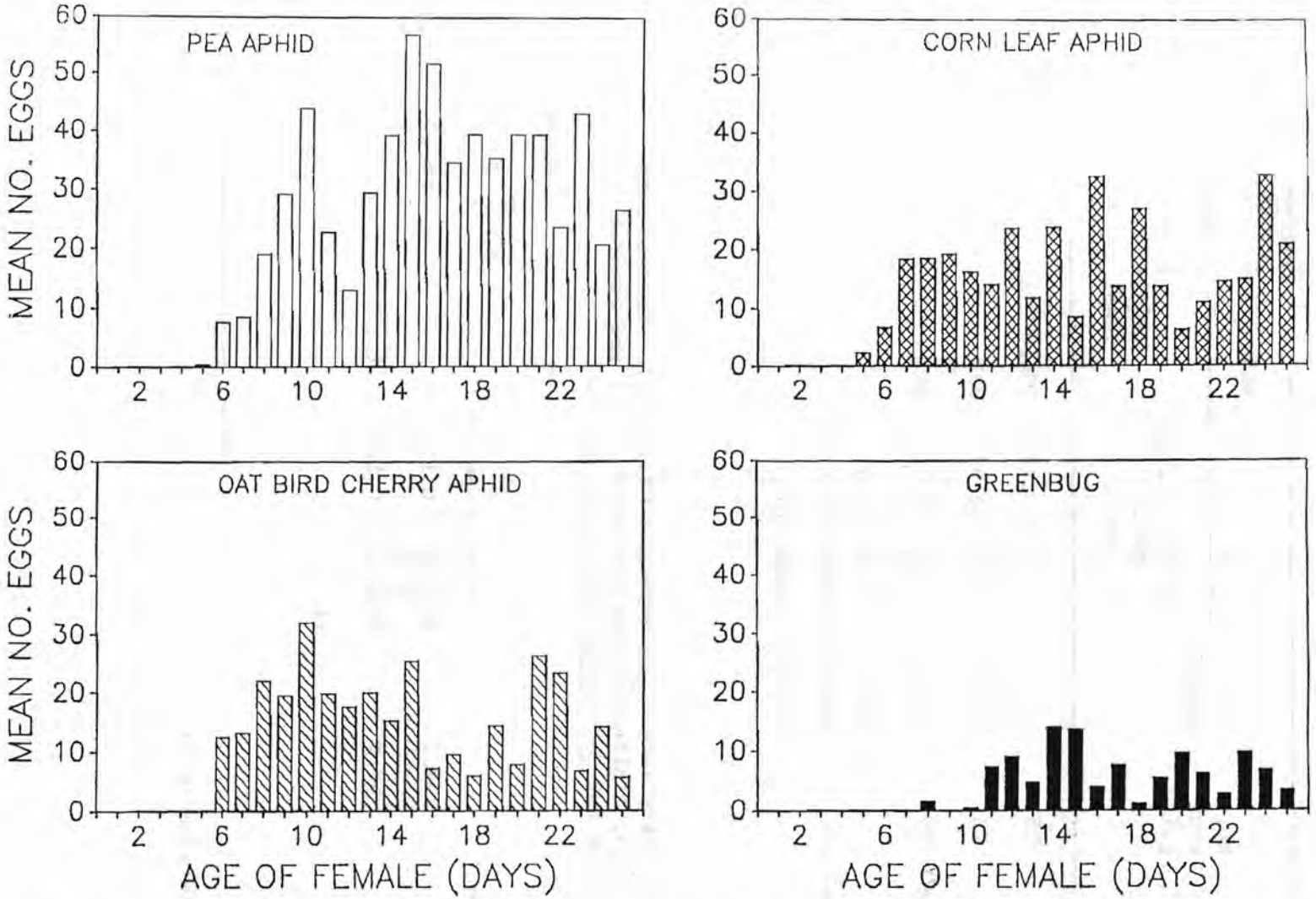


Figure 1. Ovipositional schedule for Coccinella septempunctata adults as a factor of aphid prey.

Project Number: ABC 87.1.2
Project Subtitle: Field Determination of Predation Levels for Coccinella septempunctata and a Native Coccinellid in Selected Crops; Cooperative State Agreements
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: William C. Kauffman (Project Coordinator), in conjunction with Frank Gilstrap and Jerry Michels (Texas A&M University), Timothy Kring (University of Arkansas) and John Obrycki (Iowa State University)

This report is a summary of the annual meeting of the Aphid Biological Control Evaluation Team, which was held in Kansas City, Missouri on November 2 & 3,1988. The underlying purpose of this team is cooperative evaluation of predator effectiveness in aphid pest management.

Objectives: 1) To set standard predation levels and estimate propensity of Coccinella septempunctata (C7) to reduce aphid populations in cages in selected crops for each state. 2) Following standardization of experimental procedure with C7, a dominant native coccinellid was incorporated into this field study to compare its predation efficiency with that of C7. Therefore, the FY 1988 objective was to estimate the propensity of C7 and Hippodamia convergens (Hc), a native species, to reduce aphid densities in cages of alfalfa, sorghum and corn in these three states.

Rationale: Stable predator:prey relationships are generally characterized by oscillating equilibria of two populations (see p. 43 In P. Price, Insect Ecology, 1984). The goal of this Aphid Biocontrol Cooperative Project is to establish standard predation levels for C7 and to compare these with predation by a dominant native coccinellid. These levels are defined as the minimum predator:prey ratios required to initiate a prey collapse typical at the peak of a characteristic predator:prey equilibrium.

Important to evaluating C7 efficacy against aphids is the systematic measurement of predator:prey ratios and predation rates in these co-existing populations under controlled field conditions. Furthermore, this cooperative endeavor was designed to determine whether the introduction of C7 complements, antagonizes, or is of no consequence to existing predation levels by indigenous coccinellids, thus providing positive, negative or neutral influences in aphid management.

Materials and Methods: Predation by released adult C7 in the 1987 studies was far more variable than expected. Thus, the 1988 studies were modified to release eggs or larvae of C7 and Hc in order to minimize variability. Also, corn was dropped from the study in IA due to difficulty of monitoring aphids other than by destructive sampling.

Shipments of coccinellid eggs to cooperators originated from the Niles Biological Control Laboratory at Niles, Michigan. The colony of Hc reared at Niles originated in the state which could provide the earliest seasonal source of founder individuals.

Cages for these studies were purchased for each state in FY 1987. Each site conducted 2-3 replications of an 18 cage set of experiments throughout the

growing season. The following describes the construction of the walk-in field cages: 6' x 6' x 6' cage with one zippered door in one 4' end: screening material was Lumite mesh (52 cells per inch or 21 cells per cm - the smallest mesh available) made by Chicopee of Gainesville, Georgia; cage frames consisted of light gauge aluminum tubing. Cage effects were partially accounted for by use of controls in the experimental design. Cages were erected over planted plots prior to emergence of sorghum seedlings, or within a few hours after cutting for alfalfa, in order to prevent natural infestations of aphids or native coccinellids. Aphids were introduced from laboratory colonies onto plants in each cage at the 5-leaf stage for sorghum and on alfalfa 4-inches in height.

Newly-eclosed coccinellid larvae (IA, AR) or eggs and larvae (TX) were introduced (methodology described below) when aphid densities reached 100/plant on sorghum and 150-300/cage in alfalfa (based on 8 pan samples). Laboratory-produced immatures of C7 and Hc from the Niles Biocontrol Laboratory were used to initiate all cage predation studies as described in this document.

The Hypothesis for Testing: Rates of aphid predation are the same for C7 and Hc when caged on the respective crops and aphid species. This hypothesis was tested by way of factorial experiments designed to show the effects of aphid reduction caused by three density levels of C7 and three parallel densities of Hc (see Table 1 showing introduction densities for C7 and Hc). The treatment levels of coccinellids were assigned randomly to cages. The 3x3 (3 levels of C7 by 3 levels of Hc) factorial experiment was repeated during the growing season.

Table 1. Infestation rates of C7 and Hc neonates (IA, AR) or eggs and neonates (TX).

		No. Coccinellid Immatures Introduced					
	C7:Hc	Sorghum[1]			Alfalfa[2]		
Cage	designation	C7	Hc	Total	C7	Hc	Total
Cage #1	(O:O)	0	0	0	0	0	0
Cage #2	(O:L)	0	10	10	0	80	80
Cage #3	(O:H)	0	20	20	0	160	160
Cage #4	(L:O)	10	0	10	80	0	80
Cage #5	(L:L)	10	10	20	80	80	160
Cage #6	(L:H)	10	20	30	80	160	240
Cage #7	(H:O)	20	0	20	160	0	160
Cage #8	(H:L)	20	10	30	160	80	240
Cage #9	(H:H)	20	20	40	160	160	320

1 Number of coccinellids/plant.
2 Number of coccinellids/cage.

Densities of aphids, C7 and Hc were monitored in each cage on a bi-weekly basis until aphids were no longer detectable or until plants were killed. Sampling methodology for sorghum and alfalfa is described below (Table 2). The factorial design permits analysis (AOV) of the effects of aphid density on coccinellid impact. For example, the aphid reduction from hatched eggs of 1) 20 C7 with no Hc, or 2) no C7 with 20 Hc, should not be significantly different from results of studies with 10 C7 and 10 Hc if no competition exists between the species. Additivity of predation was compared to expected values (X^2 tests) within and among coccinellid treatments (thus in the above example, the aphid reduction in the cage with 10 C7 and that in the cage with 10 Hc was used as expected aphid reduction value for the cage with 10 of each coccinellid). Significantly different levels of aphid reduction would, therefore, be attributed to some level of interspecific competition or interference between these exotic and native coccinellid predators.

Table 2. Sampling methodology of aphids on grain sorghum and alfalfa.

Crop / Aphid Species / Sampling Techniques	
Arkansas	
Crop:	Grain sorghum
Aphid:	Greenbug (*Schizaphis graminum* (Rondani))
Sampling Technique:	Visual census counts of 10 plants per cage
Sampling Frequency:	Semi-weekly
Iowa	
Crop:	Alfalfa (cv. Vernal) Aphid susceptible
Aphid:	Pea aphid (*Acyrthosiphon pisum*) Harris
Sampling Technique:	Aphids and coccinellids - gently beat from plants into white enamel pans (25 x 41 x 6cm)
Sampling Frequency:	2-3 times/week
Texas	
Crop:	Grain sorghum
Aphid:	Greenbug (*Schizaphis graminum* (Rondani))
Sampling Technique:	Visual census counts of 10 plants/cage
Sampling Frequency:	Semi-weekly

Results and Discussion

Iowa (Obrycki): Aphid densities in the control cages increased logarithmically to a mean number of over 6000 pea aphids/cage (based on 8 pan samples from each of 2 cages) (Fig. 1). Aphid samples from July 15 through July 25 in the eight cages with predators did not show an impact of coccinellid predation on aphid densities under these field conditions (Fig. 1A). Compared to the eight treatments in which coccinellids were released, the pea aphid densities in the control cages were higher from the July 27

sample until August 5 (Table 3, Fig. 1A) In the two treatments in which only Hc were released, no differences in pea aphid numbers were observed between cages in which either 80 or 160 first instar Hc were released (Fig. 2). Compared to the control cages, aphid densities on any sampling date were reduced from 38 to 84% in these cages with Hc from July 27 to August 5 (Table 2). No differences in pea aphid numbers were observed between cages in which either 80 or 160 first instar C7 were released (Fig. 3). Compared to the control cages, aphid densities were reduced from 60 to 86% in these cages for samples from July 27 to August 5 (Table 2).

Slight differences in the numbers of pea aphids were observed in cages receiving only 160 Hc, or 160 C7 compared to 80/80 Hc/C7 (Fig. 4 and Table 3). The lowest pea aphid numbers were observed in cages receiving 80/80 Hc/C7. No clear patterns were observed in aphid densities in cages in which either 160 Hc or 160 C7 or 160/160 Hc/C7 were released (Fig. 5 and Table 3). When two opposite ratios of Hc/C7 (2:1 or 1:2) were released in field cages, no differences were observed in the densities of pea aphids over all sampling dates (Fig. 6 and Table 3).

The percentage of individual Hc surviving to adults when only Hc were released in cages ranged from 8% (160 released) to 25% (80 released). In cages in which only C7 were released, the survival ranged from 6-24% when 160 first instars were released and from 13-16% in cages receiving 80 first instars. No consistent pattern in survival to adults was observed when both C7 and Hc were released in the same cage (Table 4). In cages receiving 80 C7 and 80 Hc, an average of 14% of each species survived to adults. When 160 of each species were released, an average of 8-10% of the individuals emerged as adults. In cages where either 160/80 or 80/160 individuals of each species were released, large variations in numbers of adults collected in each cage replication was observed.

The use of the pea aphid/alfalfa system is suitable to document the ability of C7 to reduce pea aphid densities (Table 3, Fig. 3). No negative impact of C7 on Hc survival could be detected in these field cages. In these trials based upon (1) aphid densities in the cages, (2) the % reduction in aphid densities compared to control cages and (3) the % of emerging adults compared to the number of first instars released in the field cages. However, due to variability in data collected using this pan sampling technique this system may not be most conducive to evaluating the interaction between Hc and C7.

Supportive Laboratory Studies (Obrycki):

1) Laboratory studies with *Dinocampus* (=*Perilitus*) *coccinellae*, a Braconid parasitoid, developed at similar rates in C7 and three native species (*Coleomegilla maculata*, *H. convergens* and *Cyclomeda munda*). The exotics Hv and P14 were not suitable hosts. The largest parasitoid females emerged from C7, the largest host.

2) Dietary characteristics of C7, Hv and P14 were investigated using pea aphid, corn leaf aphid and European corn borer (ECB) eggs as prey. ECB eggs were not recognized by larvae as suitable prey. C7 developmental time was shorter and adults were larger when preying on pea aphid as opposed to corn leaf aphid. These results concur with Bill Kauffman's life table analyses of C7 nutrition on pea aphid and cereal aphids.

Similar, but smaller differences were observed for Hv and P14.

Table 3. The percent reduction (compared to control cages) in pea aphid numbers per cage in which various numbers of C7 and Hc were released.

	No. first instars C7/Hc	% Reduction on each sample date 7/27	7/29	8/1	8/4	8/5
Cage #1	0/0					
Cage #2	0/80	71%	38%	73%	77%	82%
Cage #3	0/160	68%	66%	81%	83%	84%
Cage #4	80/0	73%	78%	78%	86%	72%
Cage #5	80/80	81%	80%	94%	93%	91%
Cage #6	80/160	58%	42%	60%	58%	65%
Cage #7	160/0	63%	60%	78%	66%	82%
Cage #8	160/80	61%	54%	61%	61%	64%
Cage #9	160/160	74%	72%	89%	85%	86%

Table 4. The numbers of adult C7 and Hc which emerged in each cage compared to the number of first instars released in each alfalfa field cage.

	No. first instars C7/Hc	Total number adults from 2 reps. (cages) Cage 1 C7,Hc	Cage 2 C7,Hc	Mean % survival to adults C7	Hc
Cage #1	0/0				
Cage #2	0/80	0,11	0,20		19.5 ± 8
Cage #3	0/160	0,13	0,17		9.5 ± 2
Cage #4	80/0	13,0	10,0	14.5 ± 2	
Cage #5	80/80	10,10	12,12	14.0 ± 1	14.0 ± 1
Cage #6	80/160	9,20	17,11	16.0 ± 7	10.0 ± 4
Cage #7	160/0	10,0	38,0	15.0 ± 13	
Cage #8	160/80	5,10	46,28	16.0 ± 18	24.0 ± 16
Cage #9	160/160	19,25	6,5	8.0 ± 5	9.5 ± 9

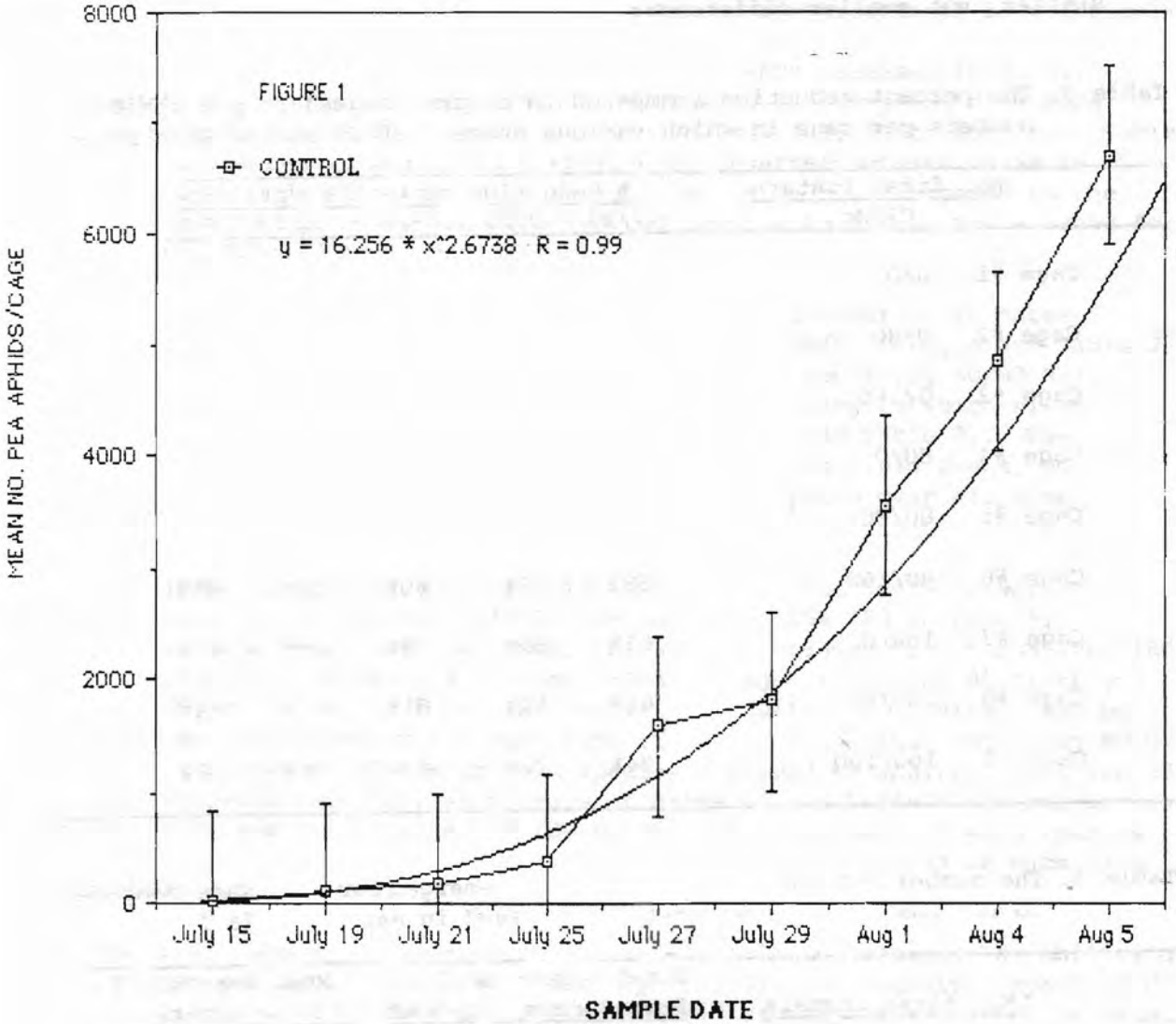


FIGURE 1
CONTROL
y = 16.256 * x^2.6738 R = 0.99
MEAN NO. PEA APHIDS/CAGE
8000
6000
4000
2000
0
July 15
July 19
July 21
July 25
July 27
July 29
Aug 1
Aug 4
Aug 5
SAMPLE DATE

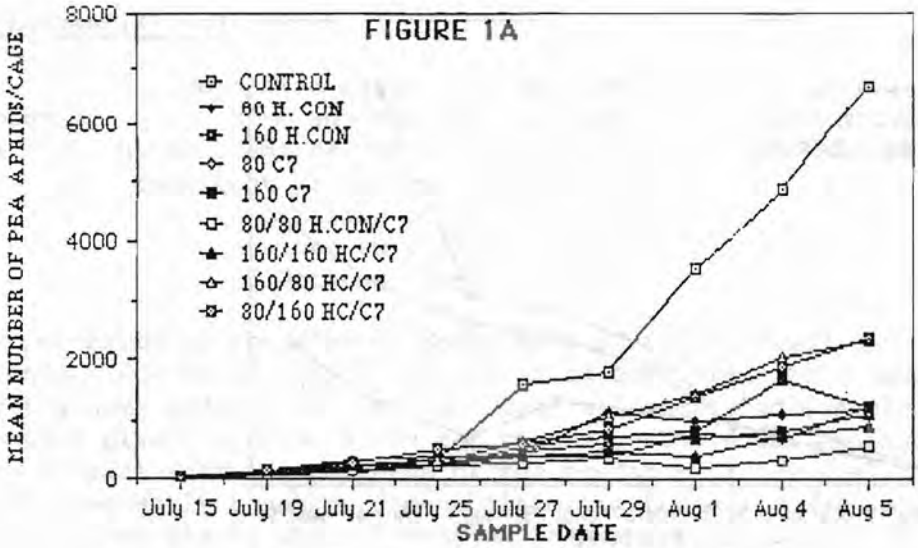


FIGURE 1A
CONTROL
80 H. CON
160 H.CON
80 C7
160 C7
80/80 H.CON/C7
160/160 HC/C7
160/80 HC/C7
80/160 HC/C7
MEAN NUMBER OF PEA APHIDS/CAGE
8000
6000
4000
2000
0
July 15
July 19
July 21
July 25
July 27
July 29
Aug 1
Aug 4
Aug 5
SAMPLE DATE

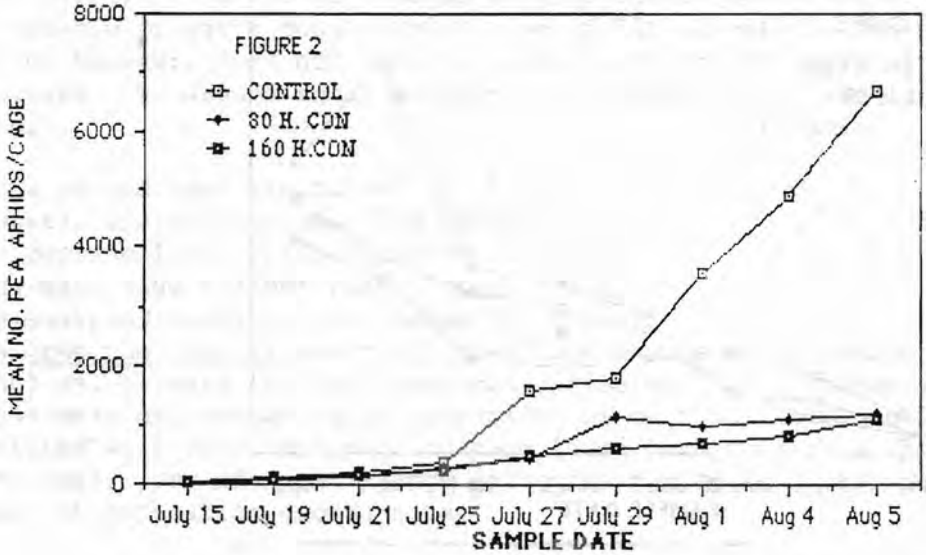


FIGURE 2
CONTROL
80 H. CON
160 H.CON
MEAN NO. PEA APHIDS/CAGE
8000
6000
4000
2000
0
July 15
July 19
July 21
July 25
July 27
July 29
Aug 1
Aug 4
Aug 5
SAMPLE DATE

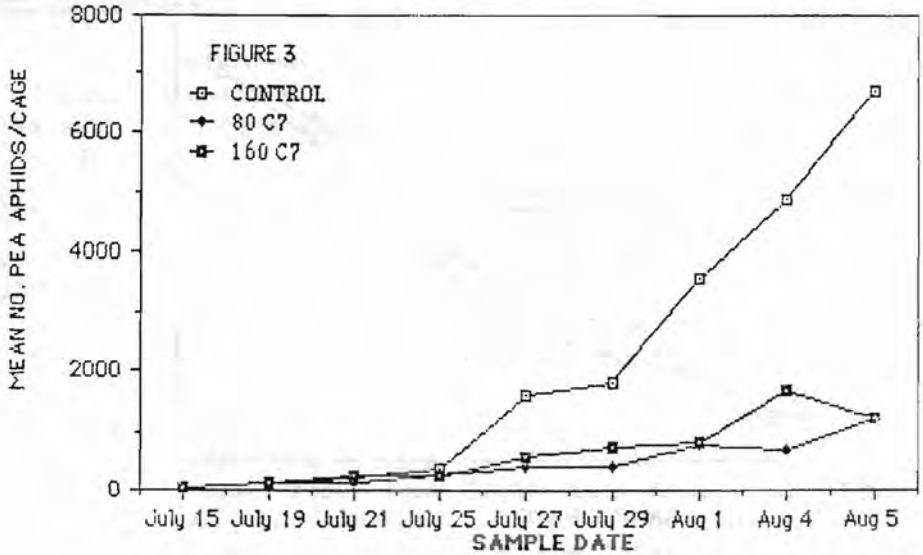


FIGURE 3
CONTROL
80 C7
160 C7
MEAN NO. PEA APHIDS/CAGE
8000
6000
4000
2000
0
July 15
July 19
July 21
July 25
July 27
July 29
Aug 1
Aug 4
Aug 5
SAMPLE DATE

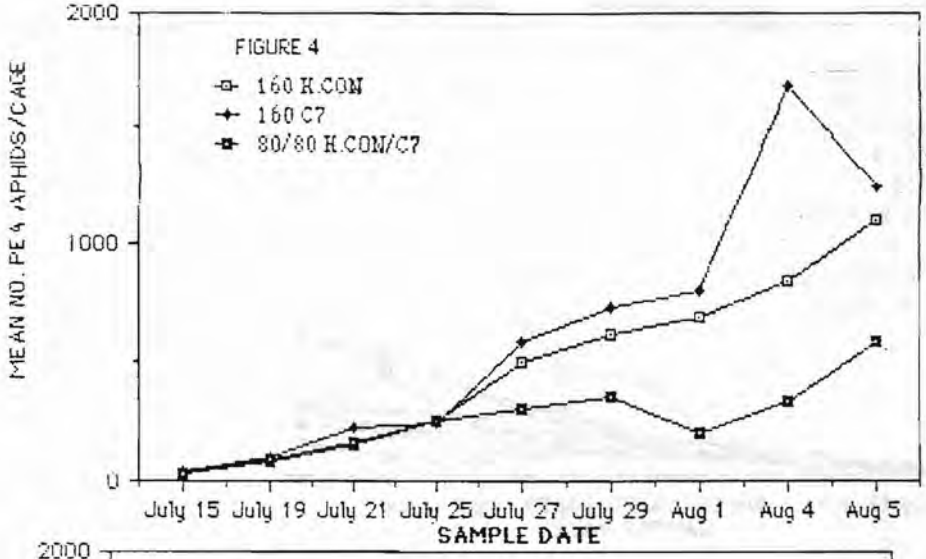


FIGURE 4
160 H.CON
160 C7
80/80 H.CON/C7
MEAN NO. PEA APHIDS/CAGE
2000
1000
0
July 15
July 19
July 21
July 25
July 27
July 29
Aug 1
Aug 4
Aug 5
SAMPLE DATE

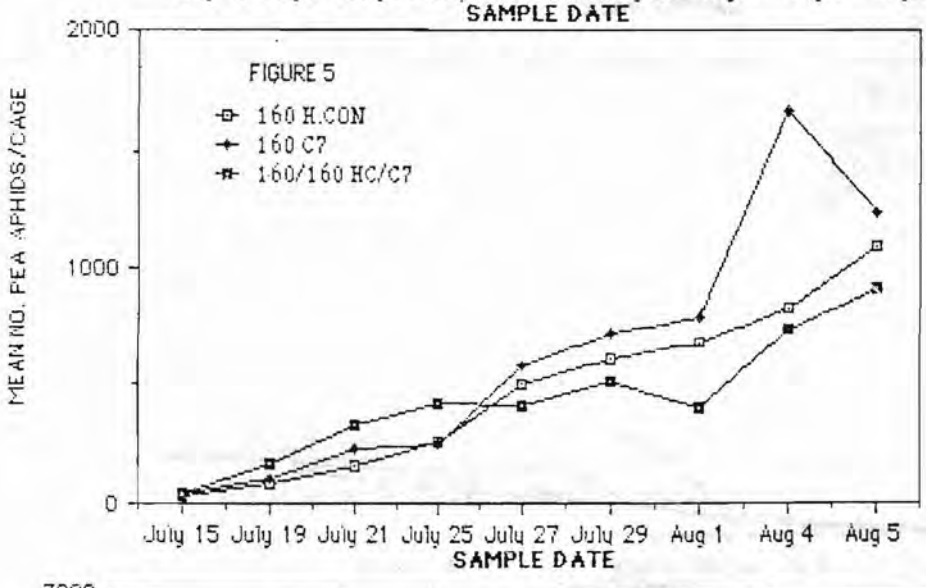


FIGURE 5
160 H.CON
160 C7
160/160 HC/C7
MEAN NO. PEA APHIDS/CAGE
2000
1000
0
July 15
July 19
July 21
July 25
July 27
July 29
Aug 1
Aug 4
Aug 5
SAMPLE DATE

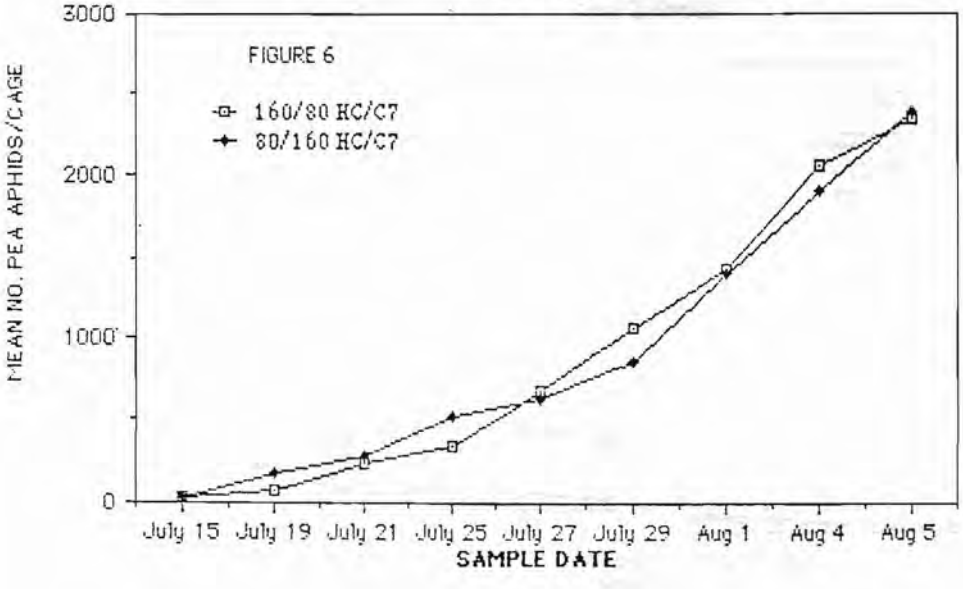


FIGURE 6
160/80 HC/C7
80/160 HC/C7
MEAN NO. PEA APHIDS/CAGE
3000
2000
1000
0
July 15
July 19
July 21
July 25
July 27
July 29
Aug 1
Aug 4
Aug 5
SAMPLE DATE

Arkansas (Kring)

The use of coccinellid larvae and an experimental technique to compare the potentials of native and the exotic predators to reduce aphid densities solved many of the problems of 1987. Three separate tests were initiated in Arkansas, each test having two replications. The first test failed due to greater than 90% mortality of Hc eggs after receipt (98% of C7 eggs in the same shipping container survived). The second and third tests were initiated on 16 August and 6 October, respectively.

As described in the Plan of Work, each of the 18 cages contained 10 sorghum plants. Counts of aphids and larvae on each plant were made on alternate days by the same individual until the test was completed (excessive damage to control plants causing aphid reductions). There was an obvious variation in the diurnal activity patterns of the predator larvae (relatively inactive and most frequently found on ground litter in the early morning, versus active foraging on plants and activity on the cage walls during the heat of day). This variation and the time required to count a cage (30-45 minutes) resulted in variable predator counts which were of little value. The experimental design, however, does not rely on predator counts, but on estimates of aphid densities. We assume equal survival of coccinellid larvae in the treatment cages.

In the second test (initiated on 16 August) and third test (initiated on 6 October), aphid densities were obviously depressed in treatment cages (i.e. with coccinellids) (Fig. 7A and 8A). For each test, regressions were run using each cage (rather than a plant) as the experimental unit. Further, data were analyzed using two estimates for the effect on greenbug density. Both mean greenbug density and mean net change in greenbug density (from previous count) values were used as dependent variables in the factorial analysis. Use of the mean net change value appears to be most suitable, as initial greenbug densities will vary from cage to cage (even though we shoot for equal densities). Use of a net change will give a more accurate indication of the impact on population growth rate.

Mean Greenbug Density

Factorial analysis of greenbug density as affected by coccinellid treatments (Model: Greenbug density = C7 + Hc + C7*Hc) provided quite variable results. The analysis was not significant for Test 2 ($F=1.81$, $P=0.10$, $R^2=0.24$), yet was significant for Test 3 ($F=12.33$, $P=0.0001$, $R^2=0.37$). In test 3, both *H. convergens* ($F=41.64$, $P=0.0001$) and *C. septempunctata* ($F=3.80$, $P=0.024$) provided significant contributions to the observed variation in greenbug density. The interaction (C7*Hc) term was not significant ($P=0.105$). Given the levels of significance, the contribution of *H. convergens* seems more critical. This is supported by the plot of greenbug density (Fig. 8A), where levels of C7-only (L:0 and H:0) provided the lowest levels of suppression when a single coccinellid species was introduced.

Mean Net Change in Greenbug Density

Analysis (Model: Mean net change = C7 + Hc + C7*Hc) again provided variable results, yet gave better explanations of the data. Analyses were significant ($F=2.93$, $P=0.015$, $R^2=0.43$ and $F=9.32$, $P=0.0001$, $R^2=0.33$) for both Tests 2 and

3, respectively. In Test 2, C. septempunctata significantly contributed to changes in greenbug density (F=9.80, P=-0.0005), but H. convergens did not (F=0.35, P=0.7095), and the interaction term (C7*Hc) was not significant. This lack of significance due to H. convergens is supported by the plot of greenbug density (Fig. 7A), where levels of Hc-only (0:L and 0:H) gave the lowest levels of suppression. Analysis of net change allowed discussion of data from Test 2, whereas analysis using mean greenbug densities did not because the analysis was not statistically significant.

In Test 3, both coccinellids (C7 barely) provided significant contributions to greenbug density fluctuations (Hc: F=31.98, P=0.0001; C7: F=3.05, P=0.05), and the interaction term was not significant. Again, given the levels of significance, the contribution of H. convergens appears more important than that of C. septempunctata, contrary to that observed in Test 2. This was visually supported by Fig. 8A, where levels of C7-only provided the lowest levels of suppression. This analysis was in agreement with that using actual greenbug density estimates.

Estimates of Coccinellid Density

It was felt that visual estimates of larval coccinellid densities were difficult to obtain and did not accurately portray actual coccinellid densities in the cages. Accordingly, it is difficult to explain how fewer than 2 larvae/cage (Test 2, Fig. 7B) or fewer than 3 larvae/cage (Test 3, Fig. 8B) had any impact on greenbug densities beyond 4 days after they were introduced. However, it is interesting to note that for both tests, cages with relatively high early coccinellid densities (Test 2-L:H, H:0, H:L; Test 3-L:H, H:L, H:H, L:L) all maintained greenbug densities at lower levels. Furthermore, in cages where suppression was obvious, some larvae were always found, indicating that though density estimates may be unreliable, presence/absence of larvae can be detected. These observations also indicate the importance of larval predation, since even low numbers of coccinellid larvae provided suppression.

In summary, these data do not indicate that an interaction between C. septempunctata and H. convergens exists. However, more data, likely from next season's studies, are needed to conclude that there is no interaction between the species as larvae. Both coccinellid species significantly reduced greenbug population growth rates, but there appears to be little difference between the impact of the two species. If the tests in FY 1989 produce results which support the conclusions from 1988, I would be satisfied with the conclusion that C. septempunctata and H. convergens have equal impact on greenbug populations without deleterious interaction, allowing an assumption of additivity in aphid reductive potential.

Supportive Laboratory Studies (Kring):

1) Coccinellid Preferences for Parasitized Aphids:

C7 consumed more aphids parasitized by Lysiphlebus testaceipes 5-7 days previously than unparasitized aphids. This preference by C7 for mummified aphids was primarily due to the reduced drop-off by these non-vagile prey, thus, a higher rate of encounter with adult C7.

2) Incidental Mortality of Foraging Coccinellids:

This study of C7, Hv and P14 adults addresses two key questions: a) How many aphids are dislodged by a predator?, and b) What is the fate of a dislodged aphid? Approximately 2-4 times as many aphids were dislodged as were consumed by C7 adults, with a lower ratio for Hv and P14. The number of aphids dying soon after being dislodged increased dramatically as soil temperatures increased. One may need to consider prey dislodgment as well as level of predation for a true determination of predator-induced aphid mortality.

Texas (Jeff Edwards, Frank Gilstrap and Jerry Michels)

Field cage studies with greenbug on sorghum were designed like those conducted in Arkansas. Generally, during this 35-39 day study, the number of coccinellid larvae sampled declined steadily until day 9 (presumably due to cannibalism, starvation or difficulty of sampling early instars). Pupation occurred between days 9-17 and emergence of adults commenced ca day 18. An abundance of aphids was a prerequisite for oviposition and development of second-generation larvae. Early, low densities of larvae in many predator treatments permitted greenbug densities to increase at similar rates as in the control cages. Acceptable levels of aphid suppression, as indicated by the number of live plants at experiment end, were realized only when significant predation by first generation larvae occurred within the first several days (Fig. 9). This early predation, although not always lowering peak aphid densities, delayed the date of peak aphid densities and, therefore, enhanced plant survival (Fig. 10). Since aphid densities increased rapidly during coccinellid pupation, a critical factor in plant protection from aphid herbivory was the aphid density attained during this quiescent period. High levels of aphids at onset of coccinellid pupation accompanied by highly synchronous pupation enabled greenbug to outrun C7 and Hc predator populations in the cages, usually resulting in plant death and aphid collapse. Introducing larger numbers of larvae did not consistently increase total predator units nor ensure adequate control of greenbug. It is likely that cannibalism, inter-specific predation of coccinellid larvae, and interference among predators may account for this lack of increased aphid suppression when more individuals were introduced.

Cooperative Research Between USDA-ARS and USDA-APHIS (Norm Elliott and Bob Kieckhefer, ARS, Brookings, SD):

Evaluation of the influence of introduced coccinellids on native aphid predators:

Background Research:

Native predators were sampled in fields of small grain, corn and alfalfa at several sites in eastern South Dakota each year for 19 years (1968-1986). From 1968-1972 sites at which sampling was done varied from year to year, and during 1986 only a single site was sampled. However, from 1973-1985 the same three sites were sampled throughout the growing season each year. Results reported here in and in unpublished manuscripts are based on sampling from 1973-1985. At each of the three sites, a small grain field and an alfalfa field were sampled throughout the growing season of each year; coccinellids, chrysopids, and nabids were sampled at ca. weekly intervals using a standard

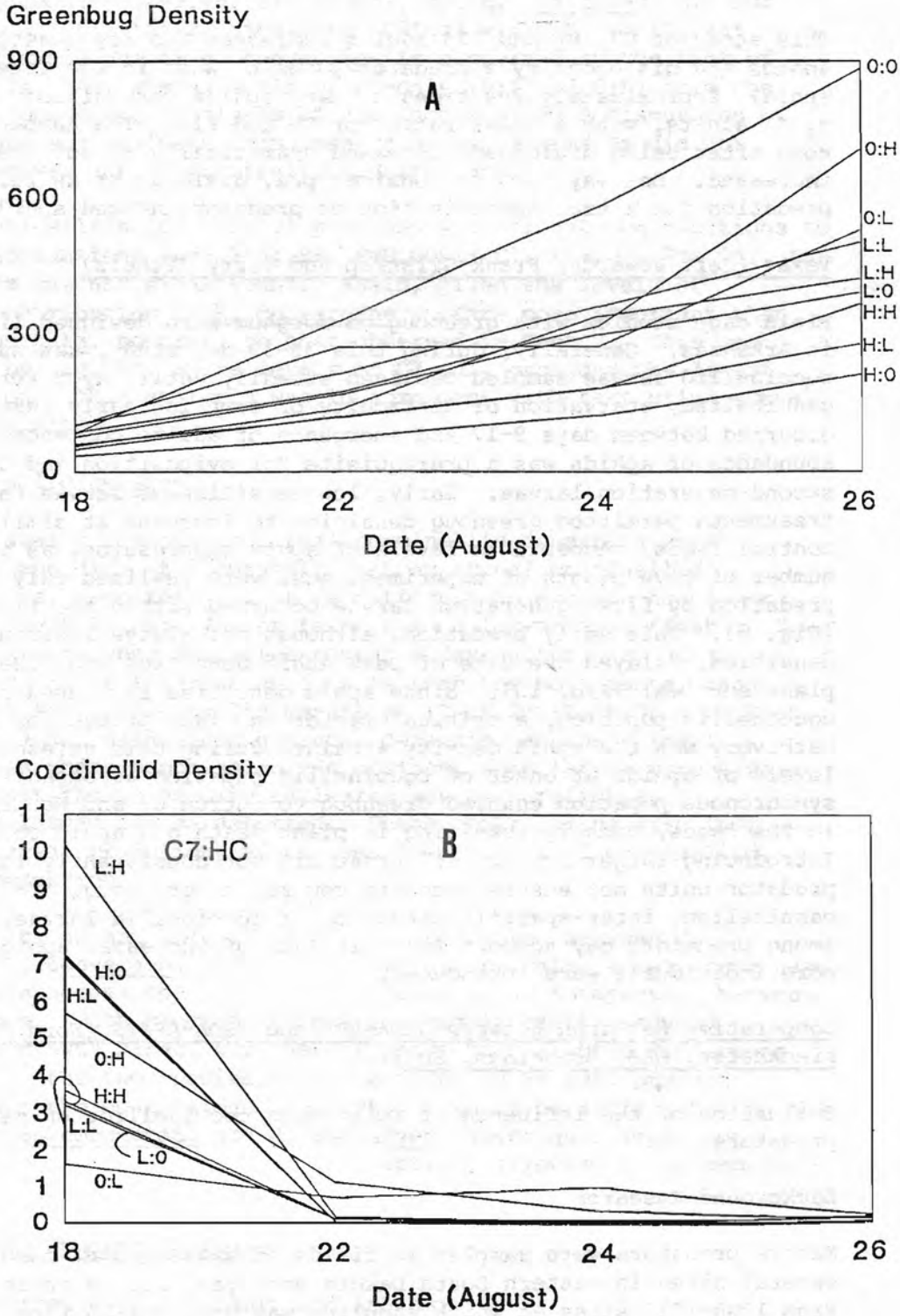
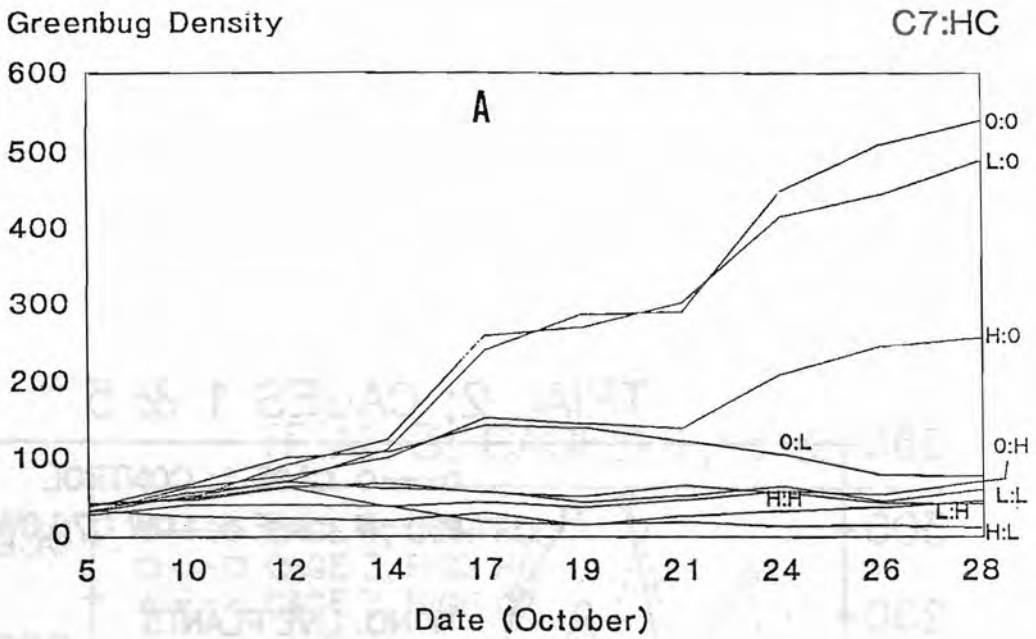


Figure 7. Greenbug and coccinellid densities on sorghum at various combinations of C7 and Hc; Arkansas, Trial 2.



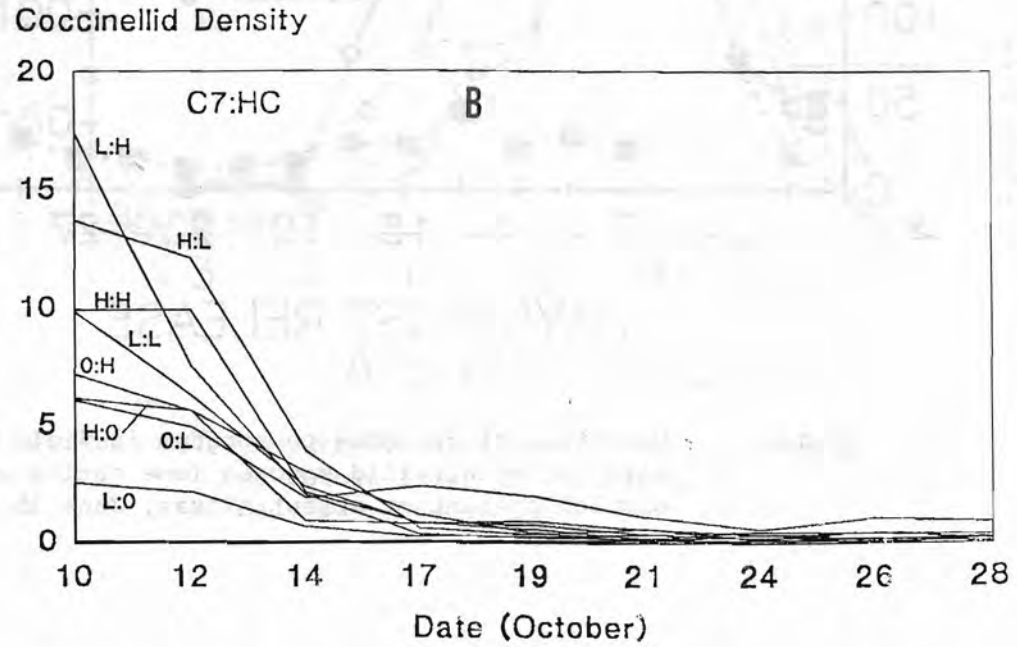


Figure 8. Greenbug and coccinellid densities on sorghum at various combinations of C7 and Hc, Arkansas, Trial 3.

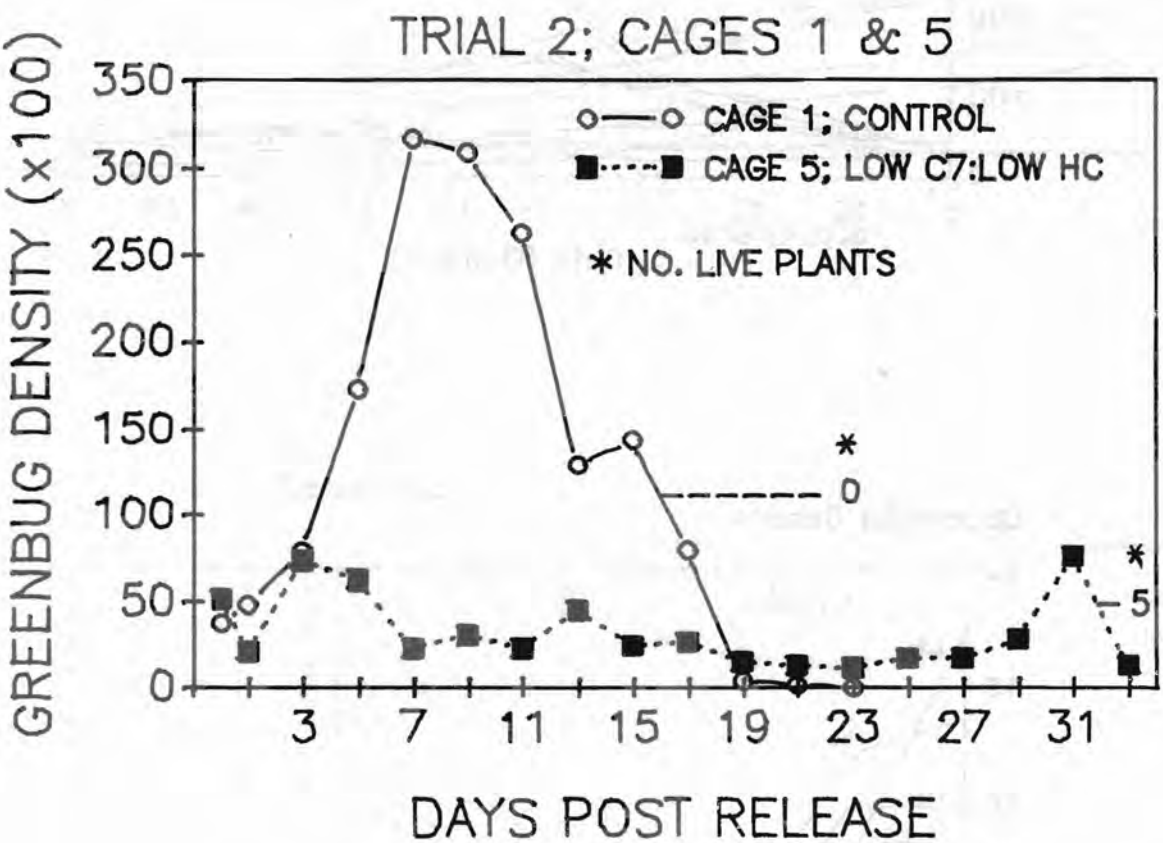


Figure 9. Densities of greenbug on sorghum in field cages with two coccinellid species (one native and one exotic) present or absent; Texas, June 25 - July 28.

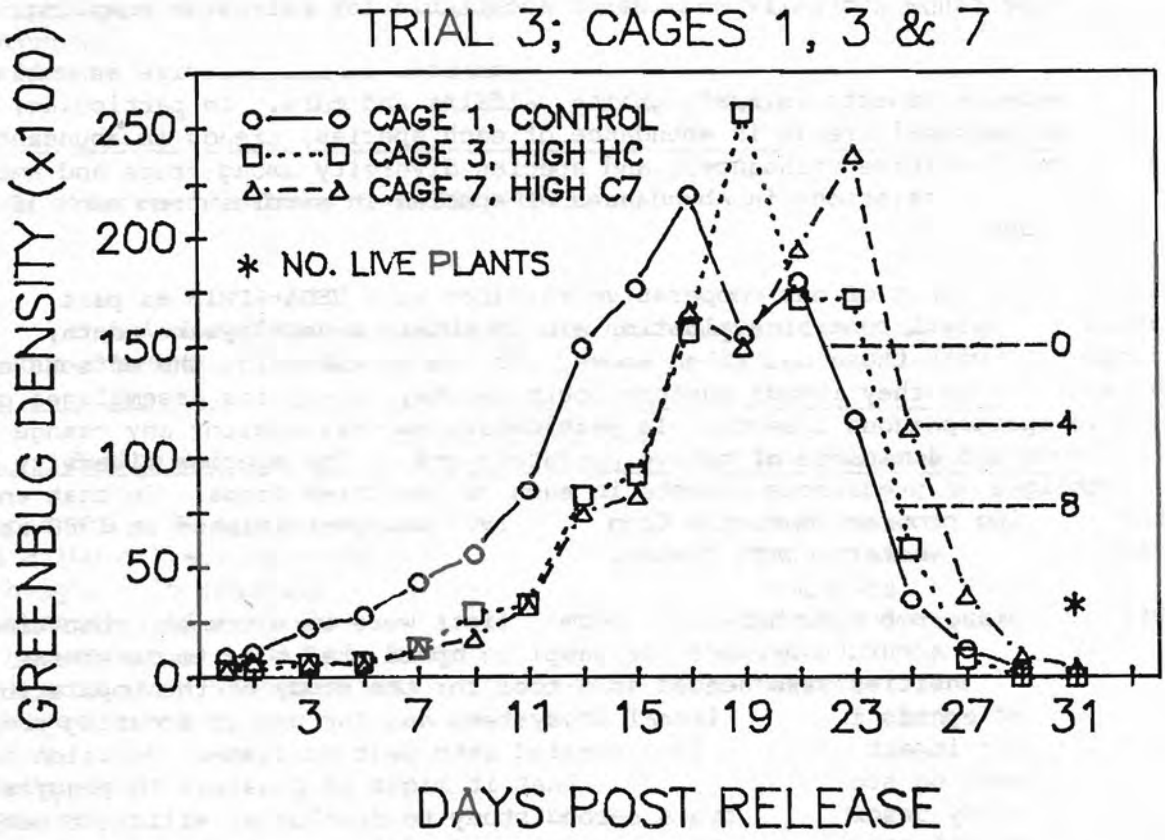


Figure 10. Comparative densities of greenbug among cages with no coccinellids or high levels of C7 or Hc; Texas July 22 - August 24.

diameter sweepnet (6 x 50 sweeps per field). Coccinellids were sampled in a single cornfield at each site at ca. weekly intervals by thorough whole plant inspections (4 x 15 minutes per field) and removal of all coccinellids with an aspirator. Predators were returned to the laboratory where they were identified to species. Coefficients of variation of weekly estimates of abundance varied depending on species, season and year but typically ranged from 0.10-0.20 for abundant species to 0.25-0.50 for uncommon species in alfalfa and small grains, and from 0.20-0.30 for abundant species and 0.40-0.60 for uncommon species in corn. Throughout these years, predator assemblages (i.e., species abundances and diversity) remained fairly constant and no one species dominated. Species composition, determined by similarity indices, was most similar in alfalfa and small grains, whereas corn was least similar to the other two. Furthermore, mean diversity indices indicated that species diversity (especially for adults) was lower in corn than in alfalfa and small grains. Levels of sampling variation observed in this study were within the range generally considered acceptable for extensive sampling.

The database derived from this sampling was used to characterize assemblages of predaceous insects in small grains, alfalfa and corn. In particular, we examined seasonal trends in abundance of each species, trends in abundance, dominance (relative abundance), and species diversity among crops and among years, and correlations in abundances of species in samples from each of the three crops.

The primary goals of our cooperative research with USDA-APHIS as part of the aphid biological control evaluation are to obtain several years' data, consistent with those described above, for use in assessing the effects of C7, Hv and P14, as they invade eastern South Dakota, on species assemblages of native aphidophagous insects. In particular, we will monitor any change in abundance and dominance of native predators and on the species diversity of assemblages of predaceous insects in each of the three crops. To that end, the sampling program conducted from 1973-1985 was re-initiated in 1988 at the same sites in eastern South Dakota.

Bill Kauffman, Bob Kieckhefer and Norm Elliott were in agreement that time-efficient yet accurate methods for sampling aphid predators to determine population densities were needed as a tool for the study of the population dynamics of aphids in agricultural ecosystems and for use in scouting programs (if predator impact is to be incorporated into pest management decision making systems based on scouting). We felt that it might be possible to piggyback onto the study described above a second study to develop an efficient sampling method for estimating population densities of adult coccinellids in small grains. This turned out not to be the case. The sampling protocol required to simultaneously satisfy all the objectives set forth above was too cumbersome for two persons (the size of the field crew assigned to the project) to accomplish efficiently. However, we still felt that it was important to develop such a sampling plan so we decided to attempt it as an additional undertaking. Therefore, the project was supplemented with funds from USDA-ARS.

The techniques we are employing for developing the sampling methodology were modified from those published recently in Canadian Entomologist (Can. Ent. 1987. 119: 815-822). By the time we got our techniques ironed out, it was too late in the small grain growing season to obtain enough data to develop the

sampling methodology. However, some preliminary results were obtained and are summarized (Tables 7 and 8).

Table 5. Relative abundances of aphidophagous predators in three crops.

Predator	Alfalfa		Small grains		Corn	
	Previous[1]	1988	Previous	1988	Previous	1988
Nabis spp.	0.62	0.66	0.39	0.31	--	--
Chrysopa spp.	0.10	0.05	0.24	0.05	--	--
H. convergens	0.14	0.15	0.19	0.45	0.44	0.32
H. tredecimpunctata	0.06	0.00	0.13	0.04	0.28	0.05
H. parenthesis	0.06	0.10	0.03	0.07	0.00	0.00
C. maculata	0.02	0.01	0.03	0.05	0.26	0.60
C. munda	0.00	0.00	0.00	0.00	0.00	0.01
C. transversoguttata	0.00	0.00	0.00	0.00	0.01	0.00
C. septempunctata	--	0.02	--	0.02	--	0.01
A. bipunctata	0.00	0.00	0.00	0.00	0.00	0.00
Other	0.00	0.00	0.00	0.00	0.00	0.00

[1]Average relative abundance from 1973-1985.

Table 6. Physical and biological variables possibly influencing abundances of predaceous insects in small grains.

Variables Measured	
Biological	Environmental
Predators - 6 x 50 sweep samples	Wind speed
Aphids - 100 tillers (binomial)	Solar intensity
Plant height - 15 readings	Temperature
Plant density - 15 readings	Dryland/irrigated
Plant growth stage - (Zadok's scale)	Time
Quadrat sampling - (15 quadrats)	Location
Daubenmeyer's method	Land use index
crop	
bare soil	
grasses	
broadleaf weeds	
Vertical obstruction readings - (15/field)	
Other prey - 6 x 50 sweeps	
biomass in 3 size classes	

Table 7. Relative abundance of adult coccinellids in sweepnet, removal and timed samples.

Species	Sweepnet	Timed	Removal
H. convergens	0.64	0.73	0.60
C. maculata	0.09	0.08	0.12
H. tredecimpunctata	0.19	0.11	0.21
C. septempunctata	0.01	0.08	0.02
H. parenthesis	0.07	0.00	0.05
C. transversoguttata	0.00	0.00	0.00

Table 8. Correlations among number of adult coccinellids in sweepnet, timed, and removal samples and the proportion of aphid infested tillers and temperature.

	Timed	Sweepnet	Removal	Aphids	Temperature
Timed	--	0.41	0.72*	0.77*	-0.07
Sweepnet			-0.08	0.40	0.47
Removal			--	0.63	-0.02
Aphids				--	0.29

* = significant at the 0.05 level.

Relative abundances of all species in each crop were similar to the long-term averages, however, C7 now comprises a small proportion of the fauna (Table 5). Increased abundance of C7, as well as Hv and P14, may alter the indices of species dominance and species diversity in these crops in future years, particularly as these species are established for biocontrol of RWA. Therefore, this intensive survey of coccinellid predators and aphids in SD agroecosystems should be continued and supported by APHIS for the next few years, particularly since this location appears to be on the leading edge of RWA's northeastern spread. These investigators also initiated a community study of aphid predators in small grain, alfalfa and corn ecosystems to learn more about why coccinellids are distributed over the landscape as they are. USDA-ARS supplemented the project by adding additional resources to those provided by USDA-APHIS for the survey described above, so that we could expand the spatial dimensions of the sampling program and also gather data on numerous physical and biological variables that we thought might influence abundances of predaceous insects in the crops (Table 6). It appears, from preliminary data, that relative abundances of adult coccinellids (5 native species and C7) were similar for sweep netting and repeated removal with an aspirator (unbiased estimate of absolute sampling) (Table 7). Therefore, this suggests that sweep net sampling is an efficient method for estimating population densities of ladybeetle adults.

Paul Schaefer - ARS, Newark, DE

Paul Schaefer is extremely valuable as an advisor to this Aphid Biocontrol

Project, particularly due to his involvement with establishment of C7 in the United States and its eventual geographic spread.

Paul continues to study the overwintering aggregations of C7 in the bases of perennial grass clumps along the Chesapeake and Delaware Canal. A census of 1,010 grass clumps resulted in a mean of ca. three ladybeetles per clump, of which ca. 90% were C7. Paul also described a population of C7 on the Delmarva Peninsula whose high reproductive rate is supported by predation of a clover aphid. This aphid, which occupies a protected niche in the axial sheath of the plant petiole, much like RWA's protected niche within rolled tillers, is presumably vulnerable to predation by C7 during aphid dispersal to uninfested plants. Therefore, despite its large size, C7 should not be discounted a priori as an effective predator of RWA in small grains. Paul also cautioned against mistaking C. septempunctata bruchii (subspecies introduced from Japan; spots are larger) for C7 in field surveys.

Peter Kareiva - University of Washington, Seattle

Peter's ongoing research of ladybeetle behavior related to aphid predation, which is supported by NSF funding, has much relevance to how this Evaluation Group interprets coccinellid efficacy. The following conclusions summarize his ecological studies:

1) C7 and Hv show a strong positive tenure time response to aphid density, whereas P14 left the field plots regardless of prey densities. It appears that C7 is a "big colonizer" specialist (presumably due to high voracity) and Hv colonizes moderate to large populations of aphids.

2) Although highly variable among individuals, all three coccinellids responded to the degree of plant complexity (i.e., plant architecture) by greatest movement and highest feeding rates on goldenrod, followed by intermediate levels on mutant leafless peas, and lowest performance on normal peas. C7 effectiveness was most affected by plant architecture, followed by P14, then Hv. Plant influences were of greater significance to adults than to larvae.

3) Although C7, Hv and P14 showed no differences in body temperature, their levels of activity differed for a given ambient temperature. For example, flight initiation by Hv occurs at a lower body temperature than C7, and C7 "basks" more frequently than Hv. These conclusions, along with previous data, suggest that these exotic coccinellids may provide complementary predation of aphids rather than competitive displacement of one another.

In conclusion, this Evaluation Group of APHIS's Aphid Biological Control Project has made significant progress in developing methods to assess the ecological role of coccinellids in aphid management in agricultural habitats. This advisory group's common interests amid scientific diversifications, eagerness to share ideas and research conclusions, and obvious enjoyment with being together is a tribute to true cooperation by each individual. We expect that funds for the cooperative agreements (TX, AR, IA) will be continued for one additional year. Also, continued monetary support for the ongoing field survey at Brookings, SD (ARS) is a high priority for the next few years. It is hoped that this group will continue to meet annually as a Technical Advisory Committee throughout the duration of this APHIS Aphid Biocontrol Project, even in the absence of formal cooperative agreements.

Project Number: ABC 88.1.2
Project Subtitle: Suitability of Prey for Hv and P14 Coccinellids
Report Period: October 1, 1987 - September 30, 1988
Report Type: Preliminary
Project Leader: William C. Kauffman, assisted by C. Esteves

Objectives: To provide preliminary data on relative nutritional value of pea aphid and greenbug prey for Hv and P14 larvae and adults. These results will accomplish the following: 1) fulfill a request from Niles Biocontrol Laboratory for additional methods development support of the mass-rearing operations of Hv and P14, and 2) decide whether to proceed with a detailed life table analysis of prey suitability to different strains of Hv and P14. This latter plan has been discussed with Dr. John Obrycki (Iowa State University) as a possible collaborative effort.

Materials and Methods: Two species of aphids, pea aphid (PA), Acrythosiphum pisum, and greenbug (GB), Schizaphis graminum, were offered first to Hv and P14 larvae then to next-generation adults to determine relative nutritional quality of these prey for Hv and P14 development and reproduction. Aphids were provided in excess throughout the experiment. Replicated treatments were conducted under constant laboratory conditions of 27°C (± 1), 20-50 RH, and 16:8 (L:D) photoperiod.

The experimental arena used for both the larval and adult studies consisted of a clear plastic cage, 18 cm high x 11 cm diameter, with a removable lid of white netting material. This cage was placed over a pot containing one fava bean plant (for pea aphids) or 15-20 "Barsoy" barley plants (for greenbug). A foam closure surrounded the plant stems at soil level. Aphids and predators were thus maintained on live, vigorous plants which, by incorporating plant effects on predator and prey behavior, more closely resembled the natural field situations.

This trial, consisting of immature and adult generations of Hv and P14, was conducted from 11 November, 1988, through 23 December, 1988. Hv and P14 eggs (Canadian strain) sent from the Niles laboratory provided the source of predators for this experiment. The experiment was initiated with a single Hv or P14 neonate placed into an arena containing pea aphid- or greenbug-infested fava bean or barley, respectively. Each treatment combination (i.e., predator species and aphid species) was replicated with eleven arenas. The survival and stadium of each individual was recorded daily until adult emergence; pupal weights were also recorded.

Newly eclosed Hv and P14 adults were sexed and weighed. Male and female beetles from each treatment combination were randomly paired and introduced into new arenas to feed on the aphid species upon which the larvae had fed. Blue flagging tape and plastic straws have been shown by O. T. Forrester (Otis MDC) and the Niles Laboratory, respectively, to be acceptable for oviposition by P14 and Hv. These two ovipositional materials were placed onto the foam bottom of the cage to: 1) facilitate collection and counting of eggs, and 2) determine for each coccinellid species which substrate is more likely to be selected for oviposition. One piece of tape (8 cm length x 3 cm width, folded lengthwise and held with a paper clip to provide a 5 mm dia refugium) and three straws (5 mm dia, 6 cm length) were added to each arena. Straws containing coccinellid eggs were cut lengthwise to enable counting eggs via a stereoscope. All egg clutches were held in 5.5cm dia plastic petri dishes to

determine percentage hatch. Adult survival and oviposition were recorded daily through day 25.

Results: Diet of larvae did not affect the developmental times of Hv or P14; however, the immature generation of P14 was ca 1-1/2 days shorter than that of Hv due to faster development of the second and third instars (Table 1). Weights of pupae and adult females were unaffected by species of prey for Hv; P14 pupae and adult females were smaller when larvae consumed GB than with PA prey (Table 2).

The adult studies (Table 3) were conducted with 3-4 replications of each treatment combination. The reasons for this small sample size were: 1) limited space for the larval arenas due to overlap with another experiment; 2) because of #1, the number of male-female pairs available for fecundity studies was greatly reduced, and 3) the constraints of rearing enough GB and PA to ensure prey in excess at all times. In retrospect, #3 was not a limiting factor since the voracity of Hv and P14 is low compared to C7; this experiment was modeled after previous C7 lifetable studies. Because of the small sample sizes, statistical analyses were not performed on these data; thus, these results should not be considered conclusive.

The species of aphid eaten by larvae and adults did not consistently affect the fecundity of Hv and P14 (Table 3). Larger egg clutch size of P14 fed GB compared to PA-fed P14 nearly offset the smaller number of egg clutches deposited by the former. Egg viability for each coccinellid species did not appear to be affected by which aphid was eaten. Low hatch of P14 eggs compared to Hv would drastically lower intrinsic rate of increase of P14 and may explain difficulties encountered in mass-rearing this coccinellid in the laboratory. There does appear to be an interaction between prey eaten and ovipositional substrate chosen (Table 4). Both Hv and P14, when fed PA, deposited most of their eggs in plastic straws, but when fed GB a larger proportion of eggs were laid on blue flagging tape.

Conclusions: Based on this preliminary experiment, whether Hv or P14 feeds on PA or GB is of little consequence to immature development and adult fecundity. Although replications were few, any clear trends among treatments should have been detected in this experiment. Therefore, I recommend that the present practice of mass-rearing Hv and P14 on GB at Niles be continued, particularly since the target prey for these coccinellids are the Russian wheat aphid (RWA) and other cereal aphids. I also conclude that the apparent absence of a significant role of PA and GB on growth and reproduction on of the Canadian strains of Hv and P14 does not warrant an exhaustive life table analysis of prey suitability of these two aphids. Such investigations may be needed, however, for other predator strains (i.e., Turkish material) or for other prey, such as Russian wheat aphid.

Table 1. Development of Hv and P14 immature stadia when larvae consumed pea aphid or greenbug.

Predator species	Prey species	Mean duration (days)					
		Instar I	Instar II	Instar III	Instar IV	Prepupal +pupa	Neonate to adult
Hv	PA	2.2	1.6	1.4	3.1	4.1	12.4
Hv	GB	2.1	1.6	1.5	3.0	3.9	12.1
P14	PA	2.0	1.0	1.5	2.1	4.2	10.8
P14	GB	2.0	1.0	1.4	2.2	4.1	10.8

Table 2. Weights of Hv and P14 pupae and adult females when larvae consumed pea aphid or greenbug.

Predator species	Prey species	Mean weight (mg)	
		Pupa	Adult female
Hv	PA	9.5	8.8
Hv	GB	9.4	9.0
P14	PA	9.7	8.6
P14	GB	8.8	8.0

Table 3. Fecundity of Hv and P14 adults when larvae and adults were fed either pea aphid or greenbug.

Predator species	Prey species	Mean					
		Preovip. period (days)	Egg clutches/ female	Eggs/ female	Eggs/ female/ day	Eggs/ egg clutch	Egg hatch (%)
Hv	PA[1]	3.5	39.0	575.5	24.0	14.5	81.2
Hv	GB	3.5	45.2	599.8	23.0	13.2	88.9
P14	PA	4.0	44.2	327.5	13.1	8.0	35.0
P14	GB	4.7	22.0	286.3	11.5	11.7	38.8

1 Two females in this treatment died by day 5, therefore, those data were excluded from analyses.

Table 4. Choice of ovipositional substrate by Hv and P14.

Predator species	Prey species	Mean percentage		
		Straws	Tape	Other
Hv	PA	84.8	13.0	2.2
Hv	GB	57.2	40.3	2.5
P14	PA	72.9	26.0	1.1
P14	GB	31.8	60.4	7.8

Project Number: ABC 88.1.3
Project Subtitle: C7 Predation of Aphis fabae in Fava Bean
Report Period: October 1, 1987 - September 30, 1988
Report Type: Preliminary
Project Leader: William Kauffman, assisted by S. Butler, C. Esteves, S. Hennessey and D. Middleton

Objective: Describe the dynamics of established, natural populations of Coccinella septempunctata (C7) and naturally occurring aphids in a vegetable crop, and the impact of this predator-prey relationship on plant health.

Rationale: This exclusion technique (i.e., hand removal) of C7 in fava beans, Vicia fabae, will assess the direct impact of C7 adult and larval predation on bean aphid, Aphis fabae, and the immediate and long-term benefits to plant health and productivity. This crop was chosen for its reliability in harboring natural infestations of aphids, economic value as a fresh-market crop, and host plants and agro-habitats conducive for C7 aggregation and predation of aphids.

Materials and Methods: Field research was conducted at New Alchemy Institute, which is a research/educational facility at Cape Cod, Massachusetts that promotes organic gardening and sustainable agriculture. The New Alchemy site represents a small farm agroecosystem which maximizes the role of beneficial insects within the context of low inputs of synthetic chemicals and a rich ecological diversity of flora and fauna.

Although two field trials were conducted throughout the growing season, only early season plots attracted adequate levels of aphids and C7. Planting and crop maintenance procedures were standardized in all plots. Plots were 20 ft x 20 ft (ca. 6 m x 6 m) with rows oriented east-west, spaced 24 inches apart and ca. 40 seeds/row. A 5-foot high cloth barrier extending east to west through the middle of the plot subdivided the plot. Each of the paired subplots (= split plots) were randomly assigned as a control plot (C7 included) or experimental plot (C7 excluded). Two replications of plots were spatially separated by approximately 100 feet.

Following germination, plots were surveyed semi-weekly for the presence of aphids and C7 in order to determine the suitable time to initiate intensive data collection. We initiated alternate day censusing of aphids and C7, and C7 were removed daily when plants were small (5 inches tall). Data were recorded daily between 8 a.m. and 12 noon until harvest.

In each subplot, the number of aphids were recorded from all plants in a randomly selected meter of row. All stages of C7 were recorded from both sides of the barrier cloth and were removed contemporaneously from the experimental block. These daily activities presumably provided fairly equal levels of habitat disturbance in both control and experimental subplots. The middle barrier was designed to minimize cross-movement of C7 between adjacent blocks.

Plant vigor and quality was assessed throughout the experiment by measuring various plant parameters including plant height and number of nodes, flowers and pods.

At green pod harvest, the pods, stems and leaves were weighed (fresh and weights). Pods were categorized as large (≥ 3/4 in dia) or small. Aphids densely located on pods produce large quantities of honeydew which is a favorable growing medium for sooty mold, anthracnose, and other plant pathogens. Casual observations indicated a possible link between the occurrence of these secondary pathogens and high densities of A. fabae. Therefore, quality of pods was assessed on the basis of blemishes associated with these fungi.

Results: Removal of C7, particularly large larvae, significantly increased bean aphid density compared to control plots where predation virtually eliminated this aphid pest (Fig. 1). Fava bean plants on which C7 were removed - therefore, high aphid levels - had less pod biomass (expressed as fresh weight and percentage weight) than the control plants (Fig. 2). However, these plants with the predator removed appear to mitigate aphid-inflicted water and nutrient losses by increasing vegetative growth in stems and leaves. Furthermore, a greater proportion of both large and small pods from C7 removal plots were blemished by pathogens and/or aphids than observed in pods from plots with natural levels of aphid predation (Fig. 3). Plots with C7 removed were characterized by high densities of aphids on tender growing tips, pods and flowers, and experienced the following (Table 1):

1) increase in flower and pod production,
2) lesser proportion of pods attaining large size, and
3) ultimately, a 26.3% decrease in marketable pods.

In conclusion, natural populations of C7 provided sufficient control of bean aphid in fava bean at this field location to demonstrate an economic benefit of aphid predation by C7. High levels of aphid predation, particularly by large larvae, decreased plant damage from aphid herbivory, thus resulting in improved pod size and quality. This field experiment will be expanded in 1989 to include 6-8 replications at New Alchemy Institute.

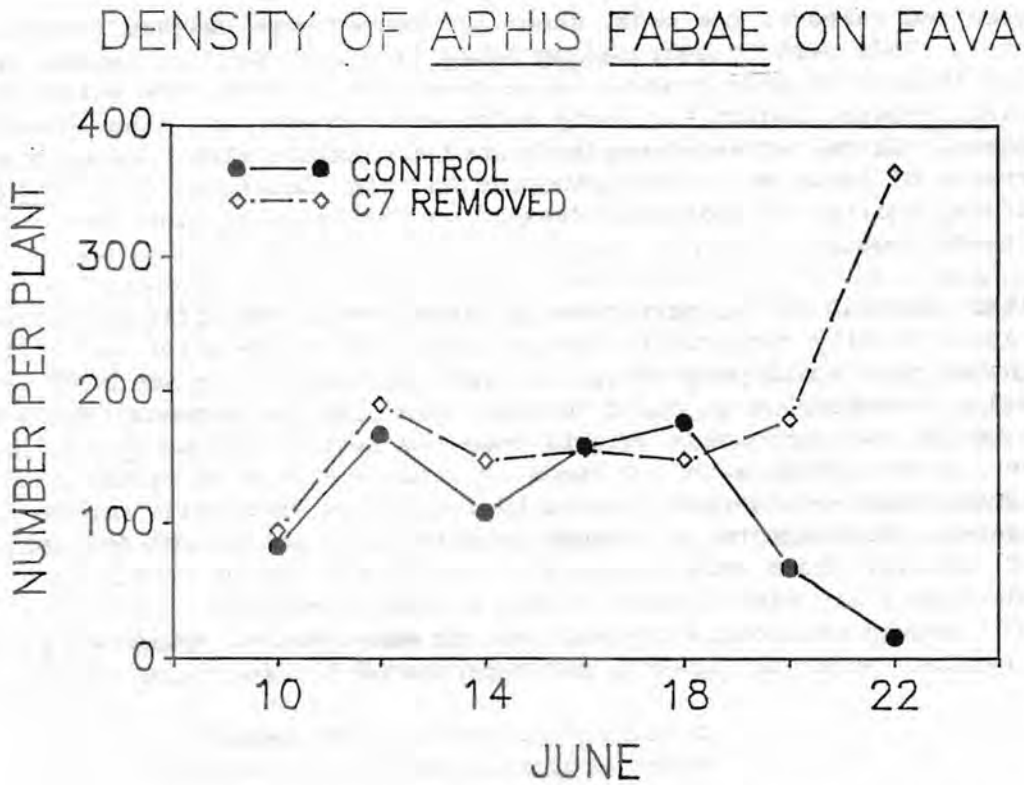


Figure 1. Comparison of bean aphid densities in plots with C7 (control) and C7 removed.

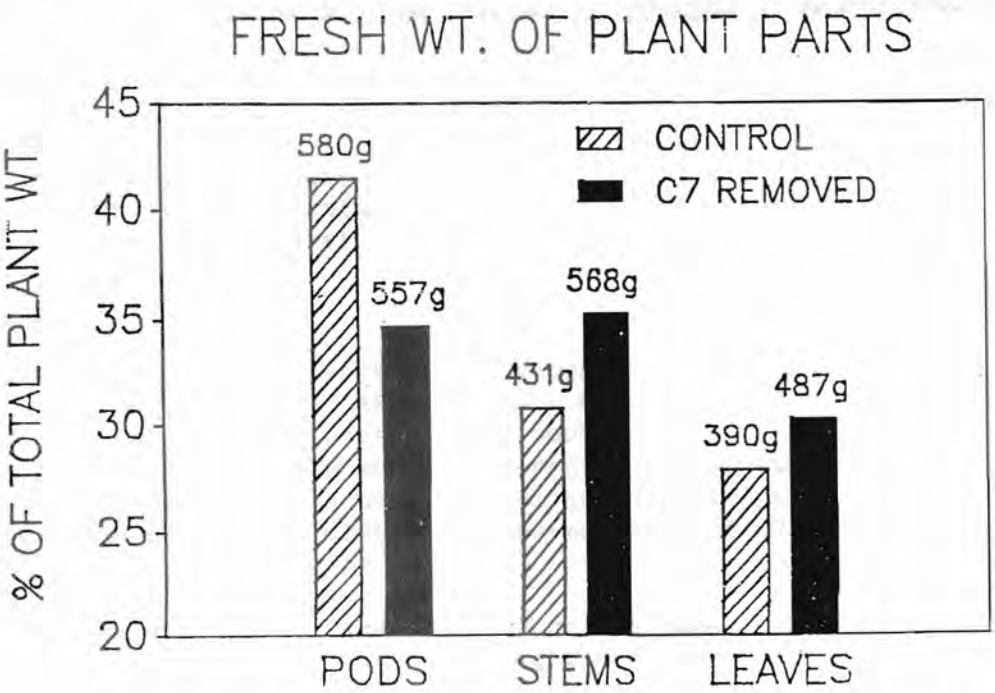


Figure 2. Weight of fava bean pods, stems and leaves resulting from high aphid densities (C7 removed) compared to lower aphid densities (control).

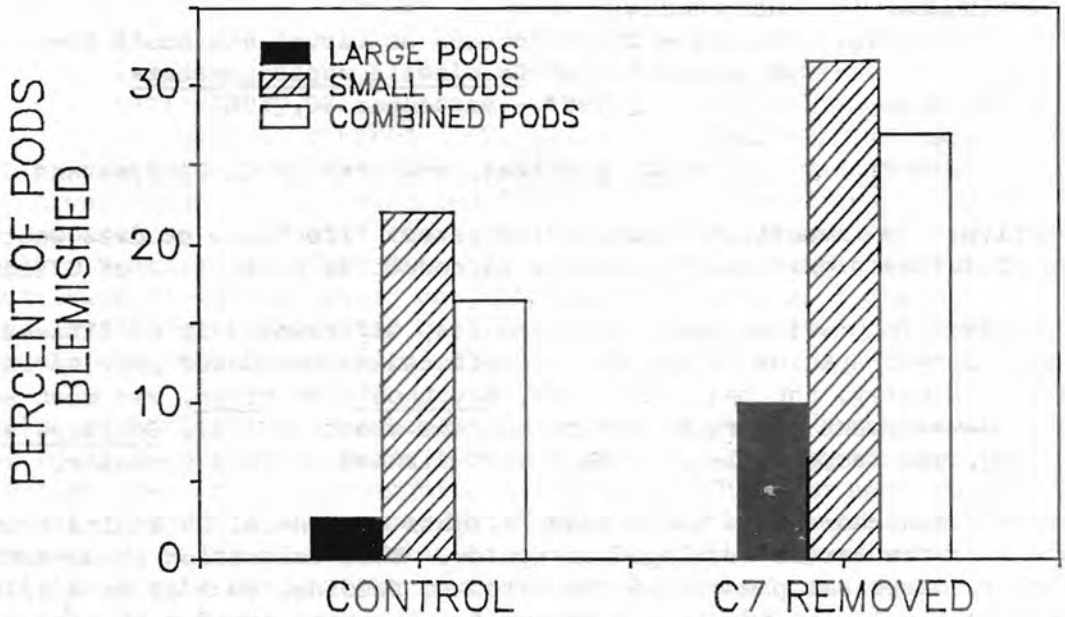


Figure 3. Surface damage of fava bean pods caused by direct aphid damage as well as secondary pathogens.

Table 1.

GREEN POD HARVEST DATA

TREATMENT	$\bar{X}$ / METER OF ROW			
	FLOWERS	TOTAL PODS	% LARGE PODS	BLEMISH-FREE LG. PODS
CONTROL	12.9	59.1	35	18.5
C7 REMOVED	20.8	70.6	23	14.1 *

* Represents a 26.3 % decrease in marketable, green pods.

Project Number: ABC 88.1.4
Project Subtitle: Relative Contributions of Larval and Adult Food to Reproduction of Coccinella septempunctata
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leader: William C. Kauffman, assisted by C. Esteves and C. Paris

Objective: To investigate through laboratory life table studies whether the diet of larvae significantly affects reproductive potentials of C7 adults.

Rationale: In previous laboratory studies, differences in C7 fitness resulted when different species of aphids were offered as no-choice prey to larvae and adults. Clearly, the pea aphid (PA), Acyrthosiphum pisum, was most suitable for C7 development and reproduction whereas greenbug (GB), Schizaphis graminum, was responsible for the lowest fitness of this predator.

Several researchers have noted heavy flights of teneral C7 adults from fields from which the larvae developed on aphids. This emigration phenomenon may be an innate dispersal phenomenon unrelated to feeding, such as mate selection, or alternatively, an adaptive strategy for locating aphid outbreaks favorable for oviposition and optimal recruitment rates of progeny.

How important are the origin of these beetles and the feeding history of the preceding larvae in limiting the ability of immigrant adults to regulate known aphid outbreaks? Can adult diet compensate for sub-optimal nutrition experienced by larvae?

We tested the null hypothesis (H_0) that feeding by C7 larvae on pea aphid or greenbug is not a significant factor in reproductive levels realized by C7 adults.

Materials and Methods: Experimental protocol was as indicated in laboratory study of "Laboratory Study of Aphid Suitability for C7 Larvae and Adults" with the following exceptions:

1. Two species of aphids were used: pea aphid and greenbug;
2. At eclosion, half of the male-female beetle pairs resulting from the pea aphid cohort were randomly chosen to feed on pea aphid and the remaining beetle pairs were switched to feed on greenbug (Fig. 1). The same procedure of constant or alternated prey was followed with teneral adults from the larval cohort reared on greenbug. Treatment combinations (i.e., larval prey/adult prey) were replicated with 4-5 arenas and repeated over time.

Results: At initiation of fecundity studies of adult C7, both sets of females reared as larvae on greenbug were smaller than those from the pea aphid cohorts (Table 1.). This confirmed our earlier conclusion of lower suitability of greenbug than pea aphid for the predatory immature stage of this coccinellid. The greatest proportion of females were fecund when pea aphid was constantly maintained for both larvae and adults. Conversely, only half of the females from constant greenbug prey oviposited. Females fed pea aphid, regardless of larval diet, oviposited earlier than corresponding females fed greenbug. Total and daily oviposition was greater for both cohorts of adults which consumed pea aphid than those which consumed greenbug.

Therefore, although a larval diet of greenbug increased time for immature development and decreased pupal and teneral adult weight relative to pea aphid-reared individuals, diet of adult was the ultimate determinant of level of reproduction realized by C7. Clearly, inter-generational constancy of aphid prey was favored over altered diet only when pea aphid, the more nutritionally suitable prey, was eaten (PA/PA). Moreover, switching prey after the larval stage either increased (GB/PA) or decreased (PA/GB) C7 fecundity compared to C7 cohorts reared continuously on GB and PA, respectively. That is, an adult diet of pea aphid fully compensated for sub-optimal nutrition of larvae reared on greenbug. Conversely, a diet of greenbug to large adults which had been raised as immatures on pea aphid severely restricted the fecundity of these adults.

These results emphasize the importance of dispersing adults locating not simply any local outbreak of aphids, but a nutritionally superior species of prey to realize their full reproductive potentials. Thus, an understanding of local movement of coccinellids among aphid foci in a field as well as inter-field dispersal is vital to assessing seasonal population dynamics of C7 and the capability of this predator to suppress aphid populations.

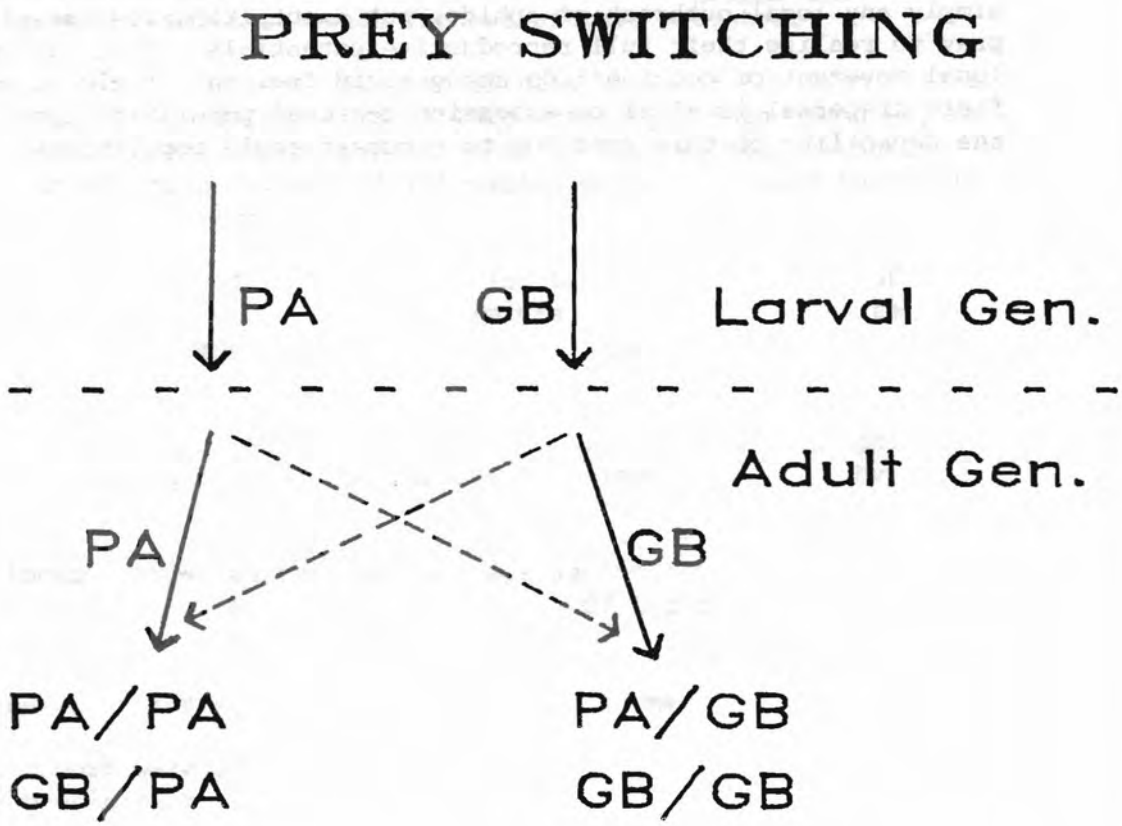


Figure 1. Larval prey/adult prey treatments using pea aphid (PA) and greenbug (GB).

Table 1. Fecundity of Coccinella septempunctata adults when aphid prey were held constant or alternated between the larval and adult generations.

Larval prey/ adult prey	No. of adult pairs	% fecund	Mean + SE[a]			
			Adult female weight (mg)	Preovip. period (days)	Eggs/ female	Eggs/ female/ day
PA/PA	15	87	37.5 ± 1.62a	7.4 ± 0.98b	540.7 ± 89.11a	23.1 ± 1.63a
GB/PA	14	79	30.0 ± 1.13b	8.7 ± 1.19b	555.1 ± 163.48a	23.7 ± 2.03a
PA/GB	15	73	39.5 ± 1.11a	13.5 ± 1.15a	89.1 ± 24.05b	3.6 ± 0.56b
GB/GB	14	50	27.4 ± 1.19b	12.9 ± 0.91a	97.6 ± 38.74b	4.1 ± 0.66b

[a] ANOVA (SAS GLM, $P \leq 0.05$) and vertical separation of means by Ryan-Einot-Gabriel-Welsch (SAS REGWF) multiple F test.

Project Number: AW 81.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasite Redistribution Program
Report Period: October 1, 1987 to September 30, 1988
Report Type: Interim
Project Leader: P. C. Kingsley

The final phase of this project was initiated in 1988 and will be brought to fruition in 1989. The number of fields sampled was reduced from 150, located in seven states in 1987, to 90 in three states in 1988. Next year we plan to survey only 60 fields. Some early trends are encouraging, especially in parasite establishment. For example, the two centrally located areas, Missouri/Iowa and Illinois have both shown a doubling in the average number of parasite species during our survey (from 1.7 to 3.8 in Missouri/Iowa and 2.2 to 4.7 species in Illinois) (Figure 2). This increase in Missouri and Iowa was mainly due to the establishment of Microctonus aethiopoides (MA) a parasite of the adult weevil (Figure 4). Parasitism of the adult stage has increased concurrently with MA establishment from 2.3% in 1981 to 34.2% in 1985 (Figure 3).

Spraying for AW control has also shown an encouraging downward trend in these Midwestern states. Out of the 60 fields surveyed each year, there has been a consistent decline in the percentage of fields treated from 33.3% in 1981 to zero in 1987 (Figure 5). Although these preliminary trends are encouraging, AW population levels have not shown a corresponding decline in these areas (Figure 1), therefore assigning a cause and effect relationship between parasites and spraying is difficult at best.

Analysis of alfalfa weevil mortality inflicted by Microctonus aethiopoides in Illinois and Ohio indicates a delayed density dependent response to its host. This type of relationship is revealed by plotting yearly parasitism rates against peak weevil population densities and then connecting the points consecutively (Figure 6). A counter clockwise spiral indicates this type of delayed response where mortality factors are having long term effects on maintaining the equilibrium of an insect population.

Work on a final publication is underway and, upon completion, will be submitted to the Bulletin of the Entomological Society of America. This paper will give an overall view of the Alfalfa Weevil Biological Control Program from start to finish.

The economic aspect of the evaluation study will also be completed this year in cooperation with Dr. Joe Moffit, Dr. Geoffrey Allen, economists at the University of Massachusetts, and their graduate student Joan White. Their approach is to incorporate the data from our grower questionnaires into a dynamic multi-sectoral model called AGSIM. With this model we will be able to analyze the change in benefits and costs to society, measured as economic surpluses, caused by the change in alfalfa production costs resulting from biological control.

Figure 1. Mean (SE) peak AW densities by area and year.

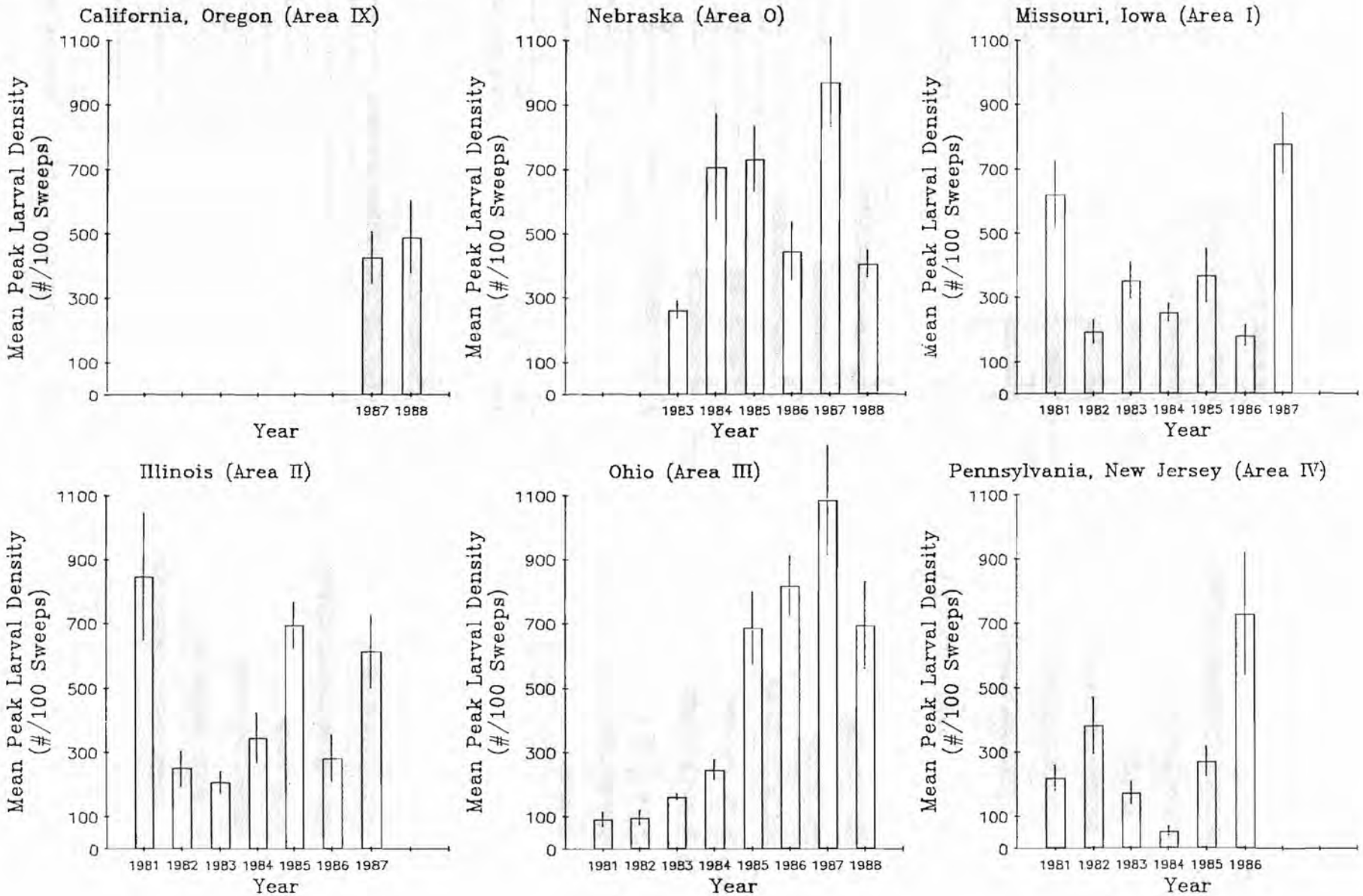


Figure 2. Average number of AW parasite species by area and year (N=6 sites).

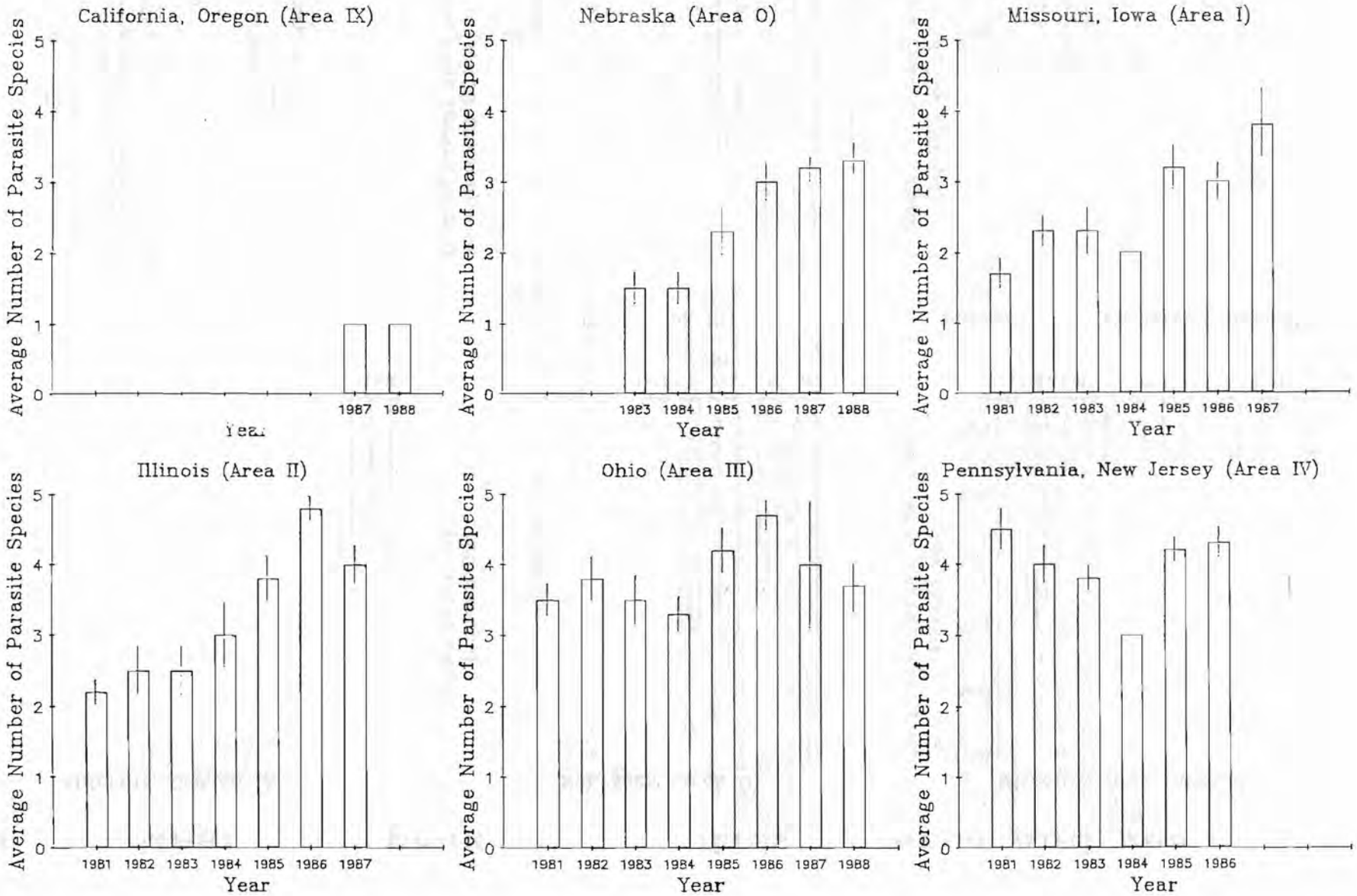


Figure 3. Percent parasitism of AW adults and larvae by area and year.

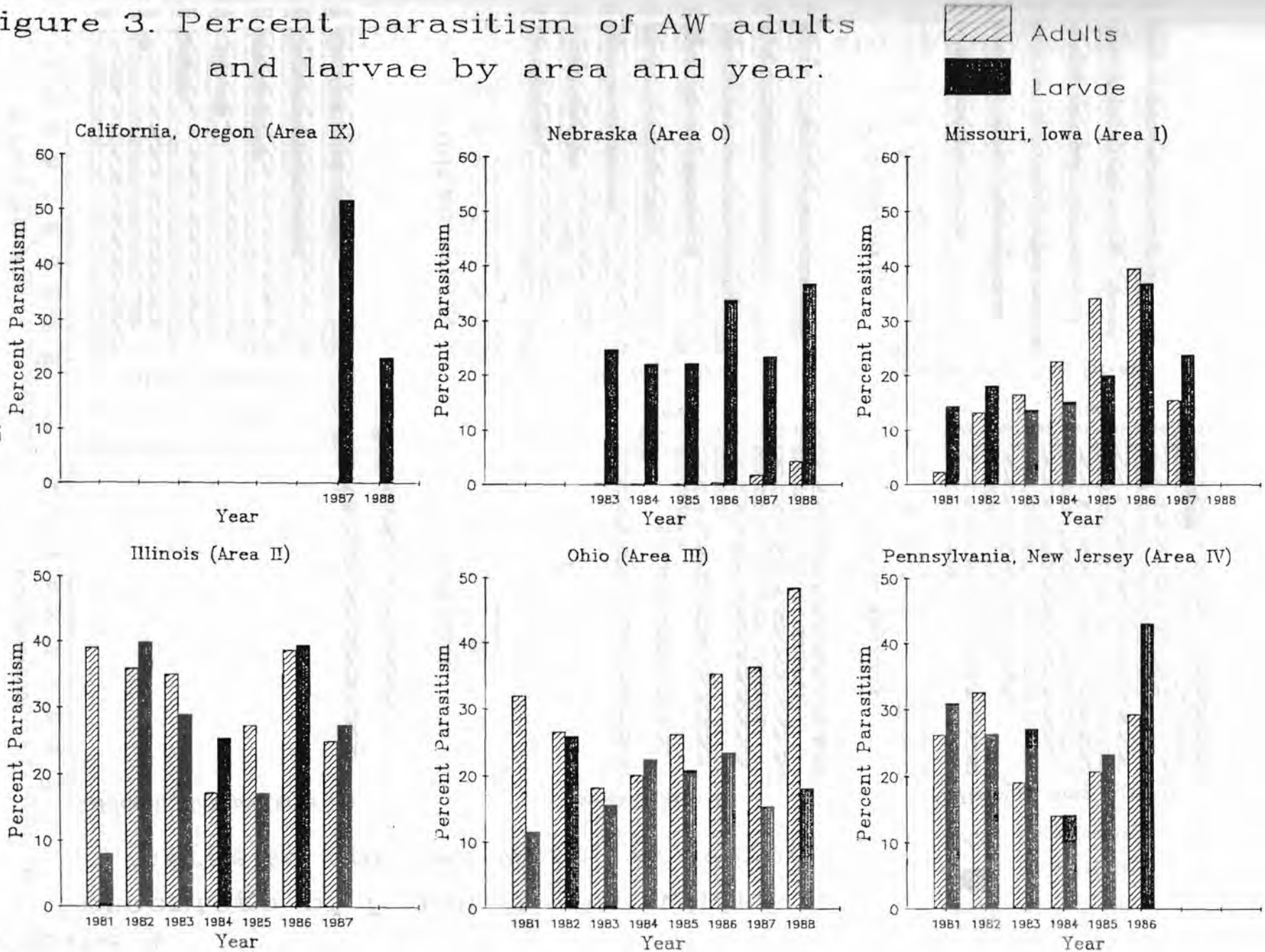


Figure 4.

Percentage of fields (N=30) where three parasite species were recovered.

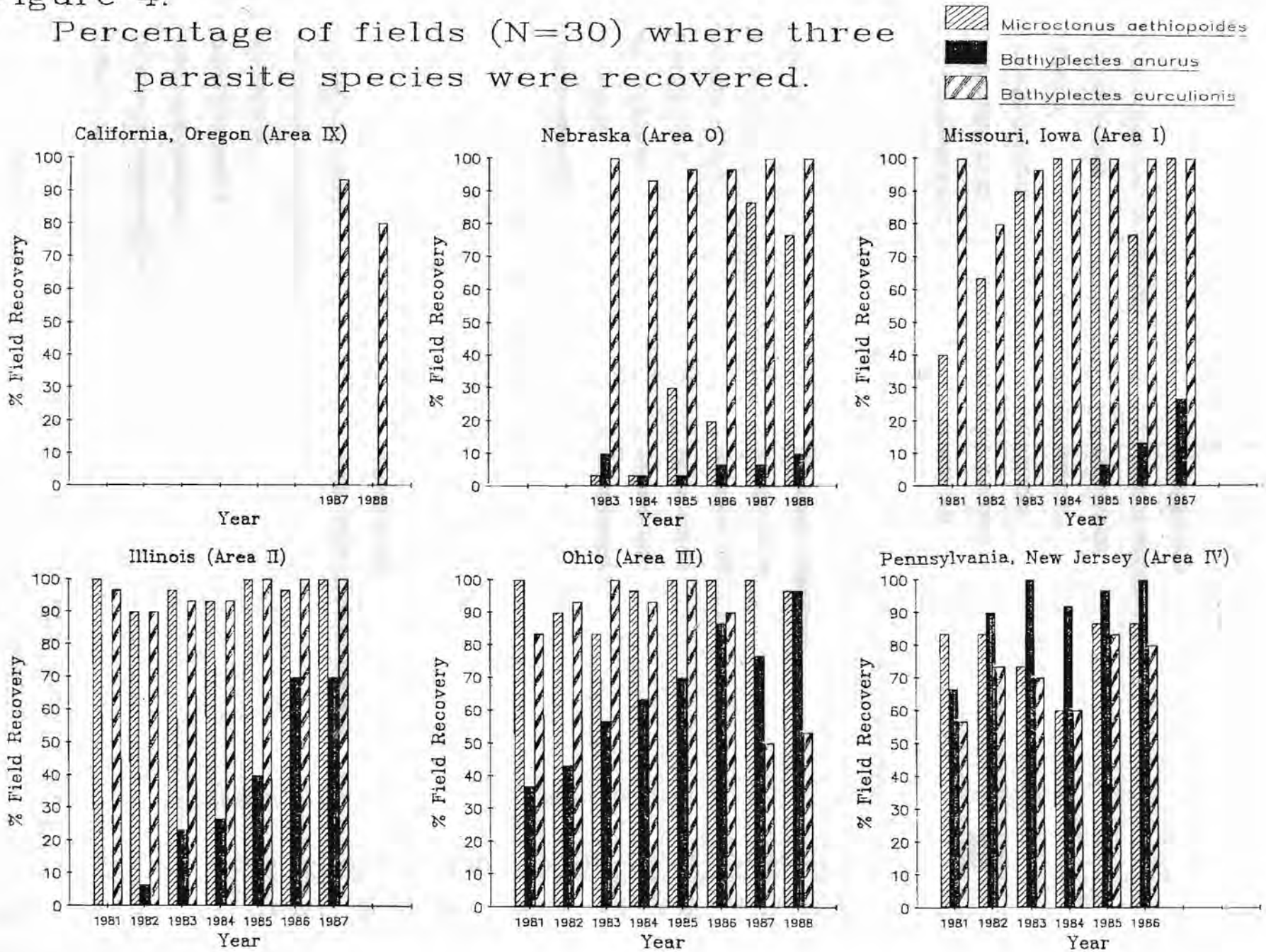


Figure 5. Percentage of fields (N=30) sprayed for AW control by area and year.

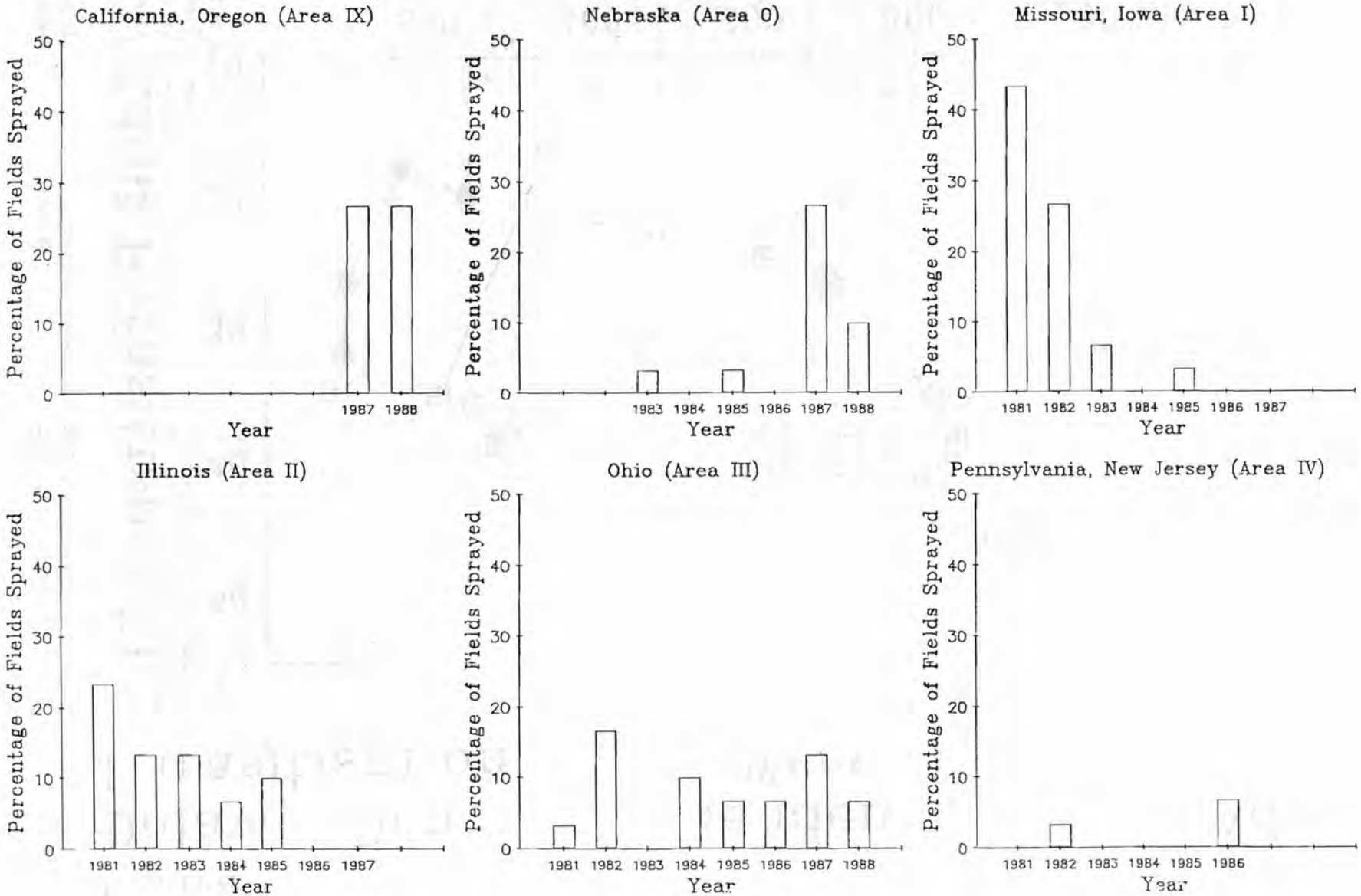
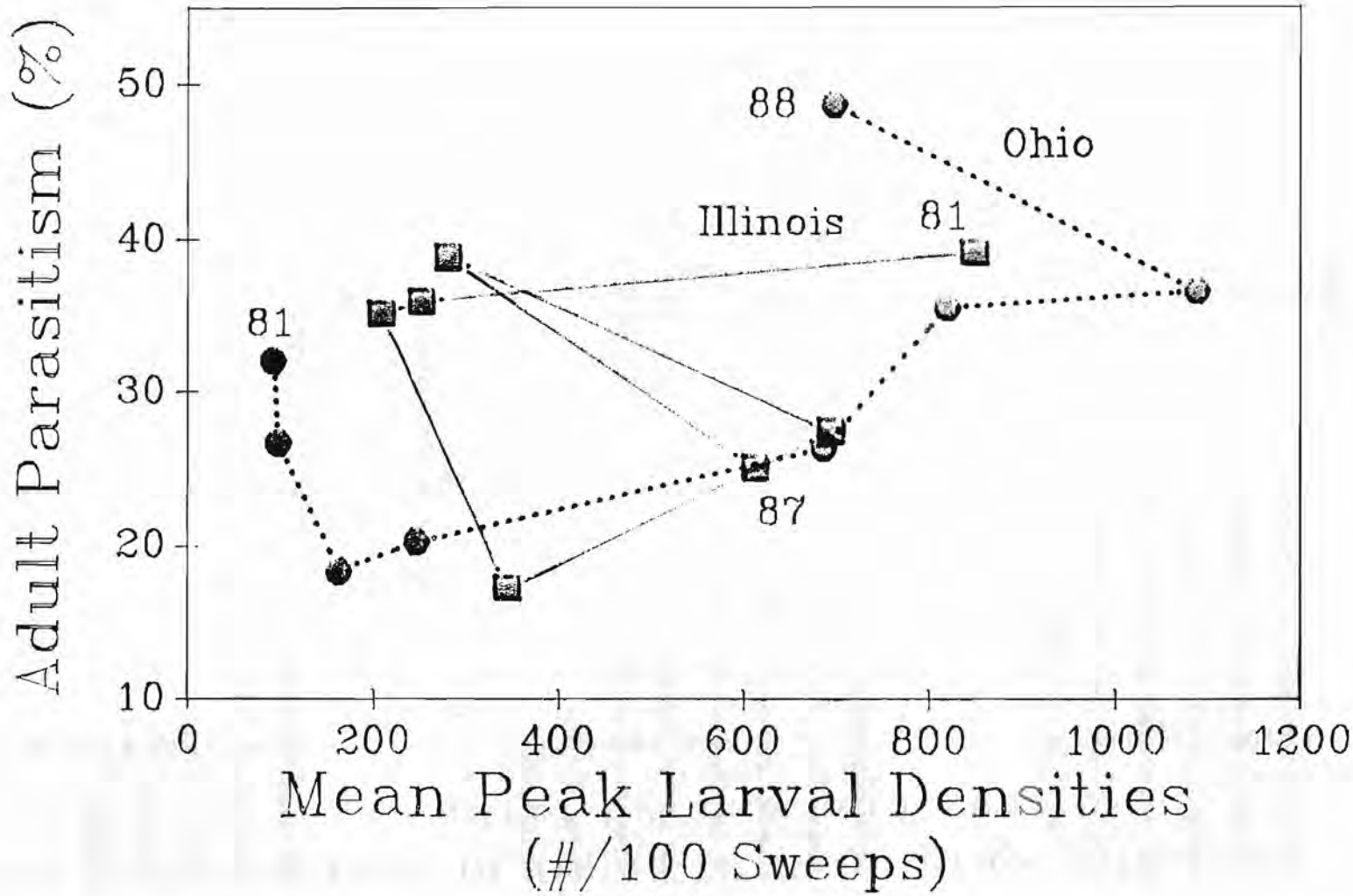


Figure 6.

Delayed Density-Dependent Effect of Adult Parasitism on Alfalfa Weevil Populations.



Project Number: CAPS 84.1.1
Project Title: Pheromone-Based Survey Technology for Early Detection of Exotic Insect Pests
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: V. C. Mastro, C. P. Schwalbe and P. C. Kingsley
Cooperators: P. Newton

Two species have been added to the survey manual: the apple ermine moth, Yponomeuta malinellus, and the pear leaf blister moth, Leucoptera malifoliella. In addition, trap density guidelines have been prepared (Appendix 1). The formulation for false codling moth (FCM), Cryptophlebia leucotreta, lures was changed based on experimental data from Dr. Phil Newton, a longtime cooperator. Following is a brief description of that formulation test.

The tests were carried out in citrus groves on the Crocodile Valley Estates in South Africa. The test design was a randomized complete block with four replicates. The standard Pherocon 1C trap was used for all lure formulations and the inter-trap spacing was 70 m. The literature indicates that the best attractant for the FCM is a 50:50 blend of E:Z 8 Dodecen-1-01 acetates E:Z 8-12:OAc). All commercial formulations are made using this blend. In earlier field tests, Dr. Newton was unable to demonstrate a dose response using this blend. These field results plus conflicting descriptions in the literature on female gland contents prompted this re-evaluation.

To determine if the ratio of the two components being used was optimum, dispensers (rubber septa) were loaded with E 8-12:OAc and Z 8-12:OAc blends. The range of ratios tested included E:Z; 1:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10. All loadings totaled 1.0 mg. A similar test was used to compare the above blends with the addition of a possible minor pheromone component (12:OAc) added at rate of 10% of the total formulations.

Highest captures were found in traps baited with 9:1 or 8:2 ratios of E8:Z 8-12:OAc. Capture rates with these blends were approximately 4 times greater than with the standard 5:5 blend. The addition of 12:OAc to any of the blends did not markedly affect capture.

Presently, work is in progress to determine if any of a number of other compounds that have been reported to be in extracts of female glands elicit any field activity either alone or in combination with the major components. At the conclusion of these studies, a paper will be prepared for publication by Dr. Newton.

Because of this redefinition of the pheromone blend for FCM that will be used for the exotic pest detection program, earlier studies with traps co-baited for other exotic or domestic species may no longer be valid. To address this question, lures for one domestic (pink bollworm, Pectinophora gossypiella (PBW)) and two exotic pests (pink spotted bollworm, Pectinophora scutigera (PS) and the pear leaf blister moth, Leucoptera malifolliella (PLBM)) were tested in combinations with the new blend for FCM.

Preliminary results (Table 1) indicate that the addition of PBW and PS lures appear to adversely affect capture of FCM males. Earlier studies indicated

that lures for PS did not adversely affect male FCM capture in traps baited for both species. When the data are complete, a statistical analysis will be performed.

Table 1. Preliminary results of testing traps baited for FCM alone and in combination with three other species' attractants.

Treatment	Mean number of males[1/] capture/trap/reading
FCM	49.0
FCM + PS	20.2
FCM + PBW	28.2
FCM + PLBM	36.4
PS	0.0
PBW	0.4
PLBM	0.3

1/ Randomized complete block design with four blocks and two readings.

Planned or ongoing studies for 1989 include trap design test with the false codling moth and the apple ermine moth.

Appendix 1.

Trapping Density Guidelines

This section provides information for planning trapping programs and estimating their effectiveness. The procedure will assist in making trap density decisions, and standardize this decision making process among cooperators. The tables and graphs were derived from a hypergeometric distribution described extensively by Victor Beal (USDA, APHIS, VS) in his Regulatory Statistics Volumes. They can be used to estimate either the number of traps needed to detect a certain number of infested acres, or reversing this, the size of an infestation detectable with an available number of traps. Here we are defining an infestation as any density of an established reproducing pest. This kind of probability distribution has been used historically to design sampling schemes for cattle disease surveys and for inspecting imported commodities for pests. At large acreage, the number of traps needed to detect a particular infestation rate levels off (Figure 1). This is because, as the total acres being sampled increases towards infinity, non-replacement becomes less important and the hypergeometric distribution then asymptotically approaches a binomial distribution.

The probabilities referred to here are based on random sampling. Under most circumstances, the random placement of traps would be a difficult proposition in exotic pest surveys. Placement using a grid pattern might be used for some cropping systems, however, a more reasonable alternative would be to calculate the number of traps to be placed in each county on a per acre basis (see example A below). Sites for individual trap placement within a county should then be selected to increase detection odds, i.e. areas with a higher likelihood of introduction (see Pathway Studies) or sites that would favor the establishment and buildup of a pest. It should be noted that these methods should be used only as a means of detecting an infestation and not to infer population densities.

The tables below were developed by Dr. Beal* using the following formula ass an approximation of the actual hypergeometric distribution:

$$n = (N - d/2 + 1/2) - (1-p)^{(1/d)} * (N - d/2 + 1/2)$$

or

$$d = (N - n/2 + 1/2) - (1-p)^{(1/n)} * (N - n/2 + 1/2)$$

where n = sample size
d = number of infested units
N = total number of units to be surveyed
p = probability of detecting at least one infested unit

These variables are described below.

* Beal, B. C., Jr. 1973. REGULATORY STATISTICS - Appendix PART III Supplement in VOLUME 2-B; p. A-3-sup-3.

Four pieces of information are required to estimate either the number of traps needed to achieve a desired detection efficiency, or the survey effectiveness with a known number of traps:

1. **Unit of a Trap's Active Area (UTAA)** - defined as the area around a trap in which its trapping efficiency is 100%. The limits of the active area are species specific, and regulated by insect behavior and trap characteristics. The major assumption is that within the UTAA the trap will capture at least one insect when any density of the pest is present. This does not consider insects randomly moving into the UTAA as this movement is species dependent and has not been defined for the target insects. We suggest that in the absence of trapping efficiency data (which is the case for species in the exotic survey project), the UTAA can conservatively be estimated as one acre.

2. **Total number of trap units to be surveyed (N)** - This value might be the total area of target host plants in a state, or in the case of a high risk urban area, simply land area.

3. **Either: The minimal number of infested trap units to be detected (d)** - The infested area could be concentrated in one location, i.e. one orchard or numerous small infestations scattered throughout the survey area. This number can be converted to an infestation rate by dividing the number of infested units to be detected by the total number of trap units to be surveyed.

   Or: The number of available traps (n)

4. **Probability (p)** - under which we would detect at least one unit of infestation, i.e. 90, 95 or 99%.

The following examples explain the use of these tables:

Example A

Question: How many traps should be placed to detect 100 acres (or more) infested by *Adoxophyes orana* in 10,000 acres of commercial orchards at a 99% level of confidence?

Pest: *Adoxophyes orana*
UTAA: one acre
Total acres (N): 10,000 acres of commercial orchard
Minimal infestation (d): 100 acres or a 1% (100/10,000) infestation rate
Confidence Level (p): 99%

From the 99% probability, Table 3, we can determine the number of traps to be used by following the 1% infestation column down to the 10,000 total units row, in this case, 448 traps or one trap for every 22 acres (10,000/448). In a county with 100 acres of apples for example, we would place 5 traps. Within the county, we can increase the odds of detection by using experience in the individual trap placement, i.e. in the case of *Adoxophyes orana*, next to, or in abandoned orchards.

Example B

Question: If 50 traps are available to survey 20,000 acres of apples for *Adoxophyes orana*, what rate of infestation can we detect at a 90% level of confidence?

Pest: *Adoxophyes orana*
UTAA: two acres (note that this is twice the size of the UTAA in Example A)
Total Acres (N): 20,000 or 10,000 UTAA
Traps (n): 50
Confidence (p): 90%

By reversing the procedure in Example 1 and using the 90% Table 1, we can say that 50 traps will detect at a 5% infestation rate, or 500 infested UTAA or 1,000 acres.

Table 1. 90% Probability.
Number of traps required to detect an infestation at eleven infestation rates (10% to .05%) in a total number of trap units ranging from 100 to 900,000 and at a confidence level of 90%. Listed are the number of infested units detectable and the number of traps required.

Total Units	Infestation Rate (# of Trap Units Infested/Total Trap Units) 10%		7.5%		5%		2.5%		1%		0.75%		0.5%		0.25%		0.1%		0.075%		0.05%	
N	Units	Traps	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n
100	10	20	8	26	5	36	3	60	1	90												
200	20	21	15	27	10	40	5	73	2	136	2	157	1	180								
300	30	21	23	28	15	42	8	78	3	160	2	192	2	235								
400	40	21	30	28	20	42	10	81	4	174	3	214	2	273	1	360						
500	50	21	38	29	25	43	13	83	5	184	4	229	3	300	1	421						
600	60	21	45	29	30	43	15	84	6	190	5	240	3	321	2	471						
700	70	22	53	29	35	43	18	85	7	195	5	248	4	337	2	512						
800	80	22	60	29	40	44	20	86	8	199	6	254	4	349	2	547						
900	90	22	68	29	45	44	23	87	9	202	7	259	5	360	2	576						
1,000	100	22	75	29	50	44	25	87	10	205	8	263	5	368	3	601	1	900				
2,000	200	22	150	29	100	44	50	89	20	216	15	284	10	410	5	737	2	1,367	2	1,569	1	1,800
3,000	300	22	225	29	150	45	75	90	30	221	23	291	15	426	8	792	3	1,607	2	1,921	2	2,353
4,000	400	22	300	29	200	45	100	90	40	223	30	294	20	434	10	822	4	1,750	3	2,143	2	2,735
5,000	500	22	375	29	250	45	125	90	50	224	38	297	25	439	13	840	5	1,844	4	2,294	3	3,009
6,000	600	22	450	29	300	45	150	90	60	225	45	298	30	442	15	853	6	1,911	5	2,402	3	3,215
7,000	700	22	525	29	350	45	175	90	70	225	53	299	35	445	18	862	7	1,961	5	2,485	4	3,374
8,000	800	22	600	29	400	45	200	90	80	226	60	300	40	446	20	869	8	2,000	6	2,549	4	3,501
9,000	900	22	675	30	450	45	225	90	90	226	68	301	45	448	23	874	9	2,031	7	2,600	5	3,604
10,000	1,000	22	750	30	500	45	250	91	100	227	75	301	50	449	25	879	10	2,056	8	2,643	5	3,690
20,000	2,000	22	1 500	30	1,000	45	500	91	200	228	150	304	100	454	50	899	20	2,174	15	2,845	10	4,113
30,000	3,000	22	2,250	30	1,500	45	750	91	300	228	225	304	150	456	75	906	30	2,215	23	2,917	15	4,268
40,000	4,000	22	3,000	30	2,000	45	1,000	91	400	228	300	305	200	457	100	909	40	2,236	30	2,954	20	4,349
50,000	5,000	22	3,750	30	2,500	45	1,250	91	500	229	375	305	250	457	125	911	50	2,249	38	2,977	25	4,398
60,000	6,000	22	4,500	30	3,000	45	1,500	91	600	229	450	305	300	458	150	913	60	2,258	45	2,992	30	4,432
70,000	7,000	22	5,250	30	3,500	45	1,750	91	700	229	525	305	350	458	175	914	70	2,264	53	3,003	35	4,456
80.000	8,000	22	6,000	30	4,000	45	2,000	91	800	229	600	305	400	458	200	915	80	2,269	60	3,011	40	4,474
90,000	9,000	22	6,750	30	4,500	45	2,250	91	900	229	675	305	450	458	225	915	90	2,272	68	3,017	45	4,488
100,000	10,000	22	7,500	30	5,000	45	2,500	91	1,000	229	750	305	500	458	250	916	100	2,275	75	3,022	50	4,500
200,000	20,000	22	15,000	30	10,000	45	5,000	91	2,000	229	1,500	306	1,000	459	500	918	200	2,288	150	3,046	100	4,551
300,000	30,000	22	22,500	30	15,000	45	7,500	91	3,000	229	2,250	306	1,500	459	750	918	300	2,293	225	3,053	150	4,569
400,000	40,000	22	30,000	30	20,000	45	10,000	91	4,000	229	3,000	306	2,000	459	1,000	919	400	2,295	300	3,057	200	4,578
500,000	50,000	22	37,500	30	25,000	45	12,500	91	5,000	229	3,750	306	2,500	459	1,250	919	500	2,296	375	3,060	250	4,583
600,000	60,000	22	45,000	30	30,000	45	15,000	91	6,000	229	4,500	306	3,000	459	1,500	919	600	2,297	450	3,061	300	4,586
700,000	70,000	22	52,500	30	35,000	45	17,500	91	7,000	229	5,250	306	3,500	459	1,750	919	700	2,298	525	3,062	350	4,589
800,000	80,000	22	60,000	30	40,000	45	20,000	91	8,000	229	6,000	306	4,000	459	2,000	919	800	2,298	600	3,063	400	4,591
900,000	90,000	22	67,500	30	45,000	45	22,500	91	9,000	229	6,750	306	4,500	459	2,250	919	900	2,299	675	3,064	450	4,592

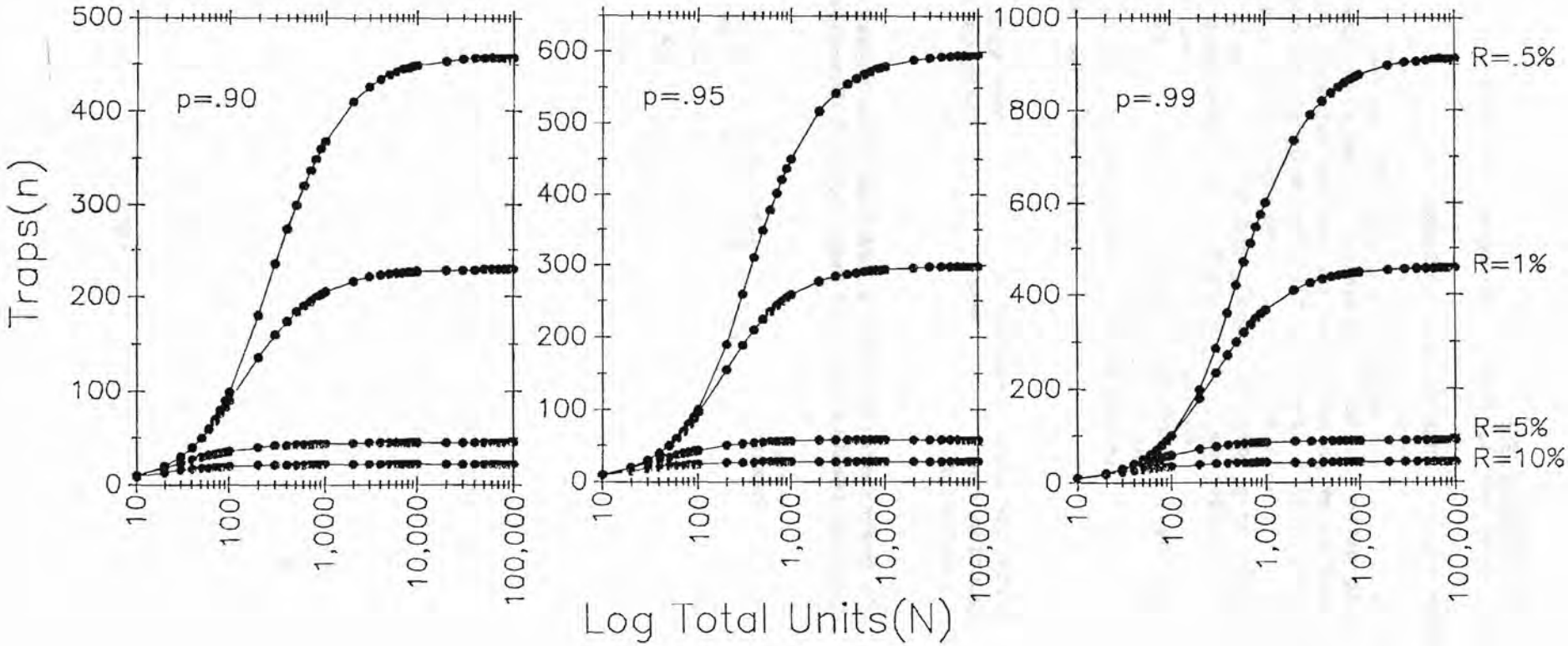
Table 2. 95% Probability.
Number of traps required to detect an infestation at eleven infestation rates (10% to .05%) in a total number of trap units ranging from 100 to 900,000 and at a confidence level of 95%. Listed are the number of infested units detectable and the number of traps required.

Total Units	Infestation Rate (# of Trap Units Infested/Total Trap Units)																							
	10%		7.5%		5%		2.5%		1%		0.75%		0.5%		0.25%		0.1%		0.075%		0.05%	
N	Units	Traps	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n
100	10	25	8	32	5	44	3	69	1	95														
200	20	27	15	35	10	51	5	89	2	155	2	173	1	190										
300	30	27	23	36	15	53	8	98	3	189	2	220	2	259										
400	40	27	30	37	20	54	10	102	4	210	3	252	2	310	1	380								
500	50	28	38	37	25	55	13	105	5	224	4	274	3	349	1	454								
600	60	28	45	37	30	56	15	107	6	235	5	291	3	378	2	518								
700	70	28	53	37	35	56	18	109	7	243	5	303	4	402	2	573								
800	80	28	60	38	40	56	20	110	8	249	6	313	4	421	2	621								
900	90	28	68	38	45	57	23	111	9	254	7	322	5	437	2	662								
1,000	100	28	75	38	50	57	25	112	10	258	8	328	5	450	3	698	1	950						
2,000	200	28	150	38	100	58	50	115	20	277	15	361	10	517	5	901	2	1,552	2	1,728	1	1,900		
3,000	300	28	225	38	150	58	75	116	30	284	23	373	15	542	8	987	3	1,894	2	2,207	2	2,593		
4,000	400	28	300	38	200	58	100	117	40	287	30	379	20	555	10	1,034	4	2,108	3	2,526	2	3,105		
5,000	500	28	375	38	250	58	125	117	50	289	38	382	25	563	13	1,064	5	2,253	4	2,750	3	3,491		
6,000	600	28	450	38	300	58	150	117	60	291	45	385	30	569	15	1,085	6	2,357	5	2,916	3	3,789		
7,000	700	28	525	38	350	58	175	117	70	292	53	387	35	573	18	1,100	7	2,436	5	3,043	4	4,025		
8,000	800	28	600	38	400	58	200	117	80	293	60	388	40	576	20	1,112	8	2,498	6	3,143	4	4,216		
9,000	900	28	675	38	450	58	225	118	90	293	68	389	45	578	23	1,121	9	2,547	7	3,225	5	4,374		
10,000	1,000	28	750	38	500	58	250	118	100	294	75	390	50	580	25	1,128	10	2,587	8	3,292	5	4,506		
20,000	2,000	28	1,500	38	1,000	58	500	118	200	296	150	394	100	589	50	1,162	20	2,781	15	3,619	10	5,176		
30,000	3,000	28	2,250	38	1,500	58	750	118	300	297	225	395	150	592	75	1,173	30	2,850	23	3,738	15	5,430		
40,000	4,000	28	3,000	38	2,000	58	1,000	118	400	297	300	396	200	593	100	1,179	40	2,885	30	3,800	20	5,563		
50,000	5,000	28	3,750	38	2,500	58	1,250	118	500	297	375	396	250	594	125	1,183	50	2,906	38	3,838	25	5,645		
60,000	6,000	28	4,500	38	3,000	58	1,500	118	600	297	450	397	300	595	150	1,185	60	2,921	45	3,863	30	5,701		
70,000	7,000	28	5,250	38	3,500	58	1,750	118	700	297	525	397	350	595	175	1,187	70	2,931	53	3,881	35	5,741		
80,000	8,000	28	6,000	38	4,000	58	2,000	118	800	298	600	397	400	595	200	1,188	80	2,939	60	3,895	40	5,771		
90,000	9,000	28	6,750	38	4,500	58	2,250	118	900	298	675	397	450	596	225	1,189	90	2,945	68	3,906	45	5,795		
100,000	10,000	28	7,500	38	5,000	58	2,500	118	1,000	298	750	397	500	596	250	1,190	100	2,950	75	3,914	50	5,814		
200,000	20,000	28	15,000	38	10,000	58	5,000	118	2,000	298	1,500	398	1,000	597	500	1,193	200	2,972	150	3,953	100	5,901		
300,000	30,000	28	22,500	38	15,000	58	7,500	118	3,000	298	2,250	398	1,500	597	750	1,194	300	2,979	225	3,966	150	5,931		
400,000	40,000	28	30,000	38	20,000	58	10,000	118	4,000	298	3,000	398	2,000	597	1,000	1,195	400	2,983	300	3,973	200	5,945		
500,000	50,000	28	37,500	38	25,000	58	12,500	118	5,000	298	3,750	398	2,500	597	1,250	1,195	500	2,985	375	3,977	250	5,954		
600,000	60,000	28	45,000	38	30,000	58	15,000	118	6,000	298	4,500	398	3,000	597	1,500	1,196	600	2,987	450	3,980	300	5,960		
700,000	70,000	28	52,500	38	35,000	58	17,500	118	7,000	298	5,250	398	3,500	597	1,750	1,196	700	2,988	525	3,981	350	5,964		
800,000	80,000	28	60,000	38	40,000	58	20,000	118	8,000	298	6,000	398	4,000	597	2,000	1,196	800	2,989	600	3,983	400	5,968		
900,000	90,000	28	67,500	38	45,000	58	22,500	118	9,000	298	6,750	398	4,500	597	2,250	1,196	900	2,989	675	3,984	450	5,970		

Table 3. 99% Probability.
Number of traps required to detect an infestation at eleven infestation rates (10% to .05%) in a total number of trap units ranging from 100 to 900,000 and at a confidence level of 99%. Listed are the number of infested units detectable and the number of traps required.

Total Units	Infestation Rate (# of Trap Units Infested/Total Trap Units)																					
	10%		7.5%		5%		2.5%		1%		0.75%		0.5%		0.25%		0.1%		0.075%		0.05%	
N	Units	Traps	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n	d	n
100	10	35	8	44	5	59	3	84	1	99												
200	20	39	15	51	10	72	5	119	2	180	2	190	1	198								
300	30	41	23	54	15	77	8	136	3	235	2	261	2	286								
400	40	41	30	55	20	80	10	146	4	272	3	313	2	360	1	396						
500	50	42	38	56	25	82	13	152	5	300	4	353	3	420	1	487						
600	60	42	45	56	30	83	15	157	6	320	5	383	3	470	2	572						
700	70	42	53	57	35	84	18	160	7	336	5	408	4	511	2	649						
800	80	43	60	57	40	85	20	163	8	349	6	427	4	546	2	720						
900	90	43	68	57	45	85	23	165	9	359	7	444	5	575	2	783						
1,000	100	43	75	57	50	86	25	166	10	367	8	457	5	601	3	841	1	990				
2,000	200	43	150	58	100	88	50	174	20	409	15	527	10	736	5	1,203	2	1,800	2	1,907	1	1,980
3,000	300	43	225	59	150	88	75	176	30	425	23	553	15	791	8	1,375	3	2,353	2	2,612	2	2,861
4,000	400	44	300	59	200	89	100	178	40	433	30	567	20	821	10	1,475	4	2,734	3	3,137	2	3,600
5,000	500	44	375	59	250	89	125	179	50	438	38	576	25	839	13	1,539	5	3,008	4	3,535	3	4,207
6,000	600	44	450	59	300	89	150	179	60	441	45	582	30	852	15	1,584	6	3,214	5	3,843	3	4,707
7,000	700	44	525	59	350	89	175	180	70	443	53	586	35	861	18	1,618	7	3,373	5	4,087	4	5,121
8,000	800	44	600	59	400	89	200	180	80	445	60	589	40	868	20	1,643	8	3,500	6	4,285	4	5,469
9,000	900	44	675	59	450	89	225	180	90	447	68	591	45	873	23	1,664	9	3,603	7	4,449	5	5,764
10,000	1,000	44	750	59	500	89	250	180	100	448	75	593	50	878	25	1,680	10	3,689	8	4,587	5	6,018
20,000	2,000	44	1,500	59	1,000	90	500	181	200	453	150	602	100	898	50	1,758	20	4,111	15	5,285	10	7,379
30,000	3,000	44	2,250	59	1,500	90	750	181	300	455	225	606	150	905	75	1,784	30	4,267	23	5,551	15	7,929
40,000	4,000	44	3,000	59	2,000	90	1,000	181	400	456	300	607	200	908	100	1,798	40	4,348	30	5,690	20	8,225
50,000	5,000	44	3,750	59	2,500	90	1,250	182	500	456	375	608	250	910	125	1,806	50	4,397	38	5,776	25	8,410
60,000	6,000	44	4,500	59	3,000	90	1,500	182	600	456	450	609	300	912	150	1,812	60	4,431	45	5,834	30	8,536
70,000	7,000	44	5,250	59	3,500	90	1,750	182	700	457	525	609	350	913	175	1,816	70	4,455	53	5,876	35	8,628
80,000	8,000	44	6,000	59	4,000	90	2,000	182	800	457	600	609	400	913	200	1,819	80	4,473	60	5,908	40	8,698
90,000	9,000	44	6,750	59	4,500	90	2,250	182	900	457	675	610	450	914	225	1,821	90	4,487	68	5,933	45	8,753
100,000	10,000	44	7,500	59	5,000	90	2,500	182	1,000	457	750	610	500	915	250	1,823	100	4,499	75	5,953	50	8,797
200,000	20,000	44	15,000	59	10,000	90	5,000	182	2,000	458	1,500	611	1,000	917	500	1,831	200	4,550	150	6,045	100	8,999
300,000	30,000	44	22,500	59	15,000	90	7,500	182	3,000	458	2,250	611	1,500	917	750	1,834	300	4,568	225	6,076	150	9,068
400,000	40,000	44	30,000	59	20,000	90	10,000	182	4,000	458	3,000	611	2,000	918	1,000	1,836	400	4,576	300	6,091	200	9,103
500,000	50,000	44	37,500	59	25,000	90	12,500	182	5,000	458	3,750	611	2,500	918	1,250	1,836	500	4,582	375	6,100	250	9,124
600,000	60,000	44	45,000	59	30,000	90	15,000	182	6,000	458	4,500	611	3,000	918	1,500	1,837	600	4,585	450	6,107	300	9,138
700,000	70,000	44	52,500	59	35,000	90	17,500	182	7,000	458	5,250	611	3,500	918	1,750	1,837	700	4,588	525	6,111	350	9,148
800,000	80,000	44	60,000	59	40,000	90	20,000	182	8,000	458	6,000	612	4,000	918	2,000	1,838	800	4,590	600	6,114	400	9,155
900,000	90,000	44	67,500	59	45,000	90	22,500	182	9,000	458	6,750	612	4,500	918	2,250	1,838	900	4,591	675	6,117	450	9,161

Figure 1. Hypergeometric distribution at four infestation rates (R) and three probabilities (R=Units Infested(d)/Total Units(N)).



Project Number: CPB 85.1.1
Project Title: Rearing Edovum puttleri, an Egg Parasite of Leptinotarsa decemlineata
Report Period: October 1, 1987 - September 30, 1988
Project Type: Interim
Project Leaders: P. C. Kingsley and R. James

The objective of our work on Edovum puttleri (EP) has been to develop efficient rearing methods. This is especially important when rearing a non-overwintering species of parasite for inundative releases as part of an integrated pest management program. With the help of the Mission laboratory, and their supply of parasitized material, we have nearly completed this objective. Methods have been developed, and materials have been designed, to efficiently handle large numbers of parasites and host eggs in a commercial rearing operation. Specifically, host eggs can now be removed from leaves, quantified easily, devitalized by irradiation, and glued in large numbers to egg cards for parasitization in oviposition cages. In addition, we have also developed parasitization schedules for synchronous emergence, determined optimal humidities for holding parasitized eggs, and continue to explore host storage techniques.

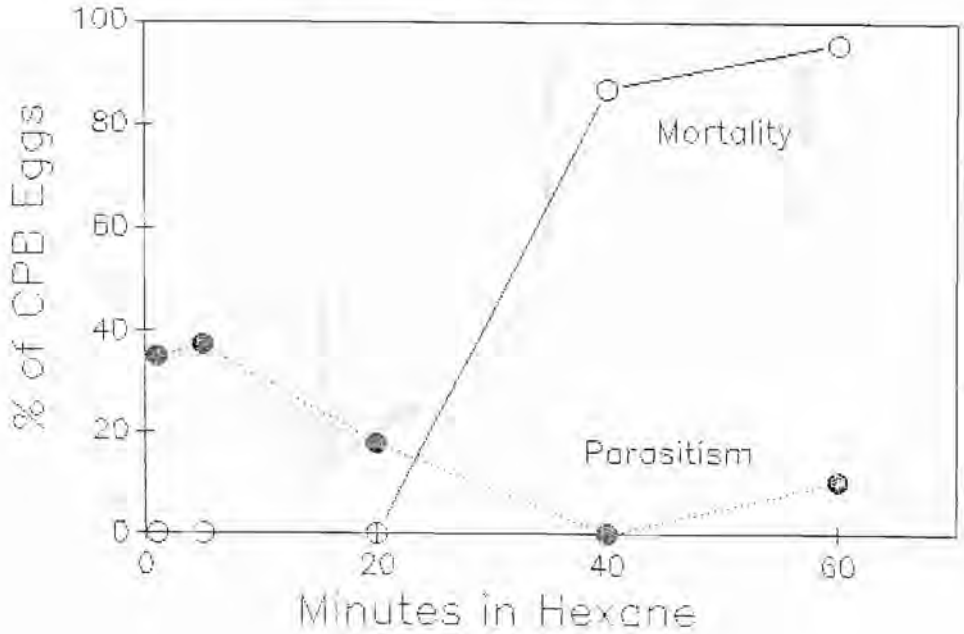
A series of experiments were conducted this fiscal year with the objective of perfecting or reducing the cost of these rearing techniques.

Hexane Wash

To determine time restraints in washing CPB eggs from leaves with hexane, we tested mortality and parasitism of host eggs held at increasing periods in the solvent.

If eggs are exposed to hexane for five minutes or less there will be no significant effects in terms of host mortality or parasitism.

Effects of washing CPB eggs in hexane on the mortality of live eggs and on parasite acceptability.



Kairomone Wash

After hexane extraction, the supernatant was routinely dripped over the host eggs in an attempt to return kairomone removed in the extraction process. This procedure was initiated because early tests showed that this kairomone was, indeed, removed from host eggs, rendering them unsuitable for parasitism. To test if this procedure was necessary in our present extraction scheme, we compared parasitism of eggs rinsed and not rinsed with kairomone in supernatant. Ten groups of eggs from each treatment were randomly glued on a single sting sheet and exposed to parasites for 12 hours. There was no significant difference between treatments.

Treatment	Total Eggs	Mean(SD) % Parasitized	Range
Kairomone Rinse	522	14.6 (7.64)	29.1 - 4.1
No Rinse	420	18.4 (11.51)	43.3 - 9.1

Frozen Egg / Kairomone

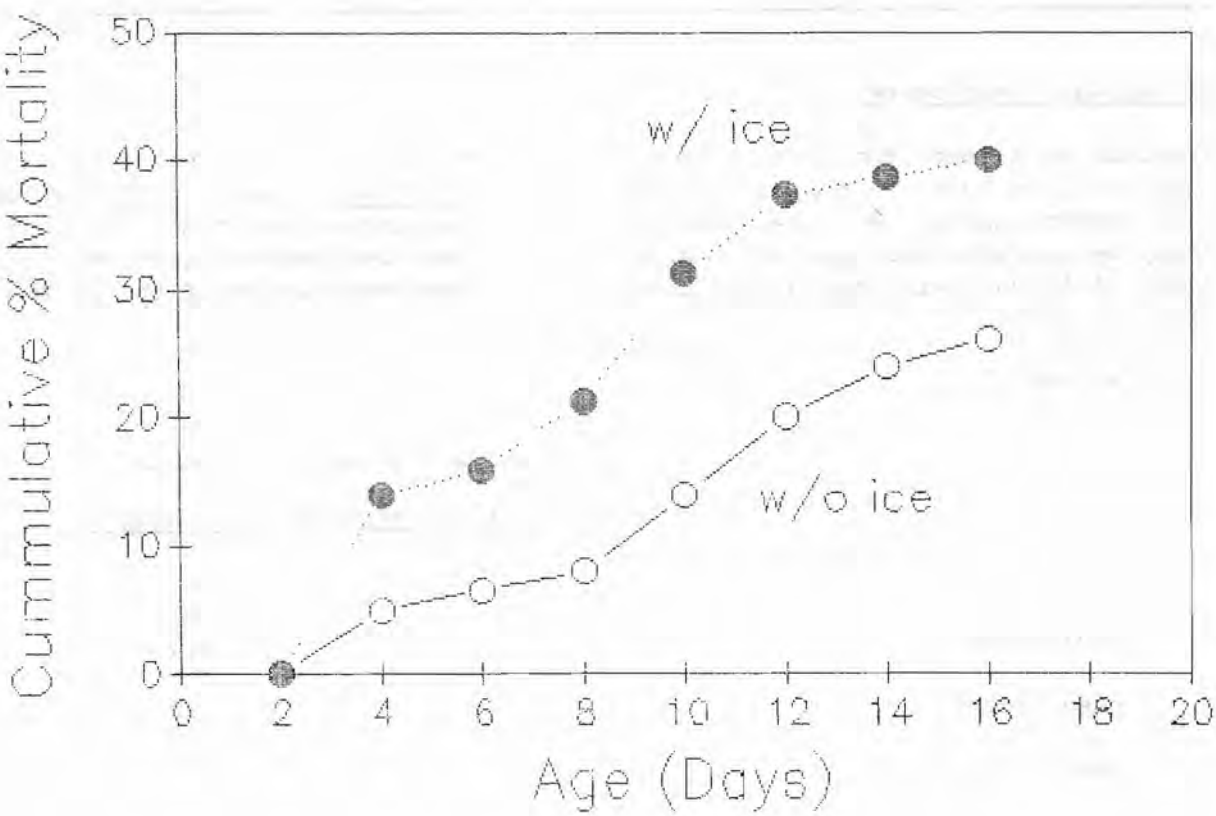
Previous work (see 1985 Interim Report) has shown conclusively that frozen eggs are less suitable for parasitism by Edovum puttleri. One hypothesis for this phenomenon may be a breakdown of host egg kairomone over time. To test this, we compared eggs stored in a freezer for over one year with freshly laid eggs. All eggs were washed with hexane, then kairomone from half the fresh eggs was placed on half the frozen eggs and visa versa, resulting in four treatments. Five reps of each treatment were then placed on a single sting sheet as above.

Treatment Eggs/Kairomone	Total eggs	Mean(SD) % parasitized	Range
Fresh/Fresh	242	24.7 (12.27)	11.7 - 40.9
Fresh/Frozen	261	37.5 (8.53)	28.6 - 44.7
Frozen/Frozen	300	0.6 (1.38)	0.0 - 3.1
Frozen/Fresh	289	1.3 (1.75)	0.0 - 3.2

Mission Shipment Test - Survival

In January Don Vacek and I looked at the effect of using "Blue Ice" to keep parasitized CPB eggs cool during shipping. In Texas parasitized eggs were divided into two groups, one was sent with ice and one without. Upon arrival, we checked for emergence and monitored mortality daily. Those parasitized eggs sent with ice emerged two days later that those without ice. After 16 days the cumulative mortality in the ice treatment was twice that in the no-ice treatment.

Effects of shipping parasitized eggs with and without ice.



Mission Shipment vs Lab Reared - Fecundity

To follow up on the previous experiment, we compared wasps reared from parasitized eggs received from the Mission Laboratory, with F1 wasps reared one generation under our mass rearing conditions. We set up 15 pairs of Otis and 30 pairs of Texas wasps, provided at least 25 eggs daily for a total of 21 days.

The number of progeny per female was significantly lower for the Texas reared wasps, but when only fertile wasps were compared, there was no difference. When females that had survived at least five days were checked for fertility during their experimental lifetime, far more Otis reared females were fertile.

		Progeny/Female				
	N	All	Fertile only	Number infertile	Number fertile	Percent infertile
Otis Reared	15	4.1(2.52)	4.5(2.83)	1	12	7.7
Texas Reared	30	1.4(0.99)	4.0(2.66)	13	9	59.1

The only clear differences between these two treatments was the shipment of the Texas wasps, and rearing conditions prior to shipment. Wasps reared at the Mission Laboratory are sometimes held under cool conditions prior to shipment to delay emergence. Further experimentation is required to identify this reduction in fitness.

Devitalization Alternatives

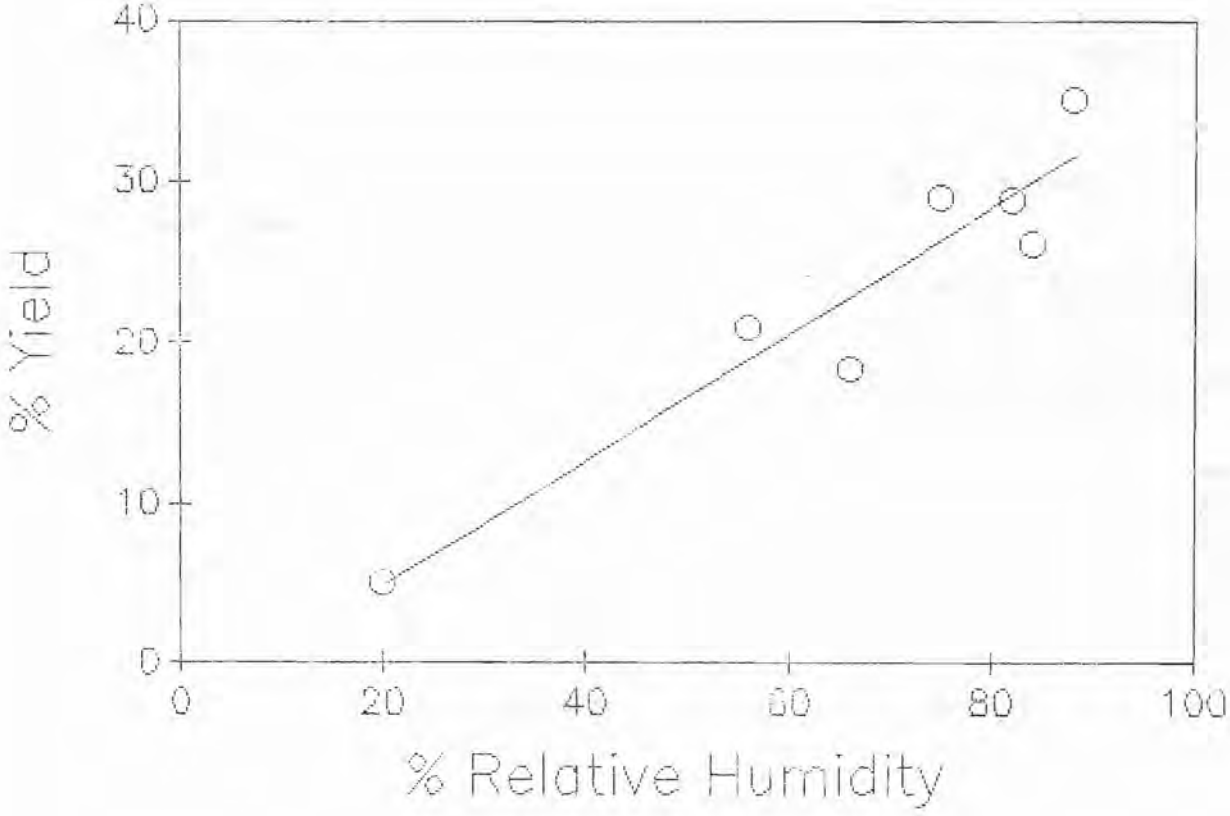
At the present time we use Gamma irradiation to successfully devitalize host CPB eggs for parasitization by EP. Since gamma irradiators are not generally available, we tested alternate methods to achieve the same result. Several chemicals fumigants were tested including formaldehyde, chloroform, hexane, ethyl acetate, and with the help of the Hoboken Laboratory, methyl bromide. Although we were able to determine a dose required to kill CPB eggs with these chemicals, such eggs were not acceptable for parasitism.

Ultraviolet light was also thoroughly tested as an alternative, without success.

Relative Humidity

Host CPB eggs removed from leaves with hexane and subsequently parasitized are apparently susceptible to drying out when reared at low relative humidities. Using various salt solutions we were able to hold parasitized extracted eggs at six relative humidities at 23°C. There were increasing yields with humidity up to 88%, the highest tested.

Average yield of parasites reared at seven relative humidities.



Project Number: CPB 86.1.1
Project Title: Development of an Artificial Diet for Colorado Potato Beetle, Leptinotarsa decemlineata
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leader: O. T. Forrester

An artificial diet was developed for rearing larvae of the Colorado potato beetle (CPB), Leptinotarsa decemlineata (Say) (Table 1). Diet-reared insects compared favorably with those reared on potato foliage in terms of days to pupation, weight and emergence (Tables 2, 3 & 4). The increased number of days required for oviposition and decreased number of eggs produced by CPB fed on the artificial diet is an indication of possible inadequate nutritional quality. Best results were obtained when the artificial diet contained plant powder of either eggplant, Solanum melongena, potato, Solanum tuberosum or silverleaf nightshade, Solanum elaeagnifolium. Adults produced from the diet were fecund when fed on potato foliage; preoviposition period was 6-7 days. Fecundity of diet reared beetles on diet was not reliable.

Rearing was accomplished by placing 10 neonate CPB on 2.2x2.2x1.5 cm cubes of diet in a 7 cm diameter x 1.7 cm deep clear plastic container with friction fitting lids. The bottom of the containers were ventilated with 2.7 cm holes and covered with 40 mesh nylon screen. Containers were held at 23°C to 25°C, 50-60% R.H. and a 15 hour photoperiod. Developing larvae were transferred to fresh diet cubes every 3-4 days. When fourth instar larvae burrowed into the diet, they were placed in 60 ml plastic cups half-full of vermiculite or potting soil for pupation. Most of the rearing problems related to diet have been solved. Contamination of the diet by either mold or bacteria can be eliminated by sanitation. The problems remaining to be solved are containerization, cannibalism and oviposition.

Table 1.

Ingredient	Original	Diet 1	Diet 2
Rolled oats	40.0	10.0	10.0
Raw wheat germ		10.0	10.0
Potato plant powder	20.0	10.0	10.0
Casein	18.0	5.0	5.0
Sucrose	16.0		
Lactalbumin Hydrolysate		15.0	15.0
Torula yeast			30.0
Brewers yeast hydrolysate		15.0	
Pollen substitute		10.0	
Tomato powder		5.0	5.0
Vitamin premix	2.5		
Vanderzant's vitamin mix		3.0	3.0
Vitamin fortification mix		3.0	3.0
Beta sitosterol	0.5	1.0	1.0
Cholesterol	0.5		0.25
Raw linseed oil		1.0	
Wheat germ oil		1.0	1.0
Soybean oil		1.0	1.0
Wesson's salt mix	4.0		
USP 13 no. 2 salt mix		2.0	2.0
Sorbic acid	0.5	0.5	0.5
Methyl paraben	0.5	0.5	0.5
Citric acid		0.5	
Ascorbic acid			0.5
Streptomycin		0.2	0.2
Agar	10.0		
Carrageenan HWG		8.0	8.0
Deionized water	550.0	450.0	450.0

(amounts in gm/ml)

Table 2. Comparison of CPB reared on diet 1 with additions of sucrose, dextrose, ascorbic acid and substitute of torula yeast for brewers yeast.

Diet	n	% survival		Developmental rate		Weight mg		
		pre-pupae	Adult	pre-pupal	adult	pre-pupal	female	male
1	50	82	44	13.0	34.2	146.2	99.6	105.4
Ascorbic acid	50	66	32	12.0	22.0	166.6	121.5	99.8
Torula yeast	50	96	24	12.7	24.1	160.6	102.0	89.9
Sucrose	50	74	36	12.8	23.0	137.3	101.2	99.6
Dextrose	50	52	32	13.3	23.6	133.3	93.9	97.7

Table 3. Comparison of CPB reared on diet 2 with various oils as lipid source.

Diet	n	% survival		Developmental rate		Weight mg		
		pre-pupae	Adult	pre-pupal	adult	pre-pupal	female	male
Wheat germ and soybean	50	86	60.0	11.9	20.7	146.9	104.2	90.2
Wheat germ	50	80	58.0	11.4	21.8	145.5	108.9	81.9
Sesame	50	74	69.9	13.5	23.5	121.3	88.9	69.9
Rice bran	50	82	62.0	12.0	22.5	119.3	80.8	76.8
Raw linseed	50	74	54.5	12.6	23.6	118.8	76.4	62.2
Corn and wheat germ	50	86	66.0	14.4	23.0	108.3	83.4	61.2
Rice bran	50	60	42.0	13.6	24.7	109.3	68.6	61.4

Table 4. Comparison of CPB reared on excised potato foliage and potted potato plants.

	n	% survival		Development		Weight mg.		
		pre-pupae	adult	pre-pupae	adult	pre-pupae	female	male
Excised foliage	50	96	86	8.1	18.1	156.8	121.1	105.1
Potted	50	-	28	-	18.6	-	143.2	115.6

Project Number: GM 78.1.3
Project Title: Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

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

Our main emphasis is development of new and registered materials that may improve treatments of gypsy moth in isolated infestations.

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

Five tender oak seedlings are treated with each test sample and allowed to dry for 3 hours. Twenty newly moulted, 2nd-instar gypsy moth larvae are then introduced onto each plant. Plants with test insects are then held in an environmental chamber at 80°F and 55% RH. Insect mortality and seedling defoliation are recorded over a period of time. Five untreated seedlings are used as a control.

Materials are also tested for their stability when exposed to rainfall and ultraviolet light. Approximately 3 hours following treatment, plants are exposed to rainfall and/or ultraviolet light. They are then dried under a fan if necessary, and a standard bioassay determines effects.

The number of experimental samples has decreased dramatically over the past few years. Today, most of our emphasis is directed at improving the effectiveness of the materials we have. Our main objectives are to improve the effectiveness of materials such as Bacillus thuringiensis (Bt) and Dimilin.

A number of Bt strains from Ecogen were tested in the laboratory against newly moulted, 2nd-instar gypsy moth larvae.

Table 1. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation after a 4 and 6 day exposure to various Bt strains applied at 2 different dosages.

Formulation	BIU 96oz/acre	After 4 days mortality	After 4 days defoliation	After 6 days mortality	After 6 days defoliation
Dipel 8L (oil)	2	3	44	13	53
6587-02	2	11	56	30	66
6587-08	2	0	76	2	94
6587-09	2	18	26	40	48
6587-10	2	9	46	19	52
6587-11	2	18	29	31	52
6587-12	2	31	29	45	44
Dipel 8L (oil)	4	24	24	51	27
6587-02	4	30	22	42	26
6587-08	4	28	29	55	31
6587-09	4	36	24	51	28
6587-10	4	46	19	64	22
6587-11	4	61	7	80	9
6587-12	4	38	23	58	25
CHECK	-	0	82	1	100

Table 2. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation after a 4 and 6 day exposure to various Bt strains applied at 8 BIU/96 oz/acre.

Formulation	After 4 days mortality	After 4 days defoliation	After 6 days mortality	After 6 days defoliation
Dipel 8L (oil)	54	10	89	12
6587-02	22	20	43	24
6587-08	56	14	74	14
6587-09	65	12	86	14
6587-10	54	15	91	23
6587-11	53	15	86	18
6587-12	74	9	88	11
CHECK	0	72	0	80

Table 3. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation after a 4 and 7 day exposure to various Bt strains applied at 12 BIU/192 oz/acre.

Formulation	After 4 days		After 7 days	
	mortality	defoliation	mortality	defoliation
Dipel 8L (oil)	16	30	72	40
6587-02	27	24	45	42
6587-08	12	22	62	36
6587-09	28	28	54	36
6587-10	54	14	85	28
6587-11	31	30	79	34
6587-12	30	24	78	30
CHECK	0	88	2	100

Table 4. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation after 3 and 6 day exposure to various Bt strains applied at 3 dosages.

Formulation	BIU 96oz/acre	After 3 days		After 6 days	
		mortality	defoliation	mortality	defoliation
Dipel 8L (oil)	2	1	50	18	85
	4	1	56	7	60
	8	5	46	20	48
6587-08	2	0	52	5	87
	4	2	48	11	66
	8	8	28	43	38
6587-17	2	3	90	9	95
	4	9	70	18	75
	8	5	70	13	82
6587-18	2	7	38	28	67
	4	4	46	37	52
	8	20	24	29	36
6587-19	2	0	94	0	100
	4	2	68	3	80
	8	4	60	21	70
6587-20	2	0	70	66	99
	4	5	84	16	93
	8	30	80	50	86
CHECK	0	0	82	0	100

Table 5. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following a 4 and 6 day exposure to various Bt strains applied at 8 BIU/192 oz/acre.

Formulation	After 4 days		After 6 days	
	mortality	defoliation	mortality	defoliation
Dipel 8L (oil)	18	38	56	54
6587-02	27	17	59	48
6587-08	21	29	57	48
6587-09	32	28	55	42
6587-10	26	22	75	33
6587-11	30	20	59	30
6587-12	57	9	93	14
6587-17	23	52	52	67
6587-18	44	15	85	21
6587-19	9	74	39	90
6587-20	36	78	75	96
CHECK	0	78	0	96

Table 6. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following a 4 day exposure to various Bt strains applied at 16 BIU/96 oz/acre.

Formulation	Inches rain	After 4 days	
		mortality	defoliation
Dipel 4L (oil)	-	83	17
Dipel 4L (oil)	.10	75	33
Dipel 4L (oil)	.25	81	20
Dipel 8L (oil)	-	73	15
Dipel 8L (oil)	.10	26	51
Dipel 8L (oil)	.25	5	75
6587-34	-	70	21
6587-34	.10	3	88
6587-34	.25	1	89
6587-42	-	91	6
6587-42	.10	22	47
6587-42	.25	12	44
6587-47	-	91	6
6587-47	.10	38	52
6587-47	.25	8	67
CHECK	-	0	98

Table 7. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following a 4 day exposure to various Bt strains with Bond sticker and applied at 16 BIU/96 oz/acre.

Formulation	Percent bond	Inches rain	After 4 days mortality	After 4 days defoliation
Dipel 4L (oil)	2	-	87	5
	2	.1	79	11
	2	.25	74	12
	3	-	81	6
	3	.25	50	20
Dipel 8L (oil)	2	-	74	15
	2	.1	35	28
	2	.25	23	27
	3	-	67	14
	3	.25	19	42
6587-34	2	-	93	4
	2	.1	21	52
	2	.25	12	50
	3	-	72	14
	3	.25	4	84
6587-42	2	-	99	1
	2	.1	71	34
	2	.25	34	39
	3	-	98	1
	3	.25	4	60
6587-47	2	-	99	1
	2	.1	17	52
	2	.25	23	37
	3	-	98	4
	3	.25	15	54
CHECK	-	-	0	98

Based in part on our laboratory work with the various strains, Ecogen selected a strain for development. The new strain was named Condor and was formulated as both oil and aqueous formulations. The formulations were laboratory tested before being field tested in 1988.

Table 8. Percent mortality of 2nd instar gypsy moth larvae and percent seedling defoliation after exposure to seedlings treated with 3 Bt formulations.

Material	Dosage BIU/gal/acre	Mortality after 2 days	Mortality after 3 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 3 days	Defoliation after 4 days
Condor 32 BIU/gal	2	7	9	18	54	56	78
Condor 32 BIU/gal	4	13	21	33	36	50	63
Condor 32 BIU/gal	8	32	40	52	14	25	32
Condor 32 BIU/gal	12	70	81	94	4	5	5
Condor 32 BIU/gal	16	59	79	91	4	7	7
Condor 32 BIU/gal	20	59	76	85	11	12	14
Dipel 8L Aqueous	2	1	2	2	34	58	73
Dipel 8L Aqueous	4	6	8	13	25	46	84
Dipel 8L Aqueous	8	9	13	27	16	32	40
Dipel 8L Aqueous	12	48	66	89	7	16	16
Dipel 8L Aqueous	16	55	74	89	5	11	13
Dipel 8L Aqueous	20	41	52	79	5	13	15
Thuricide 48LV	2	3	8	8	26	42	52
Thuricide 48LV	4	6	12	29	18	24	33
Thuricide 48LV	8	38	56	75	8	15	18
Thuricide 48LV	12	31	48	71	6	13	13
Thuricide 48LV	16	24	54	87	7	9	10
Thuricide 48LV	20	77	92	98	2	2	2
CHECK	-	0	0	0	85	93	94

Table 9. Percent mortality of 2nd-instar gypsy moth larvae and percent seedling defoliation after exposure to seedlings treated with 3 Bt formulations and treated with rainfall.

Material	Dosage BIU/gal/acre	Inches rain	Mortality after 2 days	Mortality after 3 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 3 days	Defoliation after 4 days
Condor 32 BIU/gal	16	-	53	67	84	6	11	15
Condor 32 BIU/gal	16	.10	6	6	8	32	62	67
Condor 32 BIU/gal	20	-	71	82	85	6	8	12
Condor 32 BIU/gal	20	.10	5	6	14	29	48	77
Dipel 8L Aqueous	16	-	49	73	87	7	9	10
Dipel 8L Aqueous	16	.10	10	17	39	20	22	32
Dipel 8L Aqueous	20	-	48	65	75	3	6	8
Dipel 8L Aqueous	20	.10	15	21	42	13	27	33
Thuricide 48LV	16	-	26	51	75	3	8	10
Thuricide 48LV	16	.10	10	18	36	14	22	26
Thuricide 48LV	20	-	35	50	80	4	9	13
CHECK	-	-	0	0	0	88	100	100

Table 10. Percent mortality of 2nd-instar gypsy moth larvae and percent seedling defoliation after exposure to seedlings treated with Condor (32 BIU/gal) and stickers and exposed to rainfall.

Dosage/rate	Inches rain	Sticker	Mortality after 2 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 4 days
16 BIU/gal/acre	-	-	37	74	12	18
16 BIU/gal/acre	.10	-	4	31	33	56
16 BIU/gal/acre	-	2% Bond	36	65	17	27
16 BIU/gal/acre	.10	2% Bond	3	13	33	54
16 BIU/gal/acre	-	2% PLYAC	57	75	7	15
16 BIU/gal/acre	.10	2% PLYAC	5	7	39	54
16 BIU/64oz/acre 1/	-	-	58	72	7	16
16 BIU/64oz/acre	.25	-	2	12	50	86
16 BIU/gal/acre	-	-	35	48	14	31
16 BIU/gal/acre	.25	-	1	8	38	61
16 BIU/gal/acre	-	2% Bond	50	83	7	15
16 BIU/gal/acre	.25	2% Bond	4	12	48	69
16 BIU/gal/acre	-	2% PLYAC	49	76	7	18
16 BIU/gal/acre	.25	2% PLYAC	3	13	32	63
CHECK	-	-	0	0	100	100

1/ Applied undiluted at 64 ounces per acre.

Table 11. Percent mortality of 2nd-instar gypsy moth larvae and percent seedling defoliation after exposure to oak seedlings treated with Condor (32 BIU/gal) and stickers and exposed to rainfall.

Dosage/rate	Inches rain	Sticker	Mortality after 2 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 4 days
16 BIU/64oz/acre 1/	-	-	58	82	6	17
16 BIU/64oz/acre	.10	-	3	5	30	64
16 BIU/64oz/acre	.10	-	0	6	31	61
16 BIU/64oz/acre	.10	5% Bond	0	3	18	67
16 BIU/gal/acre	-	-	47	75	7	22
16 BIU/gal/acre	-	-	39	77	6	18
16 BIU/gal/acre	.10	-	4	5	30	60
16 BIU/gal/acre	.10	-	2	4	27	57
16 BIU/gal/acre	.10	4% Bond	4	11	18	52
16 BIU/gal/acre	.10	5% Bond	5	10	14	42
16 BIU/gal/acre	.10	4% PLYAC	3	3	23	59
16 BIU/gal/acre	.10	5% PLYAC	4	8	18	48
16 BIU/gal/acre	.10	4% NuFilm	7	24	27	61
16 BIU/gal/acre	.10	5% NuFilm	1	10	18	52
CHECK	-	-	0	0	82	94

1/ Applied undiluted at 64 ounces per acre.

Table 12. Percent mortality of 2nd-instar gypsy moth larvae and percent seedling defoliation after exposure to seedlings treated with 3 Bt formulations.

Material	Dosage BIU/gal/acre	Mortality after 2 days	Mortality after 3 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 3 days	Defoliation after 4 days
Condor 64 BIU/gal	2	1	3	10	36	60	65
Condor 64 BIU/gal	4	4	8	17	25	46	67
Condor 64 BIU/gal	8	7	12	29	20	39	49
Condor 64 BIU/gal	12	7	8	28	30	38	43
Condor 64 BIU/gal	16	10	15	28	16	29	46
Condor 64 BIU/gal	20	9	12	23	15	27	39
Dipel 8L Aqueous	2	4	4	15	32	49	65
Dipel 8L Aqueous	4	2	7	21	22	35	48
Dipel 8L Aqueous	8	15	28	49	21	26	38
Dipel 8L Aqueous	12	32	40	66	13	14	20
Dipel 8L Aqueous	16	20	26	40	10	11	21
Dipel 8L Aqueous	20	33	47	64	6	10	15
Thuricide 48LV	2	3	3	7	22	32	44
Thuricide 48LV	4	21	33	44	18	28	37
Thuricide 48LV	8	9	27	52	7	11	15
Thuricide 48LV	12	32	27	55	6	7	10
Thuricide 48LV	16	12	17	46	9	10	13
Thuricide 48LV	20	24	42	72	5	8	10

Table 13. Percent mortality of 2nd-instar gypsy moth larvae and percent seedling defoliation after exposure to seedlings treated with Condor (64BIU/gal) and sticker and exposed to rainfall.

Dosage/rate	Inches rain	Sticker	Mortality after 2 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 4 days
16 BIU/32oz/acre 1/	-	-	33	58	7	16
16 BIU/32oz/acre	-	-	23	69	10	28
16 BIU/32oz/acre	.10	-	0	7	74	88
16 BIU/32oz/acre	.25	-	0	5	50	86
12 BIU/gal/acre	-	-	10	22	21	65
12 BIU/gal/acre	-	-	32	72	17	28
12 BIU/gal/acre	.10	-	2	28	44	65
12 BIU/gal/acre	.10	-	0	0	75	95
16 BIU/gal/acre	-	-	4	27	11	34
16 BIU/gal/acre	-	-	48	85	8	18
16 BIU/gal/acre	-	-	30	63	7	21
16 BIU/gal/acre	.10	-	0	36	38	81
16 BIU/gal/acre	.10	-	0	12	42	72
16 BIU/gal/acre	.10	-	3	17	43	82
16 BIU/gal/acre	-	2% Bond	25	67	12	27
16 BIU/gal/acre	.10	2% Bond	1	16	43	84
16 BIU/gal/acre	-	2% PLYAC	9	65	18	45
16 BIU/gal/acre	.10	2% PLYAC	2	10	49	82
16 BIU/gal/acre	-	2% NuFilm	16	67	16	41
16 BIU/gal/acre	.10	2% NuFilm	0	12	36	76
20 BIU/gal/acre	-	-	15	36	36	31
20 BIU/gal/acre	-	-	55	96	4	8
20 BIU/gal/acre	.10	-	0	13	48	74
20 BIU/gal/acre	.10	-	0	3	82	95
Check	-	-	0	0	96	100

1/ Applied undiluted at 32 ounces per acre.

Table 14. Percent mortality of 2nd-instar gypsy moth larvae and percent seedling defoliation after exposure to oak seedlings treated with 4 Bt formulations.

Material	Dosage BIU/gal/acre	Mortality after 2 days	Mortality after 3 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 3 days	Defoliation after 4 days
Condor 64 BIU/gal	12	30	53	68	4	10	14
Otis Lab Sample	16	26	53	74	7	10	10
	20	23	40	69	4	8	18
Condor 64 BIU/gal	12	17	35	66	8	15	19
Texas Sample	16	15	26	45	18	24	46
	20	28	44	67	10	16	22
Dipel 8L Oil	12	0	1	7	33	44	66
	16	15	28	43	17	26	38
	20	18	29	51	5	8	17
Dipel 8L Aqueous	12	20	35	48	9	13	20
	16	44	56	86	4	9	11
	20	43	53	78	2	7	9
Thuricide 48LV	12	19	45	78	2	3	3
	16	12	37	79	3	4	5
	20	42	67	83	2	2	2
Check	-	0	0	0	98	100	100

Table 15. Percent mortality of 2nd-instar gypsy moth larvae and percent seedling defoliation after exposure to oak seedlings treated with Bt.

Material	Dosage BIU/gal/acre	Mortality after 2 days	Mortality after 3 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 3 days	Defoliation after 4 days
Condor 64 BIU/gal/Otis	2	1	2	16	16	40	48
Condor 64 BIU/gal/TX	2	2	5	34	23	55	60
Dipel 8L Aqueous	2	4	10	42	12	36	40
Dipel 8L Oil	2	0	3	6	45	68	78
Thuricide 48LV	2	3	13	40	9	42	52
Condor 64 BIU/gal/Otis	4	3	4	24	26	50	62
Condor 64 BIU/gal/TX	4	5	6	21	21	44	46
Dipel 8L Aqueous	4	5	9	36	13	38	48
Dipel 8L Oil	4	0	0	0	44	70	85
Thuricide 48LV	4	10	18	45	7	26	30
Condor 64 BIU/gal/Otis	8	4	5	22	16	42	48
Condor 64 BIU/gal/TX	8	7	11	37	18	34	40
Dipel 8L Aqueous	8	12	29	56	6	22	24
Dipel 8L Oil	8	1	1	4	30	56	80
Thuricide 48LV	8	19	59	89	4	10	10
Check	-	0	0	0	84	100	100

Table 16. Percent larval mortality and seedling defoliation following exposure of 2nd-instar gypsy moth larvae to oak seedlings treated with various Bt formulations at 16 BIU/gallon/acre.

Formulation	Mortality after			Defoliation after		
	2 days	3 days	4 days	2 days	3 days	4 days
Condor 64 BIU/gal Otis 1/	27	59	86	12	22	25
Condor 64 BIU/gal TX 2/	17	28	59	15	32	35
Condor 32 BIU/gal	39	57	69	6	24	34
Thuricide 48LV	26	68	88	4	5	5
Dipel 8L Oil (Old Std)	37	63	92	4	7	8
Dipel 8L Oil (New Std)	23	36	54	8	21	25
Dipel 8L Aqueous	45	68	84	4	7	7
Dipel 12L OIl (New Std)	2	3	18	10	32	43
Check	0	0	0	83	87	93

1/ Original sample of Condor 64 BIU/gallon received at Otis
2/ A sample of Condor 64 BIU/gallon used at Mission, TX in January, 1988

Table 17. Percent larval mortality and seedling defoliation following exposure of 2nd-instar gypsy moth larvae to oak seedlings treated with Condor at 16 BIU/gallon/acre, dried for various periods of time, then exposed to .10 inches of rainfall.

Formulation	Hours drying time	Rain .10 in.	Mortality after 2 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 4 days
Condor 64 Otis 1/	2	-	21	69	22	28
Condor 64 Otis	2	yes	0	0	47	82
Condor 64 Texas 2/	2	-	38	73	14	24
Condor 64 Texas	2	yes	1	14	40	69
Condor 64 Otis	4	-	24	57	9	48
Condor 64 Otis	4	yes	2	16	29	67
Condor 64 Texas	4	-	43	80	4	12
Condor 64 Texas	4	yes	2	8	25	73
Condor 64 Otis	6	-	30	64	6	20
Condor 64 Otis	6	yes	0	5	42	66
Condor 64 Texas	6	-	55	75	5	12
Condor 64 Texas	6	yes	0	0	33	76
Condor 64 Otis	24	-	71	87	4	7
Condor 64 Otis	24	yes	5	10	50	85
Condor 64 Texas	24	-	40	84	5	20
Condor 64 Texas	24	yes	2	3	35	72
Condor 64 Otis	48	-	29	54	9	35
Condor 64 Otis	48	yes	1	31	22	57
Condor 64 Texas	48	-	39	75	8	33
Condor 64 Texas	48	yes	0	21	31	70
Check	2	-	0	0	84	99
Check	4	-	0	0	82	100
Check	6	-	0	0	82	95
Check	24	-	0	0	95	100
Check			1	1	82	96

1/ Original sample of Condor 64 BIU/gallon received at Otis.
2/ A sample of Condor 64 BIU/gallon used at Mission, TX in January, 1988.

Table 18. Percent larval mortality and seedling defoliation following exposure of 2nd-instar gypsy moth larvae to oak seedlings treated with various Bt formulations at 12 BIU/gallon/acre.

Formulation	Mortality after			Defoliation after		
	2 days	3 days	4 days	2 days	3 days	4 days
Condor 64 BIU/gal Otis 1/	6	14	41	30	60	64
Condor 64 BIU/gal Texas 2/	6	11	35	17	46	60
Condor 32 BIU/gal	35	48	74	10	30	32
Thuricide 48LV	10	21	66	5	7	7
Dipel 8L Oil (Old Std)	2	3	9	50	72	84
Dipel 8L Oil (New Std)	2	5	29	21	50	75
Dipel 8L Aqueous	11	21	45	15	38	46
Dipel 12L Oil (New Std)	0	0	12	42	80	89
Check	0	0	0	92	100	100

1/ Original sample of Condor 64 BIU/gallon received at Otis.
2/ A sample of Condor 64 BIU/gallon used at Mission, TX in January, 1988.

Table 19. Percent larval mortality and seedling defoliation following exposure of 2nd-instar gypsy moth larvae to oak seedlings treated with Condor at 16 BIU/quart/acre and sticker, dried for various periods of time, then exposed to .10 inches of rainfall.

Hours drying time	Sticker	Rain .10 in	Mortality after			Defoliation after		
			2 days	3 days	4 days	2 days	3 days	4 days
2	-	-	87	98	99	1	2	2
2	-	yes	0	0	4	62	82	84
2	2% PLYAC	-	66	78	87	7	9	15
2	2% PLYAC	yes	0	1	2	76	89	92
2	2% BOND	-	69	84	92	4	4	6
4	2% BOND	yes	1	1	10	68	79	83
4	2% PLYAC	yes	0	0	3	81	97	99
4	2% BOND	yes	2	3	6	61	83	88
6	-	yes	0	1	4	65	95	98
6	2% PLYAC	yes	0	3	17	59	80	86
6	2% BOND	yes	3	7	16	64	79	88
24	-	-	25	41	67	24	46	46
24	-	yes	0	3	9	72	97	99
24	2% PLYAC	yes	1	1	12	82	89	89
24	2% BOND	yes	1	5	13	50	80	81
48	-	-	24	57	72	14	28	41
48	-	yes	3	7	27	51	66	76
48	2% PLYAC	yes	3	11	23	49	50	78
48	2% BOND	yes	2	5	9	57	80	94
2 1/	-	-	0	0	0	95	100	100
24 1/	-	-	0	0	1	98	100	100
48 1/	-	-	0	0	0	99	100	100

1/ Untreated checks

Table 20. Percent mortality and seedling defoliation following exposure of 2nd-instar gypsy moth larvae to oak seedlings treated with Condor at 16 BIU/gallon/acre and sticker, dried for various periods of time, then exposed to .10 inches of rainfall.

Hours drying time	Sticker	Rain .10 in.	Mortality after 2 days	Mortality after 3 days	Mortality after 4 days	Defoliation after 2 days	Defoliation after 3 days	Defoliation after 4 days
2	-	-	16	36	81	31	60	60
2	-	yes	0	0	5	64	89	89
2	2% PLYAC	yes	1	1	4	60	92	94
2	2% BOND	yes	2	5	17	50	78	78
4	-	yes	0	1	2	83	93	95
4	2% PLYAC	yes	7	9	16	68	84	84
4	2% BOND	yes	4	4	26	41	65	68
6	-	yes	3	3	3	77	91	91
6	2% PLYAC	yes	2	3	17	60	79	81
6	2% BOND	yes	0	1	8	76	88	88
24	-	-	20	31	47	18	38	48
24	-	yes	5	11	27	59	82	90
24	2% PLYAC	yes	2	6	22	48	66	83
24	2% BOND	yes	4	11	31	34	57	72
48	-	-	13	20	33	34	46	52
48	-	yes	4	10	35	32	52	63
48	2% PLYAC	yes	4	5	18	54	68	79
48	2% BOND	yes	0	5	23	54	70	83
48 1/	-	-	0	0	0	100	100	100

1/ Untreated check

The Table 20 test was conducted with store supplied distilled water. Laboratory distilled water was used in the Table 19 test.

Table 21. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with Condor (oil) and Condor (aqueous) at 20 BIU/gallon/acre and then exposed to rainfall.

Material	Inches rain	Percent mortality			Percent defoliation		
		4 days	5 days	6 days	4 days	5 days	6 days
Condor (oil)	-	27	54	69	66	68	76
Condor (oil)	.1	0	1	1	100		
Condor (aqueous)	-	19	49	69	44	59	64
Condor (aqueous)	.1	9	44	56	70	74	85
Control	-	0	1	1	100		

Table 22. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with Bt at 20 BIU/gallon/acre and then exposed to rainfall.

Material	Inches rain	Percent mortality			Percent defoliation		
		2 days	3 days	4 days	2 days	3 days	4 days
Dipel 8L	-	49	64	72	6	11	19
Dipel 8L	.1	2	3	3	40	74	90
Dipel 8AF	-	56	78	87	5	6	8
Dipel 8AF	.1	28	37	49	18	24	35
EC198-15	-	79	82	93	2	2	2
EC198-15	.1	11	24	40	29	32	45
EC178-16	-	66	81	87	2	4	4
EC178-16	.1	13	17	26	25	41	2
EC178-17	-	77	92	94	1	2	2
EC178-17	.1	2	5	5	48	78	88
EC178-18	-	49	61	65	9	11	19
EC178-18	.1	6	12	12	27	49	64
EC178-19	-	70	85	89	2	4	5
EC178-19	.1	2	2	2	48	72	85
EC178-20	-	72	80	86	2	3	4
EC178-20	.1	0	1	1	56	88	96
Control	-	0	0	0	46	95	100
Control	.1	0	0	0	80	100	

Condor was field tested on 50 acre plots during 1988. Results were similar to those of Dipel 8L.

Novo Laboratories, Inc., introduced 2 new Bt formulations for testing against gypsy moth. Foray 48B and Biobit (32 BIU/gal) were tested in the laboratory against laboratory reared gypsy moth larvae. Foray has been identified as a forestry formulation and Biobit for agricultural row crops.

Table 23. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 day exposure to seedlings treated with Foray 48B and Dipel 8L.

Material	Dosage BIU/128oz/acre	Percent mortality	Percent defoliation
Foray 48B	4	46	36
Foray 48B	8	78	22
Foray 48B	12	82	15
Foray 48B	16	91	6
Foray 48B	20	95	5
Dipel 8L	4	2	86
Dipel 8L	8	7	68
Dipel 8L	12	8	61
Dipel 8L	16	48	38
Dipel 8L	20	43	22
Control	-	0	100

Table 24. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 day exposure to seedlings treated with Biobit 32B and Dipel 8L.

Material	Dosage BIU/128oz/acre	Percent mortality	Percent defoliation
Biobit	4	34	25
Biobit	8	68	16
Biobit	12	80	3
Biobit	16	84	7
Biobit	20	91	5
Dipel 8L	4	1	82
Dipel 8L	8	8	63
Dipel 8L	12	17	46
Dipel 8L	16	39	42
Dipel 8L	20	45	38
Control	-	0	90

Table 25. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 day exposure to seedlings treated with Bt at 20 BIU/gallon/acre and then exposed to 1.0 inches of rain 3 hours after treatment.

Material	Rain	Percent mortality	Percent defoliation
Foray 48B	-	97	2
Foray 48B	1.0	50	29
Biobit 32B	-	82	3
Biobit 32B	1.0	50	5
Dipel 8L	-	57	18
Dipel 8L	1.0	0	40
Control	-	0	99

Table 26. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with 4 Bt formulations at 20 BIU/gallon/acre and then exposed to rainfall.

Material	Inches rain	Percent mortality		Percent mortality	
		2 days	4 days	2 days	4 days
Foray	-	65	99	3	3
Foray	.25	39	83	10	25
Foray	.50	9	78	21	31
Foray	2.00	7	48	26	32
Biobit	-	51	96	5	7
Biobit	.25	50	83	3	3
Biobit	.50	44	98	7	12
Biobit	2.00	20	87	9	13
Dipel 8L	-	50	90	5	7
Dipel 8L	.25	1	33	18	40
Dipel 8L	.50	3	16	28	66
Dipel 8L	2.00	0	16	30	62
Dipel 8AF	-	39	98	5	6
Dipel 8AF	.25	9	57	6	19
Dipel 8AF	.50	7	56	27	38
Dipel 8AF	2.00	1	18	28	52
Control	-	0	0	54	94
Control	2.00	0	0	80	100

Table 27. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with Foray (Bt) at various dosages.

Dosage/rate	Percent mortality				Percent defoliation			
BIU/gal/acre	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days
16	2	35	56	70	2	4	7	12
24	12	65	93	99	1	2	2	2
30	15	80	93	100	1	1	1	1
35	16	63	93	100	1	1	2	2
40	16	61	87	100	2	2	2	2
48	13	67	93	99	1	2	2	2
Control	0	0	0	0	53	90	96	100

Table 28. Percent mortality of 3rd- and 4th-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with 4 Bt formulations at 20 BIU/gallon/acre.

		Percent mortality			Percent defoliation		
Material	Instar	2 days	3 days	4 days	2 days	3 days	4 days
Foray	III	62	82	96	2	2	2
	IV	44	73	91	4	8	8
Biobit	III	38	75	94	2	2	2
	IV	29	62	90	2	2	2
Dipel 8L	III	23	33	71	5	8	8
	IV	23	41	71	17	17	32
Dipel 8AF	III	42	72	93	2	4	4
	IV	47	58	83	11	14	24
Control	III	0	0	0	100		
	IV	0	0	0	100		

Foray has been registered for gypsy moth control and will be used in a number of State suppression programs in 1989.

Three Bt formulations from Mycogen Corp. were tested in the laboratory.

Table 29. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to small oaks treated with various Bt formulations.

Formulation	Dosage/Rate	After 2 days		After 4 days	
		Mortality	Defoliation	Mortality	Defoliation
MYX-5411	8 BIU/96 oz.	2	6	27	8
MYX-5411	12 BIU/96 oz.	3	5	28	5
MYX-7275	3 BIU/gallon	0	20	32	30
MYX-7275	6 BIU/gallon	5	16	35	28
Dipel 8L Aqueous	8 BIU/96 oz.	39	8	84	12
Dipel 8L Aqueous	12 BIU/96 oz.	35	6	69	12
Dipel 8L Oil	8 BIU/96 oz.	13	17	47	34
Dipel 8L Oil	12 BIU/96 oz.	26	7	68	21
Thuricide 48LV	8 BIU/96 oz.	52	3	90	4
Thuricide 48LV	12 BIU/96 oz.	25	4	83	5
Check	-	0	70	0	88

Table 30. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 and 6 day exposure to seedlings treated with 4 Bt formulations.

Material	Dosage/rate BIU/gal/acre	Percent mortality		Percent defoliation	
		4 days	6 days	4 days	6 days
MYX-9849 1/	1	26	61	46	69
	3	60	88	20	25
	6	88	100	5	7
Thuricide 48LV	1	30	86	34	26
	3	41	92	15	16
	6	61	83	7	37
Dipel 8L	1	3	9	95	99
	3	5	20	88	92
	6	12	45	57	58
Dipel 8AF	1	5	25	82	93
	3	13	48	71	84
	6	36	69	41	49
Control	-	0	1	97	100

1/ Used as a 6 BIU per gallon formulation.

Table 31. Percent mortality of 3rd-instar gypsy moth larvae and seedling defoliation following a 4 and 6 day exposure to seedlings treated with 3 Bt formulations.

Material	Dosage/rate BIU/gal/acre	Percent mortality 4 days	Percent mortality 6 days	Percent defoliation 4 days	Percent defoliation 6 days
MYX-9894 1/	12	31	65	28	28
	16	30	81	33	33
	20	27	75	32	32
MYX-9849 2/	12	25	73	22	22
	16	33	82	33	33
	20	49	93	21	21
Dipel 8AF	12	36	92	10	10
	16	47	95	12	12
	20	44	98	32	32
Thuricide 48LV	12	48	74	7	7
	16	64	99	5	5
	20	54	98	7	9
Control	-	0	0	100	

1/ MYX-9849 used as a 48 BIU per gallon formulation.
2/ MYX-9849 used as a 32 BIU per gallon formulation.

Table 32. Percent mortality of 3rd-instar gypsy moth larvae and seedling defoliation following a 4 and 5 day exposure to seedlings treated with 2 Bt formulations.

Material	Dosage/rate BIU/gal/acre	Percent mortality 4 days	Percent mortality 6 days	Percent defoliation 4 days	Percent defoliation 6 days
MYX-9849 1/	12	17	42	38	38
	16	32	58	17	26
	20	16	47	34	34
Dipel 8AF	12	11	48	30	30
	16	28	86	28	28
	20	39	95	22	22
Control	-	0	0	100	

1/ MYX-9849 used as a 64 BIU per gallon formulation.

Table 33. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 and 7 day exposure to seedlings treated with 3 Bt formulations at 16 BIU/gallon/acre and exposed to rainfall.

Material	Inches rain	Sticker	Percent mortality 4 days	Percent mortality 7 days	Percent defoliation 4 days	Percent defoliation 7 days
MYX-9849 1/	-	-	33	90	40	42
	1.0	-	7	48	67	67
	1.0	2% Bond	42	87	30	35
MYX-9849 2/	-	-	43	89	23	33
	1.0	-	20	64	33	53
	1.0	2% Bond	50	97	31	42
MYX-9849 3/	-	-	25	87	30	51
	1.0	-	5	66	74	79
	1.0	2% Bond	37	90	29	43
Dipel 8AF	-	-	50	95	23	29
	1.0	-	22	75	49	63
	1.0	2% Bond	32	84	29	37
Thuricide 48LV	-	-	59	100	8	8
	1.0	-	14	49	51	57
	1.0	2% Bond	21	77	30	31
Control	-	-	0	0	94	100

1/ MYX-9849 used as a 32 BIU per gallon formulation.
2/ MYX-9849 used as a 48 BIU per gallon formulation.
3/ MYX-9849 used as a 64 BIU per gallon formulation.

Table 34. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 and 6 day exposure to seedlings treated with MYX-9849 with Bond and exposed to rainfall.

Sticker	Inches rain	Percent mortality		Percent defoliation	
		4 days	6 days	4 days	6 days
-	-	56	76	24	24
-	1.0	11	52	44	58
.5% Bond	1.0	60	95	20	26
1% Bond	1.0	64	93	17	20
2% Bond	1.0	71	84	21	23
-	2.0	8	40	48	52
.5% Bond	2.0	47	90	28	41
1% Bond	2.0	23	66	23	24
2% Bond	2.0	75	99	24	24
-	3.0	17	40	23	36
.5% Bond	3.0	48	74	20	30
1% Bond	3.0	37	82	23	24
2% Bond	3.0	43	78	12	17
Control	-	0	0	72	100

Some experimental formulations of Thuricide 32 and 48LV were tested in the laboratory.

Table 35. Percent larval mortality and seedling defoliation following seedling spraying with various Bt formulations and exposure of 2nd-instar gypsy moth larvae.

Formulation	Dosage BIU/gal/acre	Mortality after 1 day	Mortality after 2 days	Mortality after 3 days	Mortality after 4 days	Defoliation after 1 day	Defoliation after 2 days	Defoliation after 3 days	Defoliation after 4 days
Thuricide 32-123-126	4	2	17	25	65	2	15	28	28
Thuricide 32-123-126	8	0	8	16	35	3	9	22	33
Thuricide 32-124-36	4	0	11	26	84	3	6	14	14
Thuricide 32-124-36	8	0	28	48	68	4	6	6	9
Thuricide 32-125-116	4	0	9	29	59	3	19	22	26
Thuricide 32-135-116	8	0	11	38	64	4	5	7	7
Thuricide 32-126-136	4	0	3	14	62	4	23	34	36
Thuricide 32-126-136	8	0	8	30	52	4	8	8	19
Thuricide 48-123-12a	4	1	12	16	43	4	20	36	45
Thuricide 48-123-12a	8	0	4	17	49	3	10	17	27
Thuricide 48-124-3a	4	0	4	20	73	3	9	15	18
Thuricide 48-124-3a	8	0	22	56	84	3	3	3	4
Thuricide 48-125-11a	4	0	0	11	47	3	18	28	40
Thuricide 48-125-11a	8	1	17	28	52	3	5	5	9
Thuricide 48-126-13a	4	4	7	23	64	3	7	14	17
Thuricide 48-126-13a	8	1	12	50	90	2	4	7	7
Dipel 8L Aqueous	4	0	1	6	39	4	10	30	38
Dipel 8L Aqueous	8	1	26	43	56	3	6	10	19
Dipel 8L Oil	4	0	0	0	3	10	40	70	86
Dipel 8L Oil	8	1	5	88	10	8	33	64	77
Check	-	0	1	1	1	29	80	89	100

Table 34. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 and 6 day exposure to seedlings treated with MYX-9849 with Bond and exposed to rainfall.

Sticker	Inches rain	Percent mortality		Percent defoliation	
		4 days	6 days	4 days	6 days
-	-	56	76	24	24
-	1.0	11	52	44	58
.5% Bond	1.0	60	95	20	26
1% Bond	1.0	64	93	17	20
2% Bond	1.0	71	84	21	23
-	2.0	8	40	48	52
.5% Bond	2.0	47	90	28	41
1% Bond	2.0	23	66	23	24
2% Bond	2.0	75	99	24	24
-	3.0	17	40	23	36
.5% Bond	3.0	48	74	20	30
1% Bond	3.0	37	82	23	24
2% Bond	3.0	43	78	12	17
Control	-	0	0	72	100

Some experimental formulations of Thuricide 32 and 48LV were tested in the laboratory.

Table 35. Percent larval mortality and seedling defoliation following seedling spraying with various Bt formulations and exposure of 2nd-instar gypsy moth larvae.

Formulation	Dosage BIU/gal/acre	Mortality after 1 day	Mortality after 2 days	Mortality after 3 days	Mortality after 4 days	Defoliation after 1 day	Defoliation after 2 days	Defoliation after 3 days	Defoliation after 4 days
Thuricide 32-123-126	4	2	17	25	65	2	15	28	28
Thuricide 32-123-126	8	0	8	16	35	3	9	22	33
Thuricide 32-124-36	4	0	11	26	84	3	6	14	14
Thuricide 32-124-36	8	0	28	48	68	4	6	6	9
Thuricide 32-125-116	4	0	9	29	59	3	19	22	26
Thuricide 32-135-116	8	0	11	38	64	4	5	7	7
Thuricide 32-126-136	4	0	3	14	62	4	23	34	36
Thuricide 32-126-136	8	0	8	30	52	4	8	8	19
Thuricide 48-123-12a	4	1	12	16	43	4	20	36	45
Thuricide 48-123-12a	8	0	4	17	49	3	10	17	27
Thuricide 48-124-3a	4	0	4	20	73	3	9	15	18
Thuricide 48-124-3a	8	0	22	56	84	3	3	3	4
Thuricide 48-125-11a	4	0	0	11	47	3	18	28	40
Thuricide 48-125-11a	8	1	17	28	52	3	5	5	9
Thuricide 48-126-13a	4	4	7	23	64	3	7	14	17
Thuricide 48-126-13a	8	1	12	50	90	2	4	7	7
Dipel 8L Aqueous	4	0	1	6	39	4	10	30	38
Dipel 8L Aqueous	8	1	26	43	56	3	6	10	19
Dipel 8L Oil	4	0	0	0	3	10	40	70	86
Dipel 8L Oil	8	1	5	88	10	8	33	64	77
Check	-	0	1	1	1	29	80	89	100

Table 36. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation when exposed to 8 Thuricide formulations applied to foliage with Dipel as a standard.

Formulation	Dosage BIU/gal/acre	Percent mortality 1 day	3 days	5 days	Percent defoliation 1 day	3 days	5 days
Dipel 8L	4	1	6	37	12	29	46
Dipel 8L	8	0	0	20	8	48	55
Dipel 8AF	4	2	9	90	4	15	22
Dipel 8AF	8	0	8	89	4	15	22
T48LV-35-123-12a	4	0	61	100	3	5	9
T48LV-35-123-12a	8	1	15	43	4	16	33
T48LV-35-124-3a	4	1	38	94	2	4	5
T48LV-35-124-3a	8	1	48	96	1	4	6
T48LV-35-125-11a	4	0	38	94	3	8	16
T48LV-35-125-11a	8	0	12	81	3	16	21
T48LV-35-126-13a	4	0	50	99	2	5	6
T48LV-35-126-13a	8	2	47	92	2	6	13
T32LV-35-123-12b	4	1	25	93	4	12	15
T32LV-35-123-12b	8	1	24	85	4	12	28
T32LV-35-126-13b	4	0	36	97	5	7	9
T32LV-35-126-13b	8	0	42	89	5	7	13
T32LV-35-124-3b	4	0	28	94	4	7	13
T32LV-35-124-3b	8	0	44	97	2	6	8
T32LV-35-125-11b	4	0	25	90	4	14	21
T32LV-35-125-11b	8	0	19	90	4	9	18
Control	-	0	1	1	28	90	100

The standard Thuricide 48LV formulation was tested at a number of dosages against 2nd-instar, laboratory reared gypsy moth larvae. Each test was replicated each day for 4 days for a total of 20 replications. Table 37 represents the average of the 4 tests.

Table 37. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation when exposed to a number of dosages of Thuricide 48LV.

Dosage/rate BIU/gal/acre	Percent mortality 2 days	4 days	6 days	Percent defoliation 2 days	4 days	6 days
2	4	30	83	11	21	24
4	8	38	92	7	9	14
6	11	62	98	5	9	12
8	18	63	99	4	6	8
10	12	64	98	3	6	8
12	25	69	98	3	7	8
14	23	67	99	3	7	9
16	20	76	99	3	5	6
18	26	75	99	2	4	5
20	28	80	99	2	4	5
Control	0	0	0	100	100	100

Weathering tests were conducted using Thuricide 48LV neat and at various dilutions.

Table 38. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 and 6 day exposure to seedlings treated with Thuricide 48LV and exposed to rainfall.

Dosage/rate	Inches rain	Percent mortality		Percent defoliation	
		4 days	6 days	4 days	6 days
12BIU/32oz/acre (neat)	-	52	78	11	11
12BIU/32oz/acre (neat)	1.0	0	11	68	81
16BIU/42.6oz/acre (neat)	-	92	100	10	10
16BIU/42.6oz/acre (neat)	.25	80	100	28	29
16BIU/42.6oz/acre (neat)	.10	30	74	47	56
12BIU/64oz/acre	-	64	96	2	3
12BIU/64oz/acre	1.0	1	29	20	56
12BIU/96oz/acre	0	64	91	2	3
12BIU/96oz/acre	1.0	15	52	16	18
12BIU/128oz/acre	-	59	90	3	3
12BIU/128oz/acre	1.0	18	53	13	17
16BIU/128oz/acre	-	91	100	6	7
16BIU/128oz/acre	.10	68	100	17	21
16BIU/128oz/acre	.25	78	100	27	34
12BIU/256oz/acre	-	49	89	3	4
12BIU/256oz/acre	1.0	22	61	35	29
12BIU/384oz/acre	-	79	97	2	2
12BIU/384oz/acre	1.0	20	53	37	40
Control	-	0	0	80	100
Control	1.0	0	0	85	100

A new Bt (using powder) formulation developed by Jack Henderson was tested.

Table 39. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 day exposure to seedlings treated with various Bt formulations.

Material	Dosage/rate BIU/gal/acre	Percent mortality	Percent defoliation
Henderson (old)	2	31	38
Henderson (old)	2	4	70
Henderson (old)	2	10	40
H. Yield	12	22	36
H. Yield	2	1	98
Dipel 8L	12	16	48
ABG-6222	12	8	74
ABG-6223	12	25	42
ABG-6228	12	24	36
Control	-	0	100

Table 40. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedling treated with 3 Bt formulations at 2 BIU/gallon/acre then exposed to outside conditions.

Material	Days aged outside	Percent mortality			Percent defoliation		
		2 days	3 days	4 days	2 days	3 days	4 days
Henderson (old)	0	17	36	52	4	16	17
	2	2	-	48	18	-	38
	4	1	-	2	27	-	36
Henderson (new)	0	7	10	11	27	46	58
	2	0	-	4	30	-	86
	4	0	-	1	50	-	71
Dipel 8AF	0	0	2	3	36	43	52
	2	0	-	19	32	0	76
	4	0	-	0	67	-	81
Control	0	0	0	0	70	90	96
	2	0	-	0	48	-	100
	4	0	-	0	88	-	99

A series of tests were conducted with Dipel 8L and Miller 1545 sticker. The sticker gave little protection against light to moderate rainfall 3 hours after treatment.

Table 41. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with Bt and Miller 1545 sticker.

Percent 1545 used	Inches rain	Percent mortality	Percent defoliation
-	-	49	28
-	.1	6	68
-	.25	4	68
1.0	-	44	34
1.0	.1	14	46
1.0	.25	8	66
2.0	-	39	25
2.0	.1	14	48
2.0	.25	15	56
3.0	-	46	28
3.0	.1	28	38
3.0	.25	6	54
Check	-	1	94

Table 42. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with Bt and 2% Miller sticker 1545.

Hours natural sunlight before exposure to rain	Inches rain	Percent mortality	Percent defoliation
-	-	59	9
-	.25	8	60
1	-	70	9
1	.25	4	46
2	-	70	15
2	.25	5	40
3	-	50	15
3	.25	14	40
Check	-	0	94

Table 43. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with Bt and Miller 1545 sticker.

Percent 1545 used	Inches rain	Percent mortality	Percent defoliation
-	-	68	15
-	.1	15	71
-	.25	6	69
4	-	60	22
4	.1	8	44
4	.25	5	52
5	-	45	20
5	.1	20	46
5	.25	3	66
6	-	49	19
6	.1	20	50
6	.25	15	48
8	-	46	18
8	.1	49	30
8	.25	11	70
10	-	32	34
10	.1	17	48
10	.25	16	51
15	-	40	17
15	.1	9	55
15	.25	4	74
Check	-	0	100

New samples of Dipel 8L and Dipel 12L were tested against laboratory reared 2nd instar gypsy moth larvae.

Table 44. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with Dipel.

Material	BIU gal/acre	Mortality after			Defoliation after		
		2 days	3 days	4 days	2 days	3 days	4 days
Dipel 8L	2	1	16	39	50	60	73
Dipel 12L	2	0	0	6	72	84	89
Dipel 8L	4	2	16	39	46	60	65
Dipel 12L	4	1	13	18	50	60	76
Dipel 8L	8	2	7	23	48	68	80
Dipel 12L	8	0	3	20	50	68	81
Dipel 8L	12	1	12	31	38	52	60
Dipel 12L	12	6	9	20	48	58	74
Dipel 8L	16	10	28	55	26	38	55
Dipel 12L	16	12	29	37	33	50	59
Dipel 8L	20	22	36	57	15	26	41
Dipel 12L	20	20	44	64	25	31	36
Check		0	0	3	98	100	100

Sample of Dipel 8L used in a Minnesota gypsy moth eradication program were bioassayed for efficacy.

Table 45. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following a 4 and 5 day exposure to oak seedlings treated with 3 Bt samples.

Material	BIU/gal acre	Percent mortality		Percent defoliation	
		4 days	5 days	4 days	5 days
Dipel 8L standard	4	38	72	42	46
Dipel 8AF standard	4	18	39	50	56
Dipel 8L Minnesota	4	1	18	79	84
Dipel 8L standard	8	32	60	56	56
Dipel 8AF standard	8	65	86	23	27
Dipel 8L Minnesota	8	16	37	60	62
Dipel 8L standard	12	59	80	25	27
Dipel 8AF standard	12	77	91	20	20
Dipel 8L Minnesota	12	19	37	34	44
Dipel 8L standard	16	66	89	14	14
Dipel 8AF standard	16	76	96	15	15
Dipel 8L Minnesota	16	34	63	38	44
Dipel 8L standard	20	70	83	14	16
Dipel 8AF standard	20	87	91	4	4
Dipel 8L Minnesota	20	55	80	43	43
Control	-	1	4	94	100

Table 46. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following a 4 and 5 day exposure to oak seedlings treated with 3 Bt samples.

Material	BIU/gal acre	Percent mortality		Percent defoliation	
		4 days	5 days	4 days	5 days
Dipel 8L New	4	0	4	80	100
Dipel 8AF New	4	14	27	44	62
Dipel 8L Minnesota	4	3	4	45	93
Dipel 8L New	12	9	21	58	67
Dipel 8AF New	12	42	63	16	17
Dipel 8L Minnesota	12	39	58	28	32
Dipel 8L New	20	57	71	9	15
Dipel 8AF New	20	87	99	5	5
Dipel 8L Minnesota	20	53	70	11	14
Control	-	0	0	84	100

Table 47. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following a 4 and 5 day exposure to oak seedlings treated with 3 Bt samples at 8 BIU/gal/acre.

Material	Percent mortality		Percent defoliation	
	4 days	5 days	4 days	5 days
Dipel 8L New	46	59	19	23
Dipel 8AF New	86	97	4	4
Dipel 8L Minnesota	73	79	13	13
Control	0	0	87	99

Thirty-five tender Northern red oak seedlings were sprayed with each of 8 commercially produced Bt formulations. Each seedling was treated in a laboratory spray chamber at 16 BIU/gallon/acre. Two hours after treatment, 5 plants from each series were exposed to newly moulted 2nd-instar laboratory reared gypsy moth larvae. Each seedling was infested with 20 larvae and 5 untreated seedlings were used as a control. Plants and test insects were then placed in an environmental chamber at 27°C and 50% RH. Insect mortality and seedling defoliation was then recorded over a period of time. When larvae completely defoliated a seedling, they were placed onto artificial diet.

The remaining 30 plants treated with each material and control plants were placed outdoors in an open area where they were completely exposed to the elements. Over the 14 day period, 5 plants of each treatment and control were returned to the laboratory at various time intervals for bioassay. Twenty 2nd instar gypsy moth larvae were exposed to each plant. Mortality readings were made over a period of time.

Weather conditions were recorded during the period seedlings were held outside. During the 14 day period, only a trace of rain was recorded. There were approximately 46 hours of sunlight. The average temperature during the period was: 8:00 a.m. (84.7°F), 1:00 p.m. (95.8°F) and 4:00 p.m. (92.5°F).

Foray was most active of the materials following outside aging, followed by Condor (oil) and Biobit. Condor aqueous was the least effective following outside exposure.

Table 48. Percent mortality of 2nd-instar gypsy moth larvae following a 6 day exposure to oak seedlings treated with 8 Bt formulations at 16 BIU/gallon/acre, then exposed to various amounts of outdoor exposure.

	Number of days plants aged outside						
Material	0	2	4	7	9	11	14
Biobit	100	99	92	22	26	6	4
Condor (Oil)	83	92	71	32	36	1	1
Condor (Aqueous)	90	90	8	1	2	0	1
Dipel 8AF	100	66	75	21	10	0	1
Dipel 8L	58	49	40	23	13	1	2
Foray	99	100	90	66	58	13	27
San-415 (NRD-12)	100	100	66	10	7	0	0
Thuricide 48LV	99	93	77	4	0	0	1
Control	0	0	0	0	1	1	1

Table 49. Percent seedling defoliation following a 6 day exposure of seedlings treated with Bt at 16 BIU/gallon/acre to gypsy moth larvae.

Material	Number of days plants aged outside						
	0	2	4	7	9	11	14
Biobit	9	13	21	78	36	62	72
Condor (oil)	40	39	18	62	45	88	65
Condor (aqueous)	38	35	56	78	45	72	80
Dipel 8AF	12	37	36	63	52	100	94
Dipel 8L	54	46	59	37	54	76	72
Foray	9	12	19	27	46	56	81
SAN-415 (NRD-12)	4	12	57	68	80	100	89
Thuricide 48LV	1	9	20	54	68	85	89
Control	100	100	100	100	100	100	100

Laboratory tests were conducted with 3 formulations of Dimilin to identify any differences in efficacy. Newly moulted 2nd instar gypsy moth larvae were used as test insects.

Table 50. Percent larval mortality and seedling defoliation following a 4 and 6 day exposure to foliage treated with Dimilin 25W and Dimilin 2F.

Formulation	lbs. AI/gal/acre	Inches rain	After 4 days		After 6 days
			mortality	defoliation	mortality
25W	.0312	-	49	77	100
	.0312	.25	73	81	96
	.0156	-	59	84	100
	.0078	-	63	63	99
2F	.0312	-	68	83	99
	.0312	.25	60	82	98
	.0156	-	56	81	98
	.0078	-	54	81	84
Check	-	-	0	80	1

Table 51. Percent larval mortality following exposure to Dimilin 25W and Dimilin 2F applied to oak foliage at .0312 lbs. AI/gal/acre.

Formulation	Inches rain	After 3 days mortality	After 5 days mortality	After 10 days mortality
25W	-	62	94	100
	1.0	58	97	100
2F	-	51	87	100
	1.0	51	98	100
Check	-	0	0	3

Table 52. Percent larval mortality following exposure to oak foliage treated with 3 Dimilin formulations at .0312 lbs. AI/gal/acre.

Formulation	Inches rain	After 3 days mortality	After 5 days mortality	After 10 days mortality
25W	-	47	98	100
	1.0	47	98	99
	2.0	65	100	-
	3.0	63	100	-
2F	-	43	96	100
	1.0	51	98	100
	2.0	57	99	100
	3.0	59	100	-
4F	-	62	99	100
	1.0	68	100	-
	2.0	65	98	100
	3.0	66	100	-
	-	0	0	5
Check	3.0	0	4	5

Two new experimental insect growth regulators from Rohm and Haas were tested in the laboratory and found to be as effective as Dimilin against the gypsy moth.

Table 53. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with RH-5849.

Dosage/rate	Percent mortality				Percent defoliation	
AI./gal.acre	4 days	7 days	11 days	19 days	4 days	7 days
.125	2	97			15	20
.062	10	100			22	22
.031	6	99			30	32
.015	6	97			34	34
.007	15	99			36	38
.003	24	85			57	57
.0019	6	49			70	80
.0009	8	-	29	30	95	100
.0004	0	-	2	4	87	100
.0002	0	-	1	6	91	98
.0001	0	-	8	21	87	100
Control	1	1	1	1	100	

Table 54. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with RH-5992.

Dosage/rate	Percent mortality				Percent defoliation	
AI./gal.acre	4 days	7 days	11 days	19 days	4 days	7 days
.125	1	89			21	23
.062	7	99			38	38
.031	4	99			30	34
.015	7	99			30	30
.007	19	91			28	33
.003	25	79			57	63
.0019	18	67			70	76
.0019	5	-	78	90	88	100
.0004	2	-	52	66	97	100
.0002	0	-	1	5	90	100
.0001	0	-	0	2	100	
Control	1	1	1	1	100	

Table 55. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with Dimilin 2F.

Dosage/rate AI./gal.acre	Percent mortality 4 days	7 days	11 days	19 days	Percent defoliation 4 days	7 days
.125	67	100			63	65
.062	50	99			86	88
.031	46	97			89	94
.015	49	99			78	87
.007	52	96			75	79
.003	59	98			79	79
.0019	54	97			87	89
.0009	7	-	84	93	95	100
.0004	0	-	67	80	80	100
.0002	0	-	37	45	86	100
.0001	1	-	2	5	89	100
Control	1	1	1	1	100	

Table 56. Percent mortality of 2nd-instar gypsy moth larvae and seedling defoliation following exposure to seedlings treated with RH-5849, RH-5992 and Dimilin 2F at .03 lbs. AI/gal/acre and then exposed to rainfall.

Material	Inches rain	Percent mortality 3 days	6 days	8 days	Percent defoliation 3 days	6 days	8 days
RH-5849	-	1	98	100	32	32	32
RH-5992	-	0	96	100	24	24	24
Dimilin 2F	-	0	64	97	69	69	69
RH-5849	1.0	0	5	13	90	100	
RH-5992	1.0	1	82	100	24	26	26
Dimilin 2F	1.0	0	51	100	92	92	100
RH-5849	3.0	0	0	0	100		
RH-5992	3.0	0	85	94	32	32	32
Dimilin 2F	3.0	0	53	99	92	100	
Control	-						

Three experimental formulations from FMC Corporation were tested in the laboratory. Two synthetic pyrethroids, F6308 and F7869 were found to be very effective in the laboratory against gypsy moth larvae. A Bt formulation, F7123 appeared to be as effective as the presently used HD1 strain.

Table 57. Percent mortality of 2nd-instar gypsy moth larvae and oak seedling defoliation following a 3 and 4 day exposure to F6308 and F7869.

Dosage/rate	Percent mortality				Percent defoliation			
	F6308		F7869		F6308		F7869	
lbs. AI/gal/acre	3 day	4 day	3 day	4 day	3 day	4 day	3 day	4 day
.833	100				0			
.416	99		100		0		0	
.208	100		100		0		0	
.104	98		99		0		0	
.052	97	100	100		1	1	0	
.026	78	99	96		1	1	0	
.013	58	93	95	100	1	2	1	1
.006	42	85	70	100	2	5	1	1
.003	17	57	72	97	11	16	3	4
.0016	1	11	39	84	41	58	7	8
.0008	0	0	41	86	56	86	17	19
Control	0	0	0	0	100		100	

Some treated seedlings used in test one (Table 57) were retained and exposed to larvae 3 more times over the next 28 days. Plants were held in the environmental chamber throughout this time periods. They were not exposed to ultraviolet light or rainfall.

Table 58. Percent mortality of 2nd-instar gypsy moth larvae following a 3 day exposure to seedlings treated with F6308 and F7869 and aged for various amounts of time in an environmental chamber.

Dosage/rate	Percent mortality					
	F6308			F7869		
lbs. AI/gal/acre	6 1/	14	28	6	14	28
.833	99	99	97			
.416	98	100	94	100	100	99
.308	99	96	88	100	99	99
.104	98	95	83	100	89	90
.052	96	82	85	96	88	81
.026				100	90	94
Control	0	0	0	0	0	0

1/ Days following treatment that test insects were re-introduced.

Table 62. Percent mortality of gypsy moth larvae hatched from Beauveria treated egg masses and reared on artificial diet.

Strain	Dosage	Percent mortality after					
		3 days	6 days	10 days	17 days	24 days	30 days
32	7.4×10^{10}	9	18	25	29	31	34
	3.7×10^{10}	10	17	24	25	25	26
	7.4×10^{10}	6	13	19	21	22	24
155	3.2×10^{11}	10	28	35	36	42	43
	1.6×10^{11}	14	19	25	25	27	29
	0.8×10^{11}	14	22	26	28	47	56
201		23	57	71	72	72	72
		18	41	61	61	61	61
		19	39	51	52	52	53
299	5.8×10^{10}	4	9	11	12	13	13
	2.9×10^{10}	5	9	11	11	11	11
	1.4×10^{10}	6	9	10	11	13	13
356	3.4×10^{11}	20	35	44	47	48	48
	1.7×10^{11}	10	27	43	50	52	52
	0.8×10^{11}	27	38	48	50	51	51
Control	-	15	15	15	16	17	18

Each egg mass was treated with a total of .025 grams of material.

No dosage was received for sample 201.

Table 63. Percent mortality of 2nd-instar gypsy moth larvae after exposure to red oak seedlings dusted with Beauveria then reared on artificial diet.

Strain	Dosage	Percent mortality after					
		3 days	6 days	10 days	17 days	24 days	30 days
32	7.4×10^{10}	2	2	4	4	4	13
	3.7×10^{10}	0	0	1	2	2	5
	1.8×10^{10}	1	1	1	1	1	2
155	3.2×10^{11}	2	3	7	7	7	8
	1.6×10^{11}	0	0	1	2	3	4
	0.8×10^{11}	0	0	1	3	3	3
201		0	0	8	8	8	9
		1	2	4	6	8	11
		0	0	0	0	0	4
299	5.8×10^{10}	0	0	0	1	1	3
	2.9×10^{10}	0	0	1	1	2	4
	1.4×10^{10}	1	1	2	5	6	7
356	3.4×10^{10}	0	0	0	2	2	2
	1.7×10^{11}	0	1	2	2	3	6
	0.8×10^{11}	1	1	1	2	3	3
Control	-	0	0	0	1	1	1

Each seedling was treated with a total of .025 grams of material.
No dosage was received for sample 201.

Table 64. Percent mortality of 2nd-instar gypsy moth larvae after being dusted with *Beauveria* dust and reared on artificial diet.

Strain	Dosage	Percent mortality after					
		3 days	6 days	10 days	17 days	24 days	30 days
32	7.4×10^{10}	0	0	0	1	2	3
	3.7×10^{10}	0	1	1	2	1	3
	1.8×10^{10}	0	0	1	1	4	7
155	3.2×10^{11}	0	0	0	0	1	1
	1.6×10^{11}	0	0	0	0	2	3
	0.8×10^{11}	0	0	0	0	0	0
201		0	0	1	5	7	9
		0	0	4	6	8	9
		0	0	1	2	2	4
299	5.8×10^{10}	0	0	1	2	3	4
	2.9×10^{10}	0	0	0	1	3	3
	1.4×10^{10}	0	0	0	0	2	2
356	3.4×10^{11}	0	1	1	5	11	12
	1.7×10^{11}	0	0	0	5	15	15
	0.8×10^{11}	0	0	0	7	11	12
Control	-	0	0	0	0	0	2

Each larva was dusted with a total of .025 grams of material.
No dosage was received for sample 201.

Table 65. Percent mortality of gypsy moth larvae hatched from Beauveria treated egg masses and reared on artificial diet.

Strain	Dosage	Percent mortality after 3 days	6 days	10 days	17 days	24 days	30 days
252	A	23	52	60	61	62	63
	B	37	50	56	60	60	61
	C	42	60	60	60	60	60
357G	A	24	49	56	58	58	58
	B	37	61	64	64	64	64
	C	36	58	58	58	58	58
721	A	20	46	52	52	52	55
	B	40	75	77	78	78	79
	C	36	67	70	70	70	70
752	A	46	52	57	58	60	66
	B	33	40	43	47	47	50
	C	71	86	86	86	86	86
809	A	11	14	18	23	24	25
	B	30	32	38	39	42	42
	C	24	29	32	35	37	37
1053	A	9	19	24	28	29	29
	B	8	12	18	20	21	22
	C	34	37	39	40	41	41
1326	A	42	57	63	64	64	64
	B	45	67	77	78	78	78
	C	37	48	49	49	49	49
1959	A	9	17	22	24	24	24
	B	8	19	24	24	24	24
	C	18	23	25	26	26	26
2054	A	14	20	25	31	32	34
	B	38	39	42	45	45	45
	C	43	45	46	46	47	47
26851	A	4	5	6	6	6	7
	B	9	10	11	11	12	12
	C	23	24	24	24	24	24
Control	-	3	4	4	7	10	16
Control	-	6	7	8	8	8	8
Control	-	11	17	20	22	22	24
Control	Talc	2	2	2	3	3	3
Control	Talc	25	26	28	29	32	33
Control	Talc	29	32	32	32	34	34

Each egg mass was treated with a total of .025 grams of material. No dosage was given for the samples.

Dosage A represents the material applied full strength.
Dosage B is half of A and C is half of B.

During February of 1987, tests were conducted with one strain of Beauveria. The material was dusted onto artificial diet and exposed to larvae. Oak seedlings were completely painted with Beauveria and water and exposed to larvae until seedlings were defoliated. Larvae were then reared on artificial diet. Seedlings were also dusted with Beauveria and then exposed to gypsy moth larvae. When seedlings were completely defoliated, all larvae were placed on artificial diet and reared for a number of days.

Table 66. Percent mortality of gypsy moth larvae following exposure to Beauveria of 1 x 10^{11} spores per gram.

Type test	Dosage grams per dish/plant	Percent mortality after				
		10 days	15 days	20 days	26 days	29 days
Diet	.2	2	6	17	43	62
Diet	.1	5	6	11	27	31
Paint-on	1 gm/5ml	2	2	7	37	44
Paint-on	.5gm/5ml	7	8	16	49	57
Seedlings dusted	.1	3	3	5	72	81
Control	Diet	6	7	19	42	47
Control	Seedlings	2	2	7	26	26

Newly moulted 2nd instar gypsy moth larvae were used for the above test.

During August, 1988, we conducted small field tests with Beauveria bassiana. Tests were with Delaware (Strain B) used against laboratory reared (NJ Strain) gypsy moth larvae. This test work was approved by Massachusetts regulatory personnel (Mr. Louis Wells), United States Department of Agriculture personnel (Mr. Phil Lima) and Otis Air National Guard personnel (Mr. Dixie Dugan).

Beauveria bassiana was tested in an oil formulation (UV protection) and in water without oil (no UV protection). Each test contained an oil/water control and untreated control.

Contact Spray Test

Eighteen northern red and white oak trees averaging 10.9 inches in diameter were selected for this test. Each tree was isolated from other trees by a minimum of 10 feet.

Two-hundred newly moulted, second instar gypsy moth larvae were placed on a cardboard flap at the base of each tree and allowed to move up the tree. A burlap band was placed around the trunk of each tree at a height of approximately 6 feet to aid in recovering the test insects.

When larvae were well dispersed over the tree trunks, each treatment tree was sprayed with 100 ml of spray mix. The complete lower portion of the trunk (0-5 feet) was sprayed by Ms. Pauline Wood with a hand-held sprayer furnished by

Myco Tech. Most gypsy moth larvae were hit directly with the spray and then allowed to move over the deposit on their way up the tree trunk to the burlap.

Five trees were treated with the oil formulation at (1×10^7 spores/ml) and 5 at (2.5×10^8 spores/ml). Five trees were treated with water and oil only and 3 trees were untreated. Tween 20 was used as an emulsifier.

All tree bark was dry at time of treatment and temperature was 78°F with 60% RH.

Approximately 15 minutes following treatment, 100 gypsy moth larvae were removed from each tree and placed onto artificial diet. Ten larvae were placed in each container of diet and were then moved from the field into an environmental laboratory chamber. The chamber was maintained at 80°F with 50% RH. Larvae mortality readings were made at various times over the next several days.

Table 67. The DBH of trees used for B. bassiana contact spray test.

Tree replication	High dosage	Low dosage	Treated control	Untreated control
1	10.6	13.2	6.7	12.3
2	7.5	13.4	7.7	14.9
3	10.4	10.2	8.6	11.6
4	20.4	12.2	6.7	
5	12.7	10.3	8	

Table 68. Percent mortality of gypsy moth larvae following exposure to B. bassiana, Delaware 2 strain applied directly to the test insect and tree trunk bark.

Days following exposure	Percent mortality			
	High dosage	Low dosage	Treated control	Untreated control
6	22	14	10	0
8	29	17	11	1
11	36	20	15	1
14	38	23	17	2
18	51	24	18	3
23	57	26	20	3
28	68	30	27	4

Foliage Spray Test

Eighteen northern red and white oak trees (6-8 feet) were selected for this test. Each tree was isolated from other trees by a minimum of 10 feet.

With gas engine powered back-pack sprayer, 5 trees were sprayed with B. bassiana in oil (UV) and 5 with B. bassiana in water (no UV). Five trees were sprayed with oil and water for a treated control. Three untreated trees were

areas where defoliating populations occur, unacceptable defoliation would occur before any mortality developed.

Project Number: GM 88.1.1
Project Title: Spray Droplet Deposition Studies
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: W. H. McLane, T. Roland, R. Reardon, M. McManus and W. Yendol

The work described is the result of a cooperative effort by APHIS, FS and Pennsylvania State University. The cooperators are identified as the "Northeast Forest Aerial Application Technology Group". Their main objective is to conduct field studies with spray materials and equipment that will lead to the selection of more effective materials, equipment and techniques to suppress the gypsy moth and other forest pests throughout the United States and Canada. Two NEFAAT Projects will be reported on here.

During January, 1988, a study was conducted at Moore Air Base, Mission, Texas, to determine the swath patterns and droplet characteristics of Condor using two different atomization systems. Spray card analysis was done at Pennsylvania State University by Karl Mierzejewski, Jonathan Bryant and William Yendol. A final report to Ecogen was prepared by the Pennsylvania State University group.

Introduction

This paper describes a series of spray trials performed with the Ecogen *Bacillus thuringiensis* product Condor at the Moore Airbase, Edinburg, Texas, in January, 1988. The trials were performed to test the effectiveness of atomization of Condor when applied by aircraft in neat and diluted formulation, using two commonly used spraying systems. The results presented show that the product demonstrates good properties when recoveries and deposited droplet spectra are analyzed by volumetric and image analytical methods.

Methods and Materials

Location of the Experimental Site - The experimental area was located at the USDA, APHIS facility, Moore Airbase, Edinburg, Texas. The experimental area was located at least one-half mile from major obstructions (aircraft hangars) which would have disturbed the wind pattern. The terrain was flat with short mown grass.

Aircraft and Application Equipment - A Cessna AgTruck (C188) was used for the aerial application. Two atomization systems were used - Micronair rotary atomizers (AU5000) and Teejet flat fan nozzles. For the rotary atomizer trials, the equipment (VRU settings, boom pressure and blade angles) were adjusted for the desired flow rate and droplet size.

For boom and nozzle application of diluted and neat formulation, 45 new Spraying Systems Teejet flat-fan 8004, 8003 and 8002 nozzles fitted with 50-mesh screens were installed and oriented at 90° and 45° angles to the flight direction for neat and dilute applications, respectively. A nominal application height of 50 feet above ground level (AGL) and an airspeed of 115 mph was used for each application.

Application system calibration data are presented in Table 1.

Table 1. Spray mix, application equipment, calibration settings for boom and nozzle and Micronair rotary atomizers.

Pass number	Tank mix (fl oz/A)	Atomizer type	Boom pressure (psi)	VRU setting	Blade setting (degrees)	Nozzle size	Nozzle direction (degrees)
1-3	Neat (32)	Micronair	46	5	35		
4-6	128	Flat Fans	40	-	-	8004	90
7	Neat (32)	Flat Fans	40	-	-	8003	45
8-10	Neat (32)	Flat Fans	40	-	-	8002	45

Formulation - Condor (EC 178-01), an oil-based emulsifiable concentrate, was used in the experiments. The physical characteristics of Condor are provided in Appendix C. In diluted form it was mixed with 3 parts water, and Rhodamine WT (Keyazine red XRS, Keystone Aniline Corp., Chicago, IL) was added at a concentration of 5 g/liter. In neat form, Rhodamine was added at a concentration of 10 g/liter.

Aircraft Calibration - A non-standard method of calibration was used for the boom and nozzle equipment because of the limited amount of material available. Micronairs were calibrated using a standard procedure, as this permits the collection and recycling of the tank mixture.

Boom and Nozzle Calibration - To establish the flow rate of the flat fan nozzle spray system, the various tank mixes to be used were put through a ground testing rig, which was previously constructed by the USDA, APHIS. A mix of one part Condor and three parts water (16 BIU/gallon) and the neat formulation (64 BIU/gallon) were tested (see Appendix A). The nozzle calibration results obtained with the ground test rig were used for setting up the aircraft spray system (nozzle numbers and boom pressure).

Micronair Atomizers - To establish the flow rate with the Micronair atomizers, the neat material was added to the spray tank and the aircraft was operated for one minute on the ground. The amount of material being dispensed by each atomizer was then collected and recorded. A total of six runs were done to establish the correct boom pressure and VRU setting for required flowrate (see Appendix B).

Meteorological Equipment - Meteorological measurements were made at two different heights (2 m and 15 m). All two meter sensors (wind speed, wind direction and dry bulb temperature, Met-1, Oregon) were mounted on a tower. The 15 meters station (wind speed, wind direction, wet and dry bulb temperature and pressure) was a tethersonde mounted on a directional balloon,

which relayed meteorological data by radio telemetry to a ground station (Atmospheric Instrument Research, Boulder, CO). Wet and dry bulb temperatures were also taken at ground level.

Collectors - KromeKote cards (4 x 5 inches) raised 6 inches above the ground were used as the collecting targets. One-half of the collecting area was covered with a clear acetate sheet for fluorescent tracer wash-off recovery measurement. Cards were mounted with paper-clips on 4 x 5 inch aluminum plates mounted on wood blocks (2 x 6 x 9 inches). Targets were laid out at 5-foot intervals in two rows of 400 feet, with each row separated by 100 feet (Figure 1a).

Spray Run Procedure - Target lines were laid out to be perpendicular to the wind direction as much as possible. Because of shifting winds, many of the runs were made with some crosswind component. Under such conditions the centerline of the aircraft pass was offset into the wind to allow sedimentation of droplets in the direction of the crosswind component.

Application height was nominally 50 feet. Flaggers were used to guide the pilot over the required centerline. Spray switch-on was approximately 500 feet before the collectors, and switch-off occurred after the aircraft had flown approximately 1500 feet upwind of the collectors.

To obtain data under different conditions of wind speed, atmospheric stability, temperature and humidity, runs were commenced early in the morning and continued until excessive wind speed, or turbulence due to thermic conditions made further runs impractical.

Spray Card Analysis - Spray cards were analyzed by two methods to obtain mass recovery data, droplet sizing, droplet density and volume recovery data. To determine the total mass of spray deposited,the acetate portions of the cards were subjected to a wash-off procedure, and the tracer in the solvent was measured fluorometrically. To determine droplet spectrum characteristics, image analysis was performed on the target cards.

Fluorometric Analysis - The acetate portions affixed to the cards were removed, placed in 200 ml plastic vials, 50 ml of 30% methanol/water was added, and the vials shaken on a wrist action shaker for 25 minutes. The solution was passed through a fluorometer (Sequoia-Turner Model 112, fitted with 546 um primary filter and 590 um secondary filter) to assay for tracer. Subjecting the acetates to a second washoff gave readings which were less than 1% of those obtained with the first one. The instrument was calibrated with standard solutions each day before use.

Image Analysis and Spread Factor Determination - An Optomax V Image Analyzer (ITI, Burlington, MA) linked to an IBM compatible PC was used to determine the droplet size and density characteristics of each collector. Each card was sampled across its surface until either 200 droplets or 45 fields had been counted. The total area of the card exposed was 64.52 cm^2 and the area of the card sampled was 0.50 cm^2. The calculated droplet sizes were allotted into one of 20 different size classes. The size classes represented a linear progression, starting with a lower limit of 20 um and increasing in 20 um increments.

An empirically derived spread factor polynomial was used to convert the stain size to the original droplet size for both dilute and neat formulations. Different sized droplets were generated in the laboratory using a spinning disc mono-sized droplet generator. Each of five classes of droplets were caught on the Kromekote cards and magnesium oxide covered slides. Because of the fixed coefficient between the MgO crater and the actual droplet size (1:1.15), a relationship could be established between droplet size and stain diameter for a wide droplet spectrum.

Results and Discussion

Meteorological Data - Tables 2 and 3 show summaries of the 2- and 15-meter height weather data, averaged over a period starting one minute before application and five minutes after passing over the collection lines. The percent relative humidity taken with a conventional sling psychrometer can also be found in Table 3 for runs 5 though 10.

Table 2. Summarized 2-meter weather data.

Pass number	Wind direction Mean direction	Std. dev.	Max.	Min.	Wind speed m/sec	Std.	Temp. (°C)
1	276	12	298	254	-	-	3.7
2	246	11	274	225	-	-	7.2
3	192	10	218	178	-	-	9.6
4	-	-	-	-	-	-	-
5	132	8	149	114	3.23	0.41	20.2
6	115	19	143	73	2.40	0.65	19.6
7	354	4	361	342	2.18	0.09	5.1
8	25	9	43	4	2.25	0.25	9.8
9	33	20	96	356	2.22	0.32	12.6
10	60	12	80	331	2.23	0.39	15.1

Table 3. Summarized 15-meter weather data collected from the tethersonde station.

Pass number	Wind direction Mean direction	Std. dev.	Max.	Min.	Wind speed m/sec	Std.	Temp. (°C)	3 ft RH (%)
1	235	8.5	278	222	2.59	3.1	4	-
2	240	8.4	263	222	1.31	0.35	5	-
3	199	9.2	217	182	2.28	0.31	8	-
4	188	13.5	215	152	4.29	0.61	9	-
5	142	12.9	170	103	4.26	0.88	20	77
6	130	17.9	154	93	3.20	0.82	20	37
7	-	-	-	-	-	-	6	92
8	30	30.0	41	1	2.62	0.26	11	81
9	57	32.0	360	5	2.05	1.30	10	72
10	49	56.0	349	5	1.55	0.47	16	67

Deposit Analysis - Deposit analysis data and swath patterns are presented graphically for each run. In sequence they are:

(1) Washoff fluorometric results. These are presented as deposit density in IU/cm^2 and indicate how much of the active ingredient has been caught by the collectors.

(2) Washoff data replotted with respect to the aircraft centerline, with the flight and wind directions illustrated.

(3) Overlap of adjacent swaths of washoff data to simulate a deposit pattern which would be obtained with aerial application performed at a lane separation of 75 feet.

(4) Image analysis data of one of the target lines plotted across the swath with respect to the volume recovered. Note that in most cases the values plotted would differ from those obtained in case (1). During deposition, droplets are subject to evaporation which would reduce their volume, but not generally the active ingredient.

(5) Image analysis of one of the target lines plotted with respect to the percentage area of the target covered by the deposit.

(6) Image analysis of one of the target lines plotted with respect to droplet density obtained across the swath.

(7) Image analysis of one of the target lines plotted with respect to volume median diameter (VMD) and number median diameter (NMD). This figure shows clearly how the smaller droplets are carried by crosswinds and aircraft wake effects. In addition, the spread between these two values can be taken as indicative of the width of the droplet spectrum; a mono-sized spectrum would have an identical NMD and VMD.

The experimental results should be interpreted with the understanding that the collecting targets are not particularly efficient at capturing small droplets (less than 100 um) in winds stronger than two meters/second (4 mph).

Comparison of Application Methods - Three passes were done for each of the three application method/application rate combinations. The replicate results for each of the three passes are discussed and the techniques compared.

Table 4 shows the total tracer recovered for each of the ten runs. Recovery is calculated as active ingredient (IU) per centimeter of flight path and is based on the washoff data. Recovery is calculated as follows: the concentration of Rhodamine tracer of the spray mixture and the target cards is known and can be directly related to the active ingredient concentration. The total dose of the active ingredient found in a cm band across the flight swath can be calculated from the flowrate of the spray system, the aircraft ground speed, and the concentration of tracer in the tank mixture. The recoveries are affected by many different factors, some of the major ones being catch efficiency of flat plates and correct positioning of targets so that most of the swath is captured.

Table 4 also shows that the performance of the Micronairs with the neat formulation. The recovery was similar to that obtained with the 1 gallon/acre application rate with 8004 flat fans. Applying the neat formulation with 8003 and 8002 nozzles gave higher recoveries than the other two methods.

Table 4. Spray dispensing system and the recovery of Condor.

Pass number	oz/ acre	Dispensing system	IU per cm flight swath A	B	mean	Ground speed (mph)	Percent recovery
1	32	AU5000	325,837	352,284	339,061	116	36.3
2	32	AU5000	533,579	544,883	539,231	116	57.7
3	32	AU5000	269,906	260,335	265,121	116	28.4
4	128	8004	314,686	266,939	290,813	116	31.1
5	128	8004	477,327	495,563	486,445	114	51.1
6	128	8004	367,774	310,677	339,226	116	36.3
7	32	8003	865,143	688,657	776,900	115	82.4
8	32	8002	692,269	672,421	682,345	118	74.2
9	32	8002	759,042	605,191	682,117	115	72.3
10	32	8002	489,621	524,871	507,246	115	53.8

Micronair AU5000 Spray Undiluted Formulation - The passes were performed under light wind conditions (1.3 to 2.6 m/sec), stable wind directions and low temperatures. The first replicate was made under crosswind conditions and a portion of the deposit was not captured. The third replicate gave low recovery in mass and volume. Data are show in Figures 1-7, Figures 8 and Figures 15-20 for passes 1, 2 and 3, respectively.

Application in crosswind conditions produces a range of droplets across the swath. Large droplets fall out close to the aircraft flight position, whereas smaller droplets drift down wind (Figures 7, 14 and 21). The volume medium diameter over the deposit pattern of this application system ranged from 60 to 180 um. The mean droplet diameter VMD was 108 um for this application system/formulation combination.

Droplet densities on the collectors averaged around 5 drops/cs^2. For a lane separation of 75 feet (standard for type of aircraft used), overlapping each swath produced mean deposits ranged from 118 to 227 IU/cm^2 (Figures 3, 10 and 17). These calculations are based on a leaf area index of 1 and would probably be lower in a forest canopy. However, if a turbulent environment existed within the canopy, a higher proportion of droplets might be caught.

Boom and Flat Fan Nozzles: Diluted Formulation - The three runs were made in higher wind speeds and higher temperatures than the Micronair runs. This would have adverse impact on recoveries as (1) there is a reduction in catch efficiency by flat collectors in stronger winds and (2) evaporation under higher temperatures would tend to reduce the size of the droplets and make them more difficult to catch. Some crosswind element was present in all three runs. The data for these passes are shown in Figures 22-28, Figures 29-35, and Figures 36-42 for passes, 4, 5 and 6, respectively. As expected, comparison with the Micronair neat treatment shows that average volumes/unit area are higher (by about 70%) and droplet densities are slightly higher, at about 6 droplets/cm^2 across the main part of the swath.

The droplet spectrum shows a mean VMD slightly larger than obtained with runs 1-3. The mean VMD was about 118 um. Overlapping each swath produced mean

deposits ranging from 130 to 197 IU/cm^2, similar to values obtained with Micronairs/neat formulation.

Flat Fans (8003/8002): Undiluted Formulation - To establish which type of nozzle to use in the neat application, one run was performed with 8003 nozzles (pass 7). The low droplet density obtained with this run suggested that a nozzle with a smaller flowrate would give a more efficient atomization. Subsequent runs (passes 8-10) were performed with 8002 nozzles. The passes were made with the nozzles facing 45 degrees forward to ensure maximum atomization in the airstream.

Passes 7 and 8 were made with some crosswind element, whereas passes 9 and 10 were made almost directly into wind. Winds were uniformly light (@ 2.2 m/sec) in all four passes. Temperatures were cool (5.1^{o}C to 15.1^{o}C) and humidity high (67-92 percent) during this part of the experiment.

The passes gave droplet spectra with VMD considerably higher than the other two applications. In pass 7, the mean VMD for the swath was 152 um. In pass 8, near the flight pass, the VMDs were between 250 and 300 um (Figure 56), with a mean VMD of 177 um for the swath pattern. In passes 9 and 10, the mean VMDs were 175 and 169 um, respectively.

Corresponding droplet densities were lower than either of the other treatments, averaging about 4.5 droplets/cm^2. The overall recoveries given by the 8002/8003 flat fans with the neat formulation were high (Table 3). However, when these data were considered in conjunction with the droplet density data and VMD/NMD data obtained with image analysis, it can be seen that this form of atomization produced a small number of medium to large sized droplets. These droplet characteristics would probably result in a poor distribution in a forest canopy.

The higher recoveries produced greater mean overlap patterns with high mean IU values when swaths were overlapped with a lane separation of 75 feet. Mean deposit densities between 213 and 284 IU/cm^2 were obtained (Figures 45, 52, 59 and 66).

Overlap and Lane Separation - To illustrate and compare a spray application made in a crosswind with an into-wind application, one of the original swath deposit patterns (pass 2A, Figure 71A) was reconstructed using the same deposit data values (Figure 71B). These deposit values were rearranged in such a manner so as to produce a hypothetical distribution that was more evenly distributed outwardly from the centerline. This swath deposit pattern (Figure 71B) would be more typical of an application made into the wind. Three lane separation distances (75, 100 and 125 feet) were then used to illustrate the process of overlapping swaths (Figures 71D, 72B and 72D). The coefficient of variation was found to be less in the reconstructed overlap swath than in the original swath pattern. As could be expected, the mean deposit decreased as the lane separation was enlarged. Using such overlap programs with deposit data of the type presented here can enable aerial applicators to establish the best possible application parameters (lane separation and flight path relative to the wind direction).

The swath deposit pattern by an aircraft will depend on the type of aircraft, its flying height, droplet size and the prevailing wind conditions. The

minimum swath is usually determined flying by the aircraft into wind. However, use is often made of crosswinds during some commercial applications. Application with a crosswind component is thought by some to produce more uniform deposits over multiple swath patterns. Sometimes aerial applicators will select a swath that is too wide, just to facilitate covering an area more quickly. This obviously reduces application costs, but may lead to inadequate coverage and ultimately poor pest control.

General Discussion - The broad objectives of the present field trials was to assess the physical effectiveness of Condor when used for aerial application. Such effectiveness can be measured by the following parameters: (1) ability to atomize efficiently using standard atomizers, without the production of large clumped droplets, (2) ability to be caught by standard collecting media, which is indicative of lack of product solvent evaporation which would cause the production of dry particles, and (3) suitability for use as neat or diluted material.

The results presented in this report indicate that Condor meets all these parameters. The wash-off recoveries obtained from the collectors (Table 4) show that in neat or diluted form, effective atomization and deposition takes place. Image analysis of the cards shows that the droplet spectra produced by both flat fans and Micronair rotary atomizers are comparable with other forms of commercial oil-formulated *Bacillus thuringiensis* products.

Additional results obtained from these experiments give directions regarding the use of different atomization systems used in aerial application for neat and diluted formulations. The 8004 flat fan nozzles give effective performance with the diluted product. However, for neat application it would seem to be advisable to use a rotary atomizer which could give more effective break-up of the material. In spraying for gypsy moth control, a distribution in all parts of the foliage is desirable. Smaller droplets in the spectrum, which are often not caught on flat targets, such as were used in this trial, and which are entrained in the turbulence inside the forest, would probably contribute to the deposition in the canopy. Use of rotary atomizers would be more likely to provide such a droplet spectrum. On the other hand, the smaller droplet spectra could, in reduced turbulence, drift out of the area being treated.

The experimental protocol presented in this paper relies on three distinct processes in estimating the suitability of the material: atomization, sedimentation through the air, and deposition and capture by a collector. Consequently, a variety of mechanisms come into play during the whole process which would tend to increase the variability of the results. The most likely of these three parameters which would be influenced by the physical properties of the material (dynamic and kinematic viscosity, size and uniformity of particle size and volatility of constituents) is the first one, atomization. As a next step, it is recommended that the droplet spectra produced by different atomizers are examined by laser imaging systems under simulated flight conditions in a wind tunnel. In this way, the complete droplet spectrum can be studied without being influenced by the stochastic actions of weather.

Table 5. Calibration data for the neat formulation of Condor at 64 BIU/gal with a range of flat fan nozzles at different temperatures using the ground equipment calibrator. The pump pressure was 40 psi.

Run[1] number	Nozzle[1] ml	Nozzle[2] ml	Total volume ml	Calculated volume ml	% needed	Temp. °F
1 (8002)	640	570	1210	1514	-	78
2	540	580	1120	1514	-	78
3	775	734	1510	1514	- .26	88
4	775	735	1510	1514	- .26	84
5	780	740	1520	1514	+ .39	90
6	780	740	1520	1514	+ .39	92
7	800	755	1555	1514	+2.70	94
8	795	755	1550	1514	+2.30	96
9	800	755	1555	1514	+2.70	97
10	795	755	1550	1514	+2.30	98
1 (8003)	1095	1120	2215	2271	-2.46	99
2 with	1105	1120	2225	2271	-2.00	100
3 slotted	1100	1120	2220	2271	-2.24	99
4 screens	1115	1120	2235	2271	-1.58	100
5	1135	1135	2270	2271	- .04	100
1 (8004)	1510	1530	3040	3028	+ .39	102
2 with	1530	1530	3060	3028	+1.05	102
3 slotted	1540	1540	3080	3028	-1.70	102
4 screens	1540	1550	3090	3028	+2.04	102
5	1540	1550	3080	3028	+1.70	100
1 (8006)	1160	1150	2310	2272	+1.67	102
2 with	1150	1120	2270	2272	-0.09	102
3 slotted	1150	1140	2290	2272	+0.79	102
4 screens	1160	1150	2310	2272	+1.67	104
5	1160	1140	23310	2272	+1.67	104
1 (8008)	1510	1530	3040	3028	+0.39	104
2 with	1515	1515	3030	3028	+0.03	104
3 slotted	1510	1020	3030	3028	+0.03	104
4 screens	1480	1510	2990	3028	-1.25	104
5	1490	1510	3000	3028	-0.92	104
1 (8010)	1840	1800	3640	3785	-3.80	104
2 with	1860	1840	3700	3785	-2.24	104
3 slotted	1860	1825	3685	3785	-2.64	104
4 screens	1870	1850	3720	3785	-1.70	105
5	1890	1860	3750	3785	-0.92	105

1 In these trials about 2 gallons of the "neat" was passed through the 50 mesh screens of the 8003 nozzles. Some of the Bt started to build up on the screens, and this could cause a potential problem.

Table 6. Calibration data with the ground equipment calibrator using Condor diluted with 3 parts water at 16 BIU/gal. The pump pressure was 40 psi.

Run[1] number	Nozzle[1] ml	Nozzle[2] ml	Total volume ml	Calculated volume ml	% needed	Temp. °F
1 (8004)	1610	1520	3130	3028	+3.36	75
2 with 50	1590	1520	3110	3028	+2.70	76
3 mesh	1550	1510	3060	3028	+1.05	76
4 screens	1540	1510	3050	3028	+0.73	76
5	1530	1500	3030	3028	+0.07	77
6	1530	1490	3020	3028	-0.26	77
7	1530	1490	3020	3028	-0.26	78

1 The formulation did not heat up as fast as the neat material. Total spray mixture was 2 gallons Bt + 5 gallons & 107 fl. oz. water + 21 fl. oz. Bond sticker.

Table 7. Calibration data with the aircraft equipment using the Micronair atomizers (AU5000) and applying neat Condor at the rate of 64 BIU/gal., with a VRU setting of 5.

Run number	Boom pressure	Nozzle number[1,2] 1	2	3	4	5	6	7	8	Total ml	Calculated	%
1	40	940	990	975	1045	1062	1020	975	920	8592	7855	-8.6
2	40	920	1000	990	1055	990	990	980	900	8592	7855	-8.9
3	42	1010	1040	1035	1095	1020	1020	1030	935	8185	7855	-4.7
4	46	1080	1130	1120	1160	1095	1130	890	1030	8635	8592	+0.5
5	46	1075	1130	1130	1175	1095	1095	1097	1010	8807	8592	+2.5
6	46	1055	1080	1090	1155	1050	1075	1015	980	8805	8592	-1.1
7	46	1080	1095	1070	1155	1075	1060	1086	1086	8691	8592	+1.1
8	46	1040	1054	1090	1135	1050	1075	1020	865	8429	8592	-1.9

1 The temperature of the formulation was 78-79°F.
2 Those numbers in parentheses () were the averages of seven other readings of the same nozzle number.

ECOGEN, INC.

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TELECOPIER (215)757-2956

Physical Characteristics of EC 178-01

Aerial Test - Mission, Texas

a) % Solids	25.00
(% Non-soluble Solids)	12.79
b) Pounds/Gallon	7.88
c) Specific Gravity	0.95
d) Particle Distribution	

```
                    Percent                                      Raw      Weight
  0       20       40       60       80       100                count      count

125                                                        oz       0          0
 63#                                                        2%     154      14323
 31#                                                        3%     396      18343
 16###                                                      7%    1811      41229
 B ######################                                  43%   22155     249241
 4 #################                                       35%   38414     201676
 2 ###                                                      6%    15560     35011
<2 ##                                                       4%    44152     22076
```

Counts per cycle (avg): 122643
Accumulated count: 3188716
Mean size: 8.5
Level of relfectance: 75
Sample cycles: 26
Sample time: 2 sec.

e) % Sieve retention (Non-soluble particles greater than 45 microns)	0.00
f) pH (25% solution in water)	5.85
g) Viscosity profile:	

rpm	cps
0.3	31,600
0.6	8,200
1.5	4,020
3.0	2,250
6.0	1,180
12.0	670
30.0	380
60.0	260

Graphs of each individual spray run displaying aircraft location and deposit are available upon request.

LOW VOLUME APPLICATIONS OF DIMILIN

A study was conducted to determine spray residue on northeastern oak foliage as a separate part of 1988 field studies with low volume applications of Dimilin (see GM 5.1.1). This work was designed and implemented by the Northeast Forest Aerial Application Technology Group with funding from Uniroyal Chemical. Spray analysis and report preparation was done by Dr. Yendol and associates at Pennsylvania State University. Spray plot establishment and application was done by the Animal and Plant Health Inspection Service, Otis Methods Development Center and Aircraft Operations, Moore Field, Mission, Texas.

INTRODUCTION

In 1988, the USDA Forest Service cooperative program provided partial funding for the suppression of gypsy moth in 473,520 acres of infested forest using Dimilin. These treatment area included ten states in the northeastern United States.

To date, no studies have been published on Dimilin residue patterns on oak forest canopies.

The objective of this study was to determine the spray residues deposited in the upper and lower portions of an oak canopy following the application of two Dimilin formulations by a fixed winged aircraft.

METHODS AND MATERIALS

Plot Selection and Formulations - The test area was located in Jefferson and Elk Counties, Pennsylvania on State Game Lands 54. Each spray block consisted of a 50 acre area. A number of these blocks had previously been established for evaluating various tank mixes of Dimilin as to its efficacy at different application rates (see GM 5.1.1A). The spray mixes considered in the trial were 128, 64, 32 and 16 fl. oz./acre. Each block received 0.0312 lbs./A of active ingredient (AI). The specific formulations used were Dimilin 25W and a special formulation designed for ultra-low volume application (ULVAD). For the residue samples, two of these blocks were selected. One block (B-40) was treated at the rate of 128 fl. oz./A (25W) and another (B-42) at 32 fl. oz./A (ULVAD).

The 128 fl. oz./A tank mix (0.03120 lbs. AI/A) provided 0.00374 ug of Dimilin/nanoliter of spray mixture. The 32 fl. oz./A tank mix resulted in 0.01495 ug of Dimilin/nanoliter of spray. A tracer, Keystone Red was premixed in water and then added to the final spray mixture at the concentration of 8 g/liter. Each tank mix was sampled and then used in the calibration procedure for the fluorometric analysis.

Application of Material - Application of material was made using a Cessna-Ag Truck (300 hp, fixed-wing aircraft with a wing span of 41 ft. 8 in.). Aircraft speed was nominally 120 mph and flown 50 ft. above the oak canopy. A

summary of the spray systems, pump pressure and swath width used in the application of Dimilin is found in Table 1.

Weather Monitoring System - Meteorological recordings were taken during the spray run, one minute before, during spraying and 5 minutes after the treatment. The station was positioned 50 ft. (15.2 meters) above the canopy, with recordings made of the wind speed, wind direction, wet and dry bulb temperature and relative humidity. The stations were positioned near each treatment area in a central location. These recordings were made with the aid of a radio transmitting tethersonde sensor (A.I.R. Inc., Boulder, CO). Temperature and relative humidity were also taken at 3 ft. (1 meter) above the ground level.

Foliage Residue Sampling - Ten sampling trees were selected in the center ten acre portion of each treatment plot. With the aid of a tree climber, four groups of ten leaves each were selected from the upper portion of the canopy and the same number from the lower portions of the canopy. The leaf samples were placed in plastic bags and returned to the laboratory for the wash-off procedure. Twenty-five of these leaves were selected at random from each canopy level and used for wash-off.

Foliage Wash-off Procedure - For fluorometric determinations, a single sample leaf was placed in a 125 ml polyethylene bottle containing 25 ml of 30% methanol and then shaken for one hour. Measurements were made with a fluorometer (Turner 112) fitted with the appropriate filters. Instrument calibration was performed with standard tracer solutions containing Dimilin and water, similar to the final spray mix. The surface area (cm^2) of each leaf was determined with a Li-Cor surface area instrument. Deposit per unit area determined by fluorometry was expressed as micrograms per cm^2.

RESULTS AND DISCUSSION

A summary of the weather data, averaged over a period of one minute before spraying, during the spray run and 5 minutes following application, is found in Table 2. The mean wind speed ranged from 5.4 to 1.0 m/sec (ca 12-2 mph) in the 128 fl. oz. plot. In the 32 fl. oz. treatment, the mean wind speed was 1.9 m/sec (ca 4 mph). Temperature remained at about 19°C, with the relative humidity at 54 percent. Weather data for the 32 fl. oz. treatment was taken during the application, but was not recorded during the period before and after spraying because of a malfunction in the ADAS unit.

The 128 fl. oz. block was sprayed from northeast to southwest using a reciprocating type pattern (see Appendix A), with moderate crosswinds generally out of the west. In the case of the 32 fl. oz. application, the block was sprayed from the north to south direction using the same type of flight pattern. During spraying, low crosswinds emanated from the west.

The mean mass of spray residue recovered from the leaf surface was determined using the geometric mean method (Table 3). With the geometric mean method, all the deposits are analyzed using log transformed data. When compared with the arithmetic mean method, the geometric mean method is a more conservative type of calculation that prevents the extreme quantities of the sample distribution from significantly effecting the estimated mean. Thus, the simple arithmetic mean most often overestimates the effective mean deposit due

to the influence of a small fraction of heavily dosed leaves.

However, a summary of the arithmetic mean data is also included for the purpose of comparison (Appendix B).

A highly significant third order interaction was found between plot, tree and level (Table 4). No interaction occurred between plot and level analysis. Thus, the significant level effects were consistent in both plots. Significantly less Dimilin spray (F=9.24, df 1,960 P>F 0.0024) was caught in the canopy receiving the 128 fl. oz. application (0.00912 micrograms/cm^2) than in the 32 fl. oz. treatment (0.01413 micrograms/cm^2) (Table 4). In each treatment, the upper portion of the canopy received more of the Dimilin spray than the lower level (F=7.85, df 1 P>F 0.0118).

With reference to spray accountability, generally low amounts of deposits were recovered from the foliage in each of the plots. A one acre of forest oak canopy is usually considered to be equivalent to a surface area of 5 acres. If this factor is employed in calculating the percent of spray recovered on a ug/cm^2 basis (AI of Dimilin), then only 13 percent of the spray applied was recovered from the foliage sprayed with the 128 fl. oz., and 20 percent from the 32 fl. oz. applications. Other sources of spray not measured would include deposits on the trunk, branches, ground and drift loss from the target area.

Because of high degree of variation in the residue samples of the upper and lower canopy portions, the deposit levels between blocks could not be compared (Table 5).

There was a significant difference in the mean foliage deposit between trees (F=2.42, df 1,18 P>F 0.0008) of each treatment (Table 6). This may reflect a combination of the different amounts of spray volumes used (Note: same AI applied per plot) and possibly different canopy structures and leaf densities that are commonly found in an oak forest.

Table 8: Nozzle type and other spray system characteristics.

Block number	Oz./acre	Nozzle size	Nozzle number	Nozzle direction	Pump pressure (psi)	Swath width (ft)
40	128	8004	45	90°	40	75
42	32	8002	23	90°	40	75

Table 9: A summary of the 15-meter weather data collected from the tethersonde station at the 128 fl. oz. treatment.

Time period	Wind direction Mean direction	Max.	Min.	Wind speed Mean m/sec	Max.	Min.	Temp. (°C)	RH (%)
			128 fl. oz. Treatment					
1 min. Before	280	296	242	3.86	4.4	3.1	19.0	54
During Spray	273	355	203	3.20	5.4	1.0	19.0	54
5 min. After	287	322	255	3.22	4.8	1.6	19.2	54
			32 fl. oz. Treatment					
During Spray	294			1.94			20.2	56

Table 10. An estimate of the Dimilin spray (micrograms/cm^2 AI) found on oak foliage when aerially applied at 128 and 32 fl. oz. per acre.

Rate	N	Tree section	Micrograms/cm^2 (a) mean(a)	Log 10 mean	error
128 fl.oz./A	500	both	0.00912	-2.04	0.04
	250	lower	0.00676	-2.17	0.06
	250	upper	0.01202	-1.92	0.05
32 fl.oz./A	500	both	0.01413	-1.85	0.04
	250	lower	0.01148	01.94	0.07
	250	upper	0.01698	-1.77	0.07

(a) Calculated using the geometric mean method.
(b) Transformed from Log10 mean value.

Table 11. Analysis of variance for canopy deposition (log ug/cm^2) of Dimilin sprays in oak forests using a factorial treatment design.

Source of variation	F	df	Pr>F
Plot	9.24	1,960	0.0024
Tree (plot)	2.42	1,18	0.0008
Level	7.85	1	0.0118
Plot*level	0.31	1,18	0.5858
Plot*Tree*Level	1.46	18,960	0.0001

Table 12. Mean Dimilin spray deposit for the upper and lower levels of individual oak tree canopies.

		ug/cm^2 of Dimilin					
		Lower			Upper		
Treat-ment error	Tree No.(a)	Mean number(b)	Log10 mean	Std. error	Mean number(b)	Log10 mean	Std. error
128 fl.oz.	1	0.00347	-2.46	0.38	0.01479	-1.83	0.06
	2	0.00794	-2.10	0.06	0.01047	-1.98	0.06
	3	0.00479	-2.32	0.38	0.01000	-2.0	0.08
	4	0.01479	-1.83	0.04	0.02570	-1.59	0.04
	5	0.01349	-1.87	0.06	0.01738	-1.76	0.07
	6	0.00269	-2.57	0.11	0.00692	-2.16	0.04
	7	0.00549	-2.26	0.07	0.00977	-2.01	0.13
	8	0.01349	-1.87	0.08	0.02089	-1.68	0.09
	9	0.00692	-2.16	0.05	0.00331	-2.48	0.05
	10	0.00603	-2.22	0.05	0.02138	-1.67	0.09
32 fl.oz.	1	0.01230	-1.19	0.02	0.01698	-1.77	0.03
	2	0.01122	-1.95	0.37	0.03631	-1.44	0.05
	3	0.03715	-1.45	0.03	0.03235	-1.49	0.05
	4	0.00489	-2.31	0.51	0.03631	-1.44	0.05
	5	0.01072	-1.97	0.38	0.01380	-1.86	0.38
	6	0.00851	-2.07	0.04	0.02399	-1.62	0.03
	7	0.01549	-1.81	0.04	0.00691	-2.16	0.36
	8	0.01513	-1.82	0.05	0.02630	-1.58	0.04
	9	0.00617	-2.21	0.36	0.00871	-2.06	0.08
	10	0.01259	-1.90	0.03	0.00501	-2.30	0.36

(a) Based on 25 leaf samples for each tree.
(b) Means based on transformed log10 data.

Table 13. Mean Dimilin spray deposit micrograms/cm^2 (ug/cm^2) for individual oak tree canopies.

Tree Number	ug/cm^2 of Dimilin 128 fl. oz. Mean number(b)	Log10 mean	Std. error	32 fl.oz. Mean number(b)	Log10 mean	Std. error
1	0.00724	-2.14	0.02	0.01445	-1.84	0.02
2	0.00912	-2.04	0.04	0.02042	-1.69	0.19
3	0.00692	-2.16	0.20	0.03467	-1.46	0.03
4	0.01949	-1.71	0.03	0.01349	-1.87	0.26
5	0.01548	-1.81	0.05	0.01230	-1.91	0.19
6	0.00427	-2.37	0.21	0.01413	-1.85	0.04
7	0.00724	-2.14	0.07	0.01047	-1.98	0.18
8	0.01659	-1.78	0.06	0.01995	-1.70	0.04
9	0.00479	-2.32	0.04	0.00741	-2.13	0.18
10	0.01122	-1.95	0.07	0.00794	-2.10	0.18

(a) Means based on transformed log10 data.

APPENDIX B

Amount of a Dimilin spray mixture (micrograms/cm^2) found on oak foliage when aerially applied at 128 and 32 fl. oz./acre (determined using an arithmetic mean).

Tree location	Mean	Standard	Variable	Maximum	Minimum
			ug/cm^2		
			128 fl. oz. per acre		
Both levels	0.01634	0.01648	0.07254	0.12671	0.00015
Upper	0.02127	0.02015	0.10866	0.12671	0.00059
Lower	0.01140	0.00927	0.02311	0.07093	0.00015
			32 fl. oz. per acre		
Both levels	0.02377	0.01765	0.02124	0.12950	0.00269
Upper	0.02796	0.02034	0.02767	0.12950	0.02241
Lower	0.01974	0.01361	0.01241	0.07896	0.00269

Project Number: GM 88.1.1 A
Project Title: Field Studies with Low Volume Applications of Dimilin - 1988
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leaders: W. McLane, J. Finney, T. Roland and W. Yendol

Introduction

Dimilin (diflubenzuron) was registered for gypsy moth control in 1976. The material can be used at a dosage of 0.25 to 1.0 ounces of active Dimilin in 0.5 to 2.5 gallons of carrier per acre. For the past 12 years, a formulation of Dimilin 25W has been used for nearly all gypsy moth treatments.

During 1988, nearly 67 percent of all acreage sprayed for gypsy moth was treated with Dimilin 25W. A dosage of 0.5 ounces active ingredient in a rate of 1.0 gallons per acre was most commonly used. In nearly all cases, applications were very successful.

Recently, there has been interest in low volume applications for gypsy moth control. A considerable amount of work has been conducted with neat (32 oz/acre) applications of Bacillus thuringiensis, Bt. Most tests have demonstrated that such a treatment can be as effective, if not superior, to high volume applications (see GM 5.1.1 D of this Laboratory Report). Often, such treatments have to be made with specialized application equipment such as Micronair or Beecomist atomizers. This enables superior break-up and fine droplets resulting in better coverage. However, droplets should not be so fine that off-target drift occurs.

By reducing rate of application, the overall cost of the application should be reduced. The aircraft can do increased acreage per load, eliminating ferrying and loading time. Often, low volume can be applied undiluted, resulting in the elimination of mixing time and cost. The end result should be increased efficacy as more acreage can be treated under more suitable spray conditions.

Limited studies have been conducted with low volume applications of Dimilin 25W. Some work has been done at 0.5 gallons per acre, (see Otis Laboratory Reports, 1976 and 1978). Although results were excellent, if was believed that lower volumes using the Dimilin 25W formulation would result in excessive evaporation and poor efficacy. Therefore, work with low volume applications was terminated until a more suitable formulation was developed.

In 1988, a study was conducted with low volume applications of Dimilin using a special formulation similar to Dimilin 2F. The material was applied at 64, 32 and 16 ounces per acre. A dosage of 0.5 ounces active Dimilin was used for each treatment. Dimilin 25W was applied at 1.0 gallons per acre as a standard. Each treatment and untreated control was replicated 4 times.

Methods and Techniques

Twenty-four woodland plots were established on state game lands in Elk and Jefferson Counties, Pennsylvania. Plots were square and 50 acres in size and located a minimum of 400 feet apart. Boundary lines were surveyed and marked

with fluorescent orange tape and each corner tree was marked with double fluorescent orange tape and a tag identifying corners and plot numbers Plots were located so that there would be a maximum number of corners on or near roadways.

Treatment evaluation consisted of pre- and post-spray egg mass counts, egg hatchability tests, post-spray larvae and egg mass counts under burlap and defoliation observations.

Within the center 10 acres of each plot, 10 prism points were established, 5 points on 2 parallel lines. During March and early April, pre-spray egg mass numbers were recorded on each prism tree and within each fixed radius plot. Prism tree DBH was also recorded. A few egg masses were collected from the field and returned to the laboratory for hatchability tests. Hatch was uniform at 77 percent with little virus load.

Burlap was placed on 5 oak trees at random in the center 10 acres of all plots. Two counts were made on the number of gypsy moth larvae under each band following treatment. After each reading, all larvae were removed from under the burlaps. All new egg masses were counted under burlaps at time of their removal.

At peak defoliation time (early July), a defoliation survey was conducted from the ground. Total defoliation of all oak species was estimated at each prism point within each experimental plot.

An APHIS Cessna Ag-truck aircraft was used to apply all treatments. Applications of 128 and 64 ounces per acre were made with 8004 flat fan nozzles with lesser amounts applied through 8002 nozzle tips. The aircraft sprayed a 75-foot swath at 120 mph, approximately 50 feet above the target foliage. Boom pressure was 40 psi.

Calibration and characterization was conducted at the airport before treatment started. A check of the delivery was conducted in the air. All mixing was conducted in the aircraft just prior to application. Aircraft operations were centered at the DuBois Airport.

All applications were made in the morning hours between 6:00-10:00 a.m. Temperatures ranged between 50° - 70°F. Application was terminated when winds exceeded 6 mph. Humidity was in the 40-60 percent range.

Dr. Yendol and colleagues at Pennsylvania State University did spray residue deposit sampling in the upper and lower portions of oak trees within the center 10 acres of 2 spray plots. Ten trees were sampled in a plot treated at the 128 ounce rate and 10 in a plot treated with 32 ounces per acre. This work was done as a NEFAAT project (see GM 88.1.1 of this laboratory report).

Results

All treatments gave exceptional gypsy moth population reduction based on pre- and post-spray egg mass counts. Rates of 32 and 64 ounces per acre were superior to all others. However, the 16 and 128 ounce per acre rates were also highly effective. The reduced kill with the Dimilin 25W standard treatment at 128 ounces per acre may indicate a much greater degree of

evaporation with the powder formulation as compared to the special low volume mix. The residue study conducted by Dr. Yendol would tend to support this theory.

Although the average gypsy moth population was low (300 egg masses per acre), the population was very healthy and building as indicated by the post-spray egg mass counts in the control plots. Similar tests should be conducted in 1989 on higher population densities (1,000 - 5,000 EM/A).

With this special formulation of Dimilin it appears that it may be possible to consistently achieve excellent population reduction using 32 or less ounces of carrier per acre. If so, it may also be possible to lower the dosage and, therefore, ease environmental concern about the material. Such reductions in dosage and rate of application would add up to dollar saving as well as fewer environmental problems.

Because of solubility problems with the dye used for residue studies, we had problems with the 16 ounces per acre treatment. When attempting to spray the first plot, the aircraft had to return to the airport 3 times to clean out clogged nozzle tips and screens. Following treatment of the first plot, the hopper was cleaned out and material screened a number of times through panty-hose. About 4 inches of solids from the formulation had settled on the hopper bottom. This material was removed and properly discarded. There may have been a good deal of active Dimilin remaining with the disposed material. The screened liquid was then sprayed on the remaining 3 plots with the result being very good control.

In summary, it appears low volume applications of Dimilin (special formulation) can be very effective. Testing at lower rates and dosages should continue with the aim being a "no mix" formulation that can be applied at a minimum dosage and rate to gypsy moth populations of any density.

Table 1. Spray system and application data

Material	Ounces per acre	Nozzle tip size	Number	Boom angle
Dimilin 25W	128	8004	45	90°
Dimilin (Special)	64	8004	23	90°
Dimilin (Special)	32	8002	23	90°
Dimilin (Special)	16	8002	11	90°
Control	-	-	-	-

Table 2. Total larvae found under burlaps following treatment and average defoliation.

Treatment	Total larvae after 18 days	Total larvae after 31 days	Average defoliation for each treatment
128 oz.	15	15	< 5%
64 oz.	0	0	< 5%
32 oz.	1	0	< 5%
16 oz.	36	25	< 5%
Control	66	160	11%

Table 3. Percent population change based on pre- and post-spray egg mass counts.

Treatment	Pre-spray EM/acre	Post-spray EM/acre	Percent change
128 oz.	1,110	50	- 95.4
64 oz.	1,101	10	- 99.0
32 oz.	1,193	4	- 99.6
16 oz.	1,103	66	- 94
Control	894	5,459	+510.6

Table 4. An estimate of the Dimilin spray (micrograms/cm^2 AI) found on oak foliage when aerially applied at 128 and 32 fl. oz. per acre.

Rate	N	Tree Section	Micrograms/cm^2 Mean(a)	Log 10 Mean(b)	Error
128 fl. oz./A	500	both	0.00912	-2.04	0.04
	250	lower	0.00676	-2.17	0.06
	250	upper	0.01202	-1.92	0.05
32 fl. oz./A	500	both	0.01413	-1.85	0.04
	250	lower	0.01148	-1.94	0.07
	250	upper	0.01698	-1.77	0.07

(a) Calculated using the geometric mean method.
(b) Transformed from Log10 mean value.

Project Number: GM 88.1.1B
Project Title: Field Studies With 2 Formulations of Condor, a New Bacillus thuringiensis, Bt Strain - 1988
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leaders: W. McLane, J. Finney and T. Roland

INTRODUCTION

During the past 3 years, a number of new Bacillus thuringiensis (Bt) strains have been laboratory tested at the Otis Methods Development Center. Most were from Ecogen, Inc., products of an aggressive development program to identify and market a strain of Bt that is superior to ones presently in use for gypsy moth and other forest defoliator control. Objectives for Bt strain improvement at Ecogen, Inc., included increasing the inherent potency of Bt against selected target insects and broadening its insecticidal spectrum against target species for specific crop applications.

Efforts were made to improve field residual activity and to optimize production stabilization of the product. One major consideration in approach to strain improvement was to develop technologies that result in products that minimize delays in obtaining regulatory approvals for field testing and registration of products. The strategies used include the screening of a large number of new isolates to characterize their insecticidal activities, the identification of extrachromosomal plasmids that carry toxin-encoding genes and the construction of new genetic combinations by employing a native conjugal plasmid exchange system that exists in Bt.

The genes encoding insecticidal proteins found in delta-endotoxins are not localized on chromosomes in Bt. Rather, they are carried on several non-essential, extrachromosomal plasmids. The toxin-encoding plasmids in Bt are self-transmissible from one Bt strain to another through a conjugation-like mechanism. The localization of genes encoding insecticidal toxins to specific plasmids was determined by using several methods. First, the loss of insecticidal crystal product was associated with the "curing", or natural loss, of a specific plasmid giving circumstantial evidence for the association of toxin encoding genes with plasmids. Secondly, the transfer of toxin-synthesizing ability from a crystal-producing strain to a non-producing strain was associated with the transfer of specific plasmids giving further evidence that the toxin-encoding genes were, in fact, associated with specific plasmids.

After numerous laboratory bioassays with laboratory reared gypsy moth larvae, the EG2348 strain was selected for development and given the name Condor.

The Condor strain (EG2348) was generated by first selecting a plasmid-cured derivative of a naturally-occurring Bt that had greater potency than the strain that constitutes most current products for lepidopteran insect control (HD-1). Although this cured strain had lost a plasmid containing an insecticidal protein gene, the several genes that remained produced crystals that were more active than those of the parental strain. The cured strain was then mated, using the natural Bt conjugation process, with another strain containing a plasmid that contained a gene that specified a protein having high gypsy moth activity. The resulting "transconjugant" produced

insecticidal crystals having greater potency than the HD-1 standard against gypsy moth larvae.

Condor OF (oil flowable) and Condor AF (aqueous flowable) formulations are available for testing under an EUP. Field tests (50 acre plots) were planned for 1987, however, an EUP was not received in time for testing. An EUP was granted in early 1988 and field tests with each formulation were conducted in 1988. Dipel 8L (HD1 strain) was used as a standard.

METHODS AND TECHNIQUES

Twenty woodland plots were established on State game lands in Jefferson County, Pennsylvania. Plots were square and 50 acres in size and located a minimum of 400 feet apart. Boundary lines were surveyed and marked with fluorescent orange tape and each corner tree was marked with double fluorescent orange tape and a tag identifying that corner and plot number. Plots were located so that there would be a maximum number of corners on or near roadways.

Treatment evaluation consisted of pre- and post-spray egg mass counts, egg hatchability tests, post-spray larval counts under burlaps, post-spray egg masses under burlaps and defoliation observations.

Within the center 10 acres of each plot, 10 prism points were established, 5 points on 2 parallel lines. During March and early April, pre-spray egg mass numbers were recorded on each prism tree and within each fixed radius plot. Prism tree DBH was also recorded. A limited number of egg masses were collected from the field and returned to the laboratory for hatchability tests. Hatch was uniform at 77 percent.

Burlap was placed on 5 oak trees at random in the center 10 acres of all plots. Two counts were made of the number of gypsy moth larvae under each burlap following treatment. After each reading, all larvae were removed from under the burlaps. New egg masses were counted under burlaps at time of their removal.

At peak defoliation time (early July) a survey was conducted from the ground and defoliation recorded. Total defoliation of all oak species was estimated at each prism point within each experimental plot.

Each material was applied at 16 BIU/gallon/acre to 5 plots. Five untreated plots were used as a control. All treatments were made between 6:30 a.m. and 12:30 p.m.

An APHIS Cessna Ag-truck aircraft was used to apply all treatments. The diluted formulations were applied through 8004 flat fan nozzles with 50 mesh screens. The aircraft sprayed a 75-foot swath at 120 mph, approximately 59 feet above the target foliage. Boom pressure was set at 40 psi. A total of 45 nozzles at 90° (straight down) were used for each application.

Calibration of each formulation was conducted in the air near the airport before treatments started. Mixing was done in a nurse tank and material was then pumped into the aircraft. Mixes were blended by agitation for at least 10 minutes before being loaded into the aircraft. The nurse tank was equipped

with a 50-mesh, in-line screen. All mixing and aircraft operations were centered at the Dubois Airport. Water with a PH of 6.7 was used in each mix. Bond sticker was used at 2 percent by volume with all mixes.

RESULTS

Condor OF and Dipel 8L gave very similar results based on only defoliation estimates and larvae under burlap counts. Condor AF was the least effective of the formulations tested. Condor OF followed closely by Condor AF were the most effective formulations based on new egg masses found under burlap bands. Using this evaluation technique, Dipel 8L was no better than the control.

Although such evaluation techniques are useful to identify trends in the study population, conclusions drawn are preliminary. Numbers of larvae under burlaps can be influenced by their size and the time of day counts are made. Post-spray egg mass counts under burlaps can be influenced by tree species. When defoliation is light, it is difficult to distinguish between slight differences. At best, defoliation estimates should be made in increments of 20 (example 0-20, 21-40). Treatments in this test all had defoliation of 20 percent or less making it difficult to analyze results.

In the final analysis, success or failure of the treatment is based on pre- and post-spray egg mass counts. This is the most important and final analysis made in nearly all operational gypsy moth spray programs.

Based on statistical analysis, there was no significant difference between any of the treatments tested. Although Dipel 8L gave a 68 percent egg mass reduction as compared to 42 and 38 for Condor OF and Condor AF, respectively, they could not be separated statistically. There was a statistical difference between the treatments and the control.

We had no problem in mixing any of the formulations. Bond was added last to the mix and appeared to be very compatible with each formulation.

In summary, the Condor (EG2348) strain was as efficacious in the field against gypsy moth larvae as the presently registered HD1 strain, (Dipel 8L). There was no statistical difference between the OF and AF formulations. It was somewhat disappointing that the performance of the EG2348 strain was not superior to that of the HD1 strain, (Dipel 8L). However, with biological insecticides, significant differences have to appear between strains in the laboratory before they can be identified in field tests.

Based on these and other investigator's test results, Condor OF should be recommended for pilot testing in 1989.

Table 1. Average number of larvae found under burlaps per plot following treatment.

Formulation	Number of larvae 20 days after spray	Number of larvae 33 days after spray
Condor OF	14	42
Condor AF	36	84
Dipel 8L	26	47
Control	61	188

Table 2. Percent post-spray defoliation and total numbers of new egg masses found under burlaps per treatment.

Formulation	Percent defoliation	Total egg masses under burlaps
Condor OF	6	32
Condor AF	20	40
Dipel 8F	10	118
Control	30	107

Table 3. Percent population change based on pre- and post-spray egg mass counts.

Formulation	Pre-spray EM/acre	Post-spray EM/acre	Percent Change
Condor OF	4,414	2,542	-42
Condor AF	4,479	2,794	-38
Dipel 8L	4,508	1,451	-68
Control	4,553	4,734	+ 4

Table 4. Statistical analysis of data using Walton technique.

Condor OF	R = 0.575894	R = 43.4%
	$Sigma^2$ = 0.033813	$Sigma^2$ = 3.4%
	Std = 0.183883	Std = 18.4%
Condor AF	R = 0.623799	R = 37.6%
	$Sigma^2$ = 0.076476	$Sigma^2$ = 7.6%
	Std = 0.276542	Std = 27.7%
Dipel 8L	R = 0.321872	R = -67.8%
	$Sigma^2$ = 0.008393	$Sigma^2$ = 0.8%
	Std = 0.091615	Std = 9.2%
Control	R = 1.127548	R = 3.9%
	$Sigma^2$ = 0.643458	$Sigma^2$ = 64.3%
	Std = 0.802158	Std = 80.2%

Project Number: GM 88.1.1C
Project Title: Field Studies With Early Dimilin Applications in Delaware - 1988
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leader: W. McLane, N. Schneeberger, L. Bradley, T. Roland and P. Bohne

The Animal Plant Health Inspection Service, Forest Service, Delaware Department of Agriculture and Uniroyal Chemical cooperated in a study to determine the usefulness of early applications of Dimilin for gypsy moth control.

Introduction

In 1986 the USDA Forest Service, Animal and Plant Health Inspection Service (APHIS), West Virginia Department of Agriculture, Pennsylvania Department of Environmental Resources and Uniroyal Chemical Company, Inc. cooperatively evaluated the effectiveness of Dimilin (diflubenzuron) applied as follows:

1. First sign of egg hatch
2. General hatch (100%)
3. Standard timing (ca. 30% foliage expansion; 1st & 2nd instar)

The results were not statistically conclusive, however, acceptable foliage protection and population reduction were achieved in several of the early-timed blocks to warrant further evaluation (McLane and Schneeberger 1986; McLane and Roland 1986).

The following year (1987) another field evaluation was carried out in West Virginia to evaluate Dimilin applied at:

1. First sign of egg hatch
2. General hatch (100% egg hatch with some dispersal)

Populations were significantly reduced in the block treated at general hatch, and no noticeable defoliation was detected. Post treatment populations were <u>not</u> significantly reduced in the area treated early, however, foliage protection was achieved in much of the block. The untreated check area was severely defoliated throughout. Based in part on these results, the Dimilin label was modified to permit treatment at general hatch (McLane 1987).

In 1988, the Forest Service and APHIS decided to again look at the effectiveness of earlier-timed Dimilin applications. The project was a cooperative effort between USDA Forest Service and APHIS, Delaware Department of Agriculture (DDA), Maryland Department of Agriculture (MDA) and Uniroyal Chemical Company, Inc. The objectives of this evaluation were to:

1. Determine if Dimilin applied at the beginning of egg hatch would protect foliage and reduce gypsy moth populations.

2. Determine the effectiveness of early-timed Dimilin applications compared to operationally timed-applications.

Methods and Materials

Project Area Selection

Nineteen (19) discrete woodlots ranging in size from 45 to 253 acres were located in Kent County, Delaware, and Caroline and Queen Annes Counties, Maryland, according to the following criteria:

1. No defoliation present the previous year.
2. Healthy, building populations with no overt evidence of virus.
3. Egg mass counts ca. 1000-3000/acre.

Fourteen (14) woodlots in Kent County, Delaware, were selected to receive the Dimilin treatments. These woodlots were previously scheduled for treatment by DDA in their planned gypsy moth suppression program. Four woodlots in Caroline and Queen Annes Counties, Maryland, and the remaining one in Kent County, Delaware served as untreated checks.

Seven (7) of the 14 woodlots were treated when about 5 percent of the egg masses showed signs of hatch (early timing). This was determined by walking the most southerly exposed edge of each woodlot daily, and observing a minimum of 100 egg masses for any sign of hatch. The woodlots were treated as they became ready until a total of 7 were treated. The treatments were made using the USDA, APHIS Cessna Ag-truck spray plane on April 11, 1988. The remaining 7 woodlots were treated on April 26 and 27 by DDA during their 1988 cooperative gypsy moth suppression project (standard timing). Applications were made using a Piper Pawnee Brave spray plane. All treatments were made at the rate of 0.03 lbs. active ingredient per acre (2 oz. Dimilin 25W) in 1.0 gallons total mix per acre.

Monitoring

Egg Quality Determinations - During March 1988, 10 egg masses, five above the one foot level and five below the one foot level, were collected and returned to the laboratory. The egg masses were held in petri dishes at 80°F and 55% relative humidity for at least 3 weeks. Emerging larvae were counted as were the number of non-eclosed larvae and the percent hatch calculated. A sample of larvae from each collection were reared on artificial diet through 4th instar to evaluate the general health of the population.

Foliage Development - At the time of treatment, foliage development estimates were made of the overstory and understory vegetation on a transect through the middle of each woodlot.

Egg Mass Counts - Ten (10) 1/40 acre fixed radius sampling plots were randomly established within the interior of each woodlot for pre- and post-treatment egg mass counts. Pre-treatment counts were conducted in March 1988 and post-treatment counts in October and November. An analysis of variance was conducted on the rate of change in the egg mass counts between treatment replicates to determine if population differences were significant.

Larval Counts - Five (5) representative chestnut or white oak trees near the center of each woodlot were banded with burlap. Each site was checked weekly and the number of larvae under each burlap band was counted and recorded.

Total larvae counted were compared between treatments.

Defoliation Estimates - Ground estimates of defoliation were made at each of the ten (10) egg mass survey plot locations in the woodlots. Two observers independently estimated oak defoliation (0-100%) at each location. The two estimates were averaged and assigned to one of the following categories: 0, 1-20 percent, 21-40 percent, 41-60 percent, 61-80 percent, and 81-100 percent. An analysis of variance test was used to determine if there were significant differences between the treatments.

Aerial detection surveys using sketch mapping techniques and hand-held photography were conducted by DDA. In addition, high altitude color, infrared optical bar camera photography was also obtained.

Results and Discussion

The stand conditions at the time of treatment are presented in Table 1. Egg hatch in the early Dimilin woodlots ranged from 4-13 percent and averaged 8% percent overall. The egg hatch was less than 10 percent in 6 of the 7 woodlots. Foliage development on the oak overstory ranged from 1-10 percent and averaged 4 percent overall. Oak leaf expansion was less than 5 percent in 6 of the 7 woodlots. In the woodlots that received the standard timed applications of Dimilin, egg hatch averaged 98 percent (range - 95-100%), and oak foliage development averaged 14 percent (range = 10-22%).

A summary of defoliation estimates and pre- and post-treatment egg mass counts is presented in Table 2. Oak defoliation averaged 5% in the standard treatments, 54% in the early treatments and 79% in the untreated check areas. Defoliation levels were significantly different (= 0.05) between all of the treatments (Figure 1, Table 3).

Post-treatment gypsy moth populations were reduced by the standard timed applications (23%) but increased in the early timed applications (446%) and the untreated checks (967%) (Figure 2, Table 3). Post-treatment egg mass counts were significantly different between the standard-timed applications and the untreated check areas, but not between the early treatments versus checks or the standard versus early treatments (= 0.05).

Under the conditions of this field evaluation, early timed applications of Dimilin provided greater foliage protection than not treating at all, but not as great as that provided by the standard timed applications. Current gypsy moth suppression projects are considered to be successful if defoliation is kept to a minimum of about 40 percent. Given this criterion, the early-timed Dimilin applications would likely fail to meet foliage protection objectives of suppression projects. The early-timed Dimilin applications failed to significantly reduce (= 0.05) residual gypsy moth populations compared to the untreated checks. Oddly enough, there was not a significant (= 0.05) difference between the standard-timed and early-timed applications either, even though egg mass counts were reduced by the former treatments in all woodlots, except one. This can probably be attributed to the limited number of woodlots comprising the project area. Additional replicates would likely have minimized the influence of any extreme intra-treatment variation.

This field evaluation did not demonstrate the expected degree of foliage

protection that was suggested by the 1987 project. Why this occurred is open to speculation. Misapplication in all seven blocks is not likely since the treatments were made during very favorable weather conditions, and spray deposit was observed on the spray cards in all of the treatment blocks. Perhaps some investigations should be initiated to determine if these disappointing results are related to limitations of the 25W formulation. The early-timed applications in 1987 were made with Dimilin 25W and Dimilin 2F formulations. We do know that the Dimilin 25W formulation is very susceptible to evaporation. It should also be noted that population reduction (23%) with the standard application was much less than one would expect. A reduction of 90-95% should have occurred.

In summary, the results of this field evaluation support the need to apply Dimilin 25W when general hatch has occurred (100%) and the larvae begin dispersing. Additional laboratory evaluations are recommended to determine the extent of Dimilin 25W formulation limitations, if any.

References

McLane, W. and N. Schneeberger. 1986. 1986 Field Trials with Early Application of Dimilin in West Virginia; pages 219-221, in the Proceedings of the 1986 Gypsy Moth Annual Review. December 1-4, 1986. Norfolk, Virginia.

McLane, W. and T. Roland. 1986. 1986 Field Trials with Early Application of Alsystin and Dimilin; pages 222-223 in the Proceedings of the 1986 Gypsy Moth Annual Review. December 1-4, 1986. Norfolk, Virginia.

McLane, W. 1987. early Application of Dimilin for Gypsy Moth Control; pages 193-199 in the Proceedings of the 1987 National Gypsy Moth Review. December 7-10, 1987. Charleston, West Virginia.

Table 1. Insect and host foliage development at the time of treatment. 1988 Early Dimilin Evaluation.

Treatment	Spray dates	Percent egg hatch	Percent oak leaf expansion
Standard	4/26-27	98	14
Early	4/11	8	4
Check	N/A	N/A	N/A

Table 2. Average oak defoliation levels and pre- and post-treatment egg mass counts by treatment. 1988 Early Dimilin Evaluation.

Treatment	N	Oak defoliation	Pre-treat. egg masses per acre	Post-treat. egg masses per acre	percent change
Standard	7	5	1549	1011	-23
Early	7	54	1709	8062	446
Check	5	79	1337	10860	967

Table 3. Interactions that show significant differences (oc=.05) between treatments. 1988 Early Dimilin Evaluation

	Comparison		
Variable	Standard vs. check	Early vs. check	Early vs. standard
Oak defoliation	yes	yes	yes
Percent change of EM/A	yes	No	No

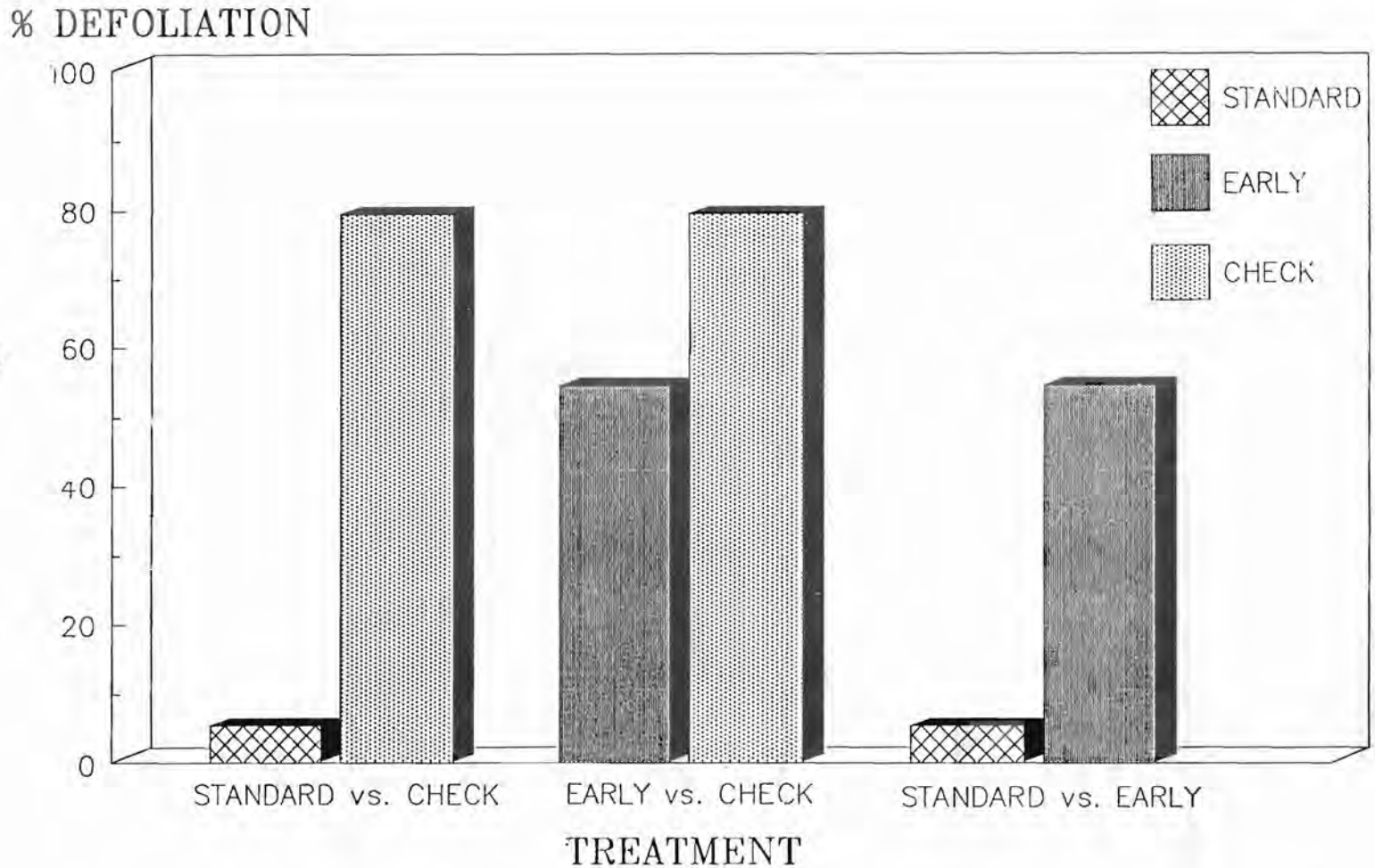


Figure 1. -- Comparison of oak defoliation between treatments.
1988 Early Dimilin Evaluation.

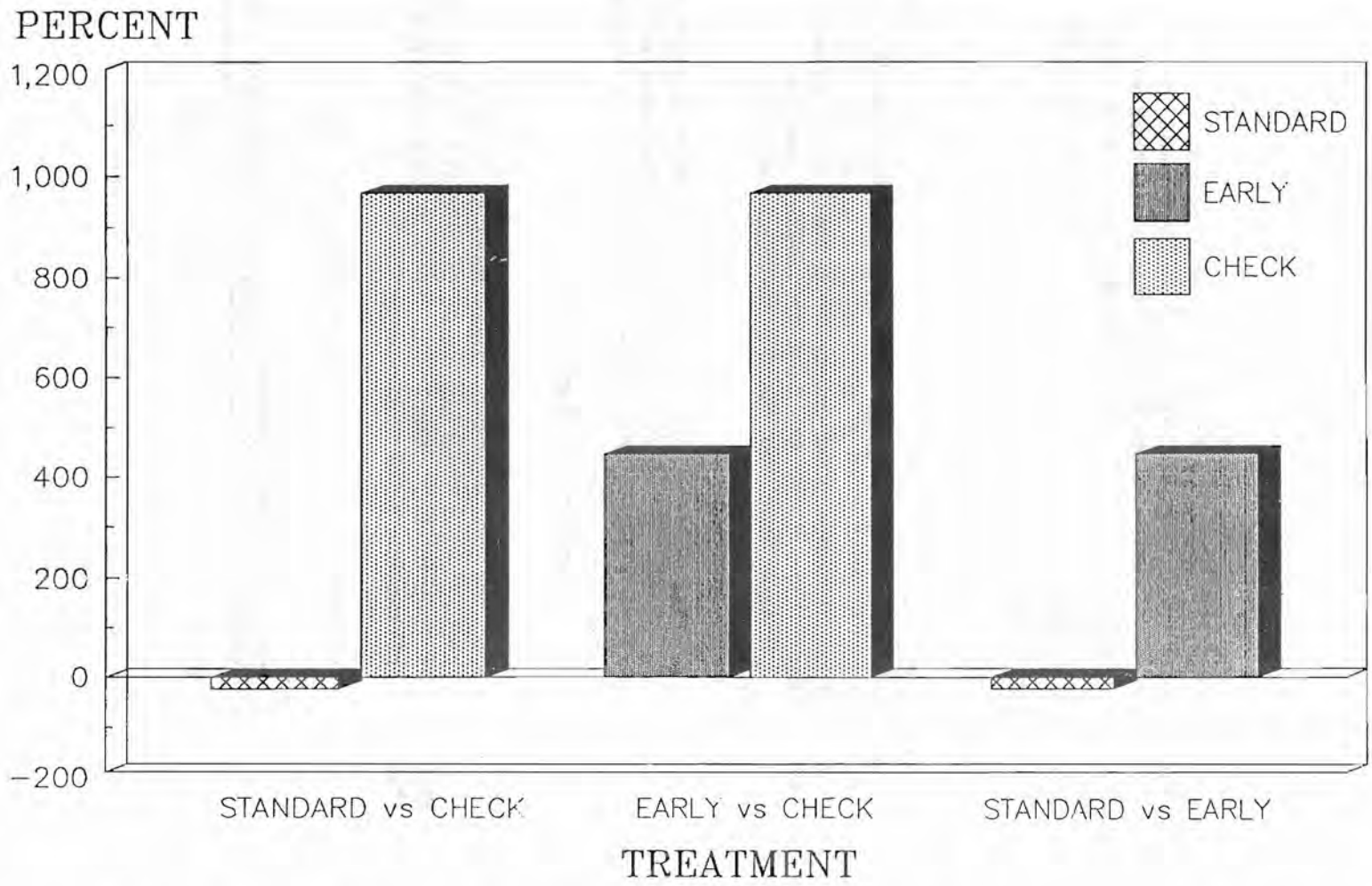


Figure 2 -- Comparison of percent change in egg masses per acre between treatments. 1988 Early Dimilin Evaluation.

Project Number: GM 88.1.1D
Project Title: Field Studies with Dipel 8AF in West Virginia to Determine Optimum Rate of Application - 1988
Report Period: October 1, 1987 - September 30,1988
Report Type: Final
Project Leaders: W. McLane, K. Mierzejewski, R. Reardon, W. Yendol and M. McManus

The work described is the result of a cooperative effort by APHIS, FS and Pennsylvania State University. The cooperators are identified as the "Northeast Forest Aerial Application Technology Group". Their main objective is to conduct field studies with spray materials and equipment that will lead to the selection of more effective materials, equipment and techniques to suppress the gypsy moth and other forest pests throughout the United States and Canada. This work was recommended by the multi-agency "Cooperative Gypsy Moth *Bacillus thuringiensis* Committee".

INTRODUCTION

For years *Bacillus thuringiensis*, Bt has been used at a gallon total volume per acre to treat gypsy moth infestations with each gallon containing 8 to 20 BIU of Bt plus water. It was believed that a minimum of a gallon of spray material per acre was needed to achieve good coverage of the target area. In most cases, a high percentage of the spray was lost to evaporation long before reaching its target. Some states even used 2 gallons per acre in hopes of making Bt more efficient.

During the past 3 years, a number of small plot tests have been conducted with undiluted (neat) applications of Bt. Micronair or Beecomist atomizers were used for application in nearly all tests. The rotary atomizers break up the small amounts of liquid used per acre (32-64 oz) and produce fine droplets. Nearly all undiluted applications have been as efficacious as diluted applications of one or more gallons per acre.

In 1988, per recommendations of the "Cooperative Gypsy Moth Bt Committee", a study was conducted in West Virginia to finalize small plot field studies with diluted and undiluted applications of Bt. Results of this NEFAAT experiment were to determine the most efficacious of 3 treatments.

Table 1. Treatments that were tested on North Mountain, West Virginia during 1988.

Material	BIU/acre	Rate/acre	Nozzles	Acres
Dipel 8AF	20	128 oz 1/	8004	300
Dipel 8AF	20	256 oz 1/	8008	300
Dipel 8AF	20	40 oz 2/	AU5000	300
Control	-	-	-	300

1/ With 2% Bond sticker
2/ Undiluted/no sticker

OBJECTIVES

1. Compare a neat application (40 ounces/acre) of Dipel AF applied by AU-5000 Micronair atomizers with treatments of Dipel AF applied at 1 and 2 gallons per acre by Tee Jet nozzles with stainless steel flat fan spray tips.

2. Establish quality and quantity of spray deposit at 3 levels within the tree canopy.

3. Identify any differences that might occur between large (250-350 micron) and small (75-100 micron) droplets as they pertain to canopy penetration and efficacy. Efficacy will be determined by defoliation prevention and post-treatment population reduction.

4. Upon completion of the test, be able to make recommendations as to what treatment rate is most effective so that states can consider our findings for possible implementation into their 1989 program.

METHODS

A. Plot Establishment

Twenty-four woodland plots of 50 acres each were established on the western slope of North Mountain where a healthy building gypsy moth population was present (600-2,000 egg masses per acre).

Plot corners and lines going to the back corners were marked with fluorescent orange surveyor tape. Each corner was marked with 2 tapes and a paper tag was attached to each corner stating plot number and location. As many of the corners as possible were located on passable roads.

Plots were randomly selected for treatment assignments.

B. Evaluation Area

All evaluations were made in the center 10 acres of each plot (Appendix IV). A grid was established in each plot where prism points were used for pre- and post-spray egg mass counts. Defoliation estimates were made along the 4 grid lines.

C. Spray Materials

Dipel 8AF without dye was the choice of material for the test. Pennsylvania State University personnel added brilliant sulphoflavine dye to the material for evaluation purposes. The Forest Service, Hamden, Connecticut, tested its efficacy on diet. The material was applied at 20 BIU per acre.

Table 2. Bioassay results with samples of Dipel 8AF used in West Virginia spring trials and laboratory standard of HD-15-1980.

Preparation	Slope (95% CI)	LC_{50} (95% CI)	PR_1	LC_{75} (95% CI)	PR_2
HD-1S-1980	4.36 (± 0.46)	43.21 (36.50-49.67)		61.69 (53.68-72.74)	
Dipel 8AF Drum #1	2.30 (± 0.31)	51.21 (38.79-62.47)	0.84	100.63 (82.25-134.56)	0.61
Dipel 8AF Drum #2	2.46 (± 0.31)	50.10 (36.82-62.09)	0.86	94.31 (76.18-127.52)	0.65
Dipel 8AF Drum #3	1.85 (± 0.30)	43.78 (27.78-56.94)	0.99	101.22 (78.66-150.79)	0.61
Dipel 8AF Drum #4	3.32 (± 0.36)	46.34 (38.10-53.96)	0.93	73.97 (63.86-87.63)	0.83
Dipel 8AF Drum #6	3.04 (± 0.34)	45.06 (33.86-54.93)	0.96	75.06 (62.00-94.23)	0.82
Dipel 8AF Drum #7	1.75 (± 0.30)	41.77 (27.18-53.79)	1.03	101.65 (80.04-147.21)	0.61
Combined N=6	x (± SE)	x (± SE)	x (± SE)	x (± SE)	x (± SE)
Dipel 8AF Lot # 16-045 BR	2.45 (± 0.26)	46.38 (± 1.49)	0.94 (± 0.03)	91.14 (± 5.37)	0.69 (± 0.0)

Reference ratios:

$$PR_1 = \frac{LC_{50}\,\text{HD-1S-1980}}{LC_{50}\,\text{Dipel 8AF}} \qquad PR_2 = \frac{LC_{75}\,\text{HD-1S-1980}}{LC_{75}\,\text{Dipel 8AF}}$$

Conclusion: (1) As per labelling regulations, Dipel 8AF (Lot #16-045-BR) appears to be labelled correctly at 64 BIU/U. S. gallon, when evaluated using gypsy moth larvae. (2) Consistent with past observations with Dipel products, the flat slope results in lower than expected potency relative to the standard HD-1S-1980 at LC_{75} or higher doses.

These data were reported by Dr. N. DuBois, Forest Service.

Table 3. Mixes used for the three treatments

Neat	40 oz. per acre
Total	40 oz. per acre
20 BIU/gal/acre	40 oz. Bt per acre
Water	85.44 oz. per acre
Bond 2%	2.56 oz. per acre
Total	128 oz. per acre
20 BIU/2 gal/acre	40 oz. Bt per acre
Water	210.88 oz. per acre
Bond 2%	5.12 oz. per acre
Total	256 oz. per acre

D. Nurse Tanks

Otis Methods Development Center furnished a 400 gallon nurse tank with a strong hydraulic agitation system. They also furnished a portable water pump with a water truck capable of holding 900 gallons at one time. Forest Service at Hamden made available their mixing rig along with other mixing equipment. All mixing equipment had in-line 50 mesh screens that were cleaned after delivering 300 gallons of material. All equipment, especially screens, were checked prior to the program for cleanliness. Equipment was on site one week prior to anticipated spray time and was operated with water to make sure everything was in operating condition.

When mixing was needed, water was added to the nurse tank followed by Bt, sticker and dye. The mix was then topped off with water. Before opening Bt drums, each drum was rolled completely over at least 10 times.

Empty containers were triple-rinsed and waste material held in 55 gallon drums until the end of the program. At that time, the material was sprayed onto a designated area well away from the plots.

Samples of 50 ml size were collected from each drum and also each nurse tank mix for bioassay on diet and seedlings. A sample was also collected from the aircraft spray pump at the time of their arrival from a spray run. A drum sample was taken from the top and after the drum had been half emptied.

E. Airport

The Martinsburg, West Virginia airport was used as a base for mixing and aircraft operations. The mixing and loading area was located away from the main airport activity. The work area was roped-off and only approved personnel were allowed within this area.

F. Aircraft and Dispersal Equipment

Two Cessna Ag-trucks (APHIS-Mission, TX) and a local contract aircraft (Cessna Ag-truck) applied the material. All aircraft were on site 3 days

prior to the anticipated spray time so that systems could be examined and calibration and characterization could take place.

Aircraft equipped with Tee Jet nozzles with flat fan tips traveled at 120 mph and dispersed their material at an altitude of 50 feet above the average height of the tree tops The ag-truck with the Micronair atomizers traveled at 115 mph with a 75 foot swath. Each aircraft was calibrated based on a 75 foot swath. Pressure was as close to 40 PSI as possible. The aircraft worked at right angles to any wind, working from down-wind to up-wind as much as the plots geographical location would allow.

One aircraft was fitted with 8 Micronair atomizers (AU-5000) and calibrated to apply a total of 40 ounces per acre. Inboard atomizers were placed approximately 53 inches from the center of the aircraft fuselage with each thereafter about 36 inches apart. Atomizers did not extend beyond 75 percent of the wing length. Each atomizer was equipped with a tachometer that recorded RPM's in the aircraft cockpit. At calibration and characterization time, blade angles were adjusted to produce a droplet VMD of between 75-100 microns. Calibration was done on the ground by holding 5 gallon plastic pails under each atomizer and dispersing material for a minimum of one minute. Collected material was then measured in plastic graduate cylinders and results recorded. The material was recorded to the atomizer that it came from and each successive reading was charged to the corresponding atomizer. This way we were able to record differences between individual atomizers. A minimum of 4 runs were made and averaged. The same personnel made all measurement readings as the graduated cylinders were positioned on a level surface. After measurement was recorded the material was dumped back into the aircraft for the next run.

The temperature of the material and outside temperature was recorded for each run. Once we approached the flow rate that we needed, a minimum of 4 runs were conducted to finalize our finding. Calibration was conducted at a number of material temperatures as flow rate with neat applications is affected by increase of temperature.

To obtain the proper droplet size (75-100 micron) the atomizer blade angles were all set initially at 35°. The aircraft sprayed over white kromekote cards held horizontally approximately 3 feet above the ground. Droplets were then sized using hand held magnification.

Two aircraft were equipped with Tee Jet nozzles with stainless steel flat fan tips. The one gallon per acre was applied with 8004 tips at 90° and the two gallon with 8008 tips at 45°. Nozzles were evenly spaced across the aircraft including drop nozzles on sturdy pipe under the fuselage. The far outward nozzle did not extend beyond 75 percent of the wing length. The last flat fan nozzle or Micronair atomizer was blocked off where it attaches to the boom and feed material by hose from the end of the boom. This was true for each side of the aircraft. Each flat fan nozzle was equipped with a 50 mesh screen. Each aircraft had an in-line screen of 50 mesh size.

The aircraft with flat fan nozzles were calibrated in the air. One for 2 gallons per acre and the other for 1 gallon per acre. After their systems were charged and pressure set they were positioned on a level area and filled to the 100 gallon mark on the hopper. The aircraft then flew at 120 mph and

sprayed for one minute and returned to the exact location where he was loaded (area was marked with tape). A measurement was made to determine how much material was put out. A minimum of 3 calibration runs were made with each aircraft.

As with Micronair atomizers, the aircraft passed over spray cards to determine drop size to (250-350 micron). All calibration and characterization was conducted with the actual sprayed formulation.

One aircraft did all neat plots. One did only 1 gallon per acre and the other 2 gallons per acre treatments. All aircraft were loaded and left the airport at the same time to treat their assigned plot. The first to return to the airport waited until all were back. They then loaded and went out together to do their second plot and continued in this manner until all plots were treated. This technique gave us assurance that each series of plots were treated under nearly the same weather, insect and foliage conditions.

G. Treatment and Aircraft Guidance

Treatments were made when 50 percent of the gypsy moth larvae had reached early second-instar. The remainder were late first-instar. This was determined by counting and sizing 100 larvae at 10 sites throughout the study area starting with the first sign of second-instar larvae. A count was made daily until spray time.

All applications were made in the morning hours starting at daylight. No afternoon or evening applications were made. When temperatures or wind speed reached 75°F of >7 mph respectively, above treetop, treatment was terminated. When material was not being recorded on spray cards and/or pilots report poor conditions, spraying was terminated. Spray cards were used to document a plot being sprayed and also analyzed at Pennsylvania State University to determine droplet size.

Yellow balloons, approximately 30 inches in diameter, were used at each corner of the spray plots to identify treatment areas. Ground crews and airport personnel had radio contact with the aircraft. A fixed wing monitor aircraft was also used to observe and direct the treatment as well as take photographs.

H. Weather Monitoring

Meteorological recordings were taken at two locations during the spray run, 30 minutes before and 30 minutes after the treatment. The stations were positioned 15.2 meters (50 feet) above the canopy, recording wind speed, wind direction, wet and dry bulb temperatures, relative humidity and barometric pressure. Periodic recordings were also taken above 3 meters above the canopy (10 feet). The measurements allow the estimation of a lapse rate and the calculation of a turbulence index in the spray passage region. Recordings were made with the aid of radio transmitting tethersonde sensor (A.I.R., Inc., Boulder, CO). Three systems were used to monitor the three treatments that were applied simultaneously. Temperature and relative humidity were also taken at the one meter (3 feet) above the ground in each plot. Gauges were positioned in the plots to determine temperatures and the amount of precipitation for a 10 day post-treatment period.

I. Foliage Residue Sampling and Droplet Characteristics

One replication of each treatment was sampled on the first spray day. Ten to 15 sampling trees were selected in each of the center plots. Two sampling areas were located in each of the selected trees, an upper and a lower canopy portion. Approximately 40 leaves were removed from each canopy portion, 10 leaves from each level were placed in a special container to prevent smearing of droplets and then returned to the laboratory for photographic purposes. These 20 photographed leaves were used for image analysis. Photography was accomplished with 100 daylight film, using a Nikon FE 35mm SLR camera. The spray droplet characteristics were determined with an Optomax V Image Analyzer, using custom software to calculate droplets per cm^2 and percentage of the total leaf area covered. The spread factors for the three tank mixes were determined and used to approximate volume per cm^2.

The remaining portion of leaves were used for fluorometric analysis. For fluorometric determinations, a single leaf was placed in a 125 ml polyethylene bottle containing 25 ml of 30% methanol and then shaken for one hour. Deposit per unit area determined by fluorometry was expressed as nanoliters per cm^2.

TREATMENT EVALUATION

Pre-treatment Evaluation

Egg Quality Determination - During April 1988 ten egg masses were removed from each plot and returned to the APHIS Laboratory to determine the viability and general health condition of that specific population. Egg masses were held individually in plastic petri dishes at 80°F and 55% RH for at least 3 weeks. The number of emerging larvae were ascertained as well as the number of non-ecloded larvae. The percent larval hatch and the general health state of the population (possible virus disease, degree of parasitism, large number small larvae and/or larvae never attaining pupation) was determined. Measurements were analyzed by standard ANOV and other appropriate statistical tests. In some cases, a pairwise multiple comparison of means were made using the Newman-Keuls technique.

Egg Masses/Plot Quantification - Pre-spray egg mass determinations were made in each plot using the prism point sample technique. For the prism point sample technique, 20 prism points were established, five per line on the four line grid within the center of each plot. For location and marking purposes, a blue ribbon line was installed leading to the grid area and pink ribbon markings were used to mark the grid lines. Each of the sample sites were marked in pink and blue.

One egg mass was collected at each of the subplots in all treatment plots when the pre- and post-spray egg mass densities were determined. The initial larval density was determined from the collected pre-spray EM. These were placed individually on moist filter pads in petri dishes and incubated at 22°C in a 50% RH with 16:8 photoperiod. The initial larval density for each plot was calculated: EM/a x No. eggs/EM x % hatch.

Post-treatment Evaluations

Defoliation Estimations - The amount of defoliation was determined from ground

observation points at each sample site in the center ten-acre grid system. The estimates were made at the same time when the burlap-larvae determinations were made. The same two individuals estimated defoliation occurring on the oak trees. Defoliation estimate were made on a percentage basis (from 0-100%) and these estimates were converted to 25% increment ranges (0-25, 26-50, 51-75, and 76-100%). The mean percent defoliation differences for each treatment were determined by Duncan's multiple range test. Percentages can be transformed to arcsin (x + 0.5) for analysis.

At the peak of defoliation, the plots were photographed from the air with infrared film.

Post-Spray Egg Mass Determinations - During early November, post-spray egg mass determinations were made at each of the sampling sites as previously described.

RESULTS

For re-transformed geometric means (data converted from log values to arithmetic values), statistical data such as standard deviations and standard errors are not shown.

Image Analysis Data

Droplet Densities: (Drops/cm)
(Geometric Means)

Treatment	Upper Canopy	Lower Canopy
1 gallon	2.614	2.225
2 gallons	3.793	2.89
Undiluted	3.243	3.078

Volume per Unit Area: (nl/cm)
(Geometric Means)

Treatment	Upper Canopy	Lower Canopy
1 gallon	6.021	4.99
2 gallons	5.272	4.145
Undiluted	0.948	0.774

Droplet Sizes

Application Rate		VMD/NMD
1 gallon	- Upper Canopy	205/149
	- Lower Canopy	211/128
2 gallons	- Upper Canopy	171/123
	- Lower Canopy	172/123
Undiluted	- Upper Canopy	103/71
	- Lower Canopy	96/66

Wash-off Data

IU per Unit Area: (IU/cm)
(Geometric Means)

Treatment	Upper Canopy	Lower Canopy
1 gallon	60.842	63.125*
2 gallons	57.795	41.192
Undiluted	50.42	44.813

* Statistically significant difference between upper lower canopy. Other rates showed no significant differences.

Biological Data

Population Change: Walton's Method
Pre-spray egg mass counts

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
Control	1045	1152	1183	1627	1655	1972
Undiluted	681	1065	1112	1359	1728	1807
1 gallon	632	657	800	1278	1541	1929
2 gallons	619	896	965	1271	1412	1437

Post-spray egg mass counts

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
Control	4435	7378	6836	5479	1769	7505
Undiluted	2487	1801	1013	480	1167	336
1 gallon	2163	2213	791	2164	1316	1166
2 gallons	733	2408	1468	1542	3048	1980

Treatment	Means + 90% Confidence Intervals
Control	286.9 ± 151.2
1 gallon	43.5 ± 78.5
2 gallons	69.4 ± 93.4
Undiluted	-6.0 ± 77.7

Defoliation Protection Effects

Treatment	Defoliation (Percent + Std)
Control	48.9 ± 15.7
Undiluted	7.5 ± 2.8
1 gallon	6.4 ± 2.7
2 gallons	9.8 ± 7.1

More fine droplets from the undiluted application with Micronair atomizers were deposited in the lower canopy than with the one or two gallons per acre applications. Substantially more undiluted spray was deposited in the upper canopy than with a gallon of diluted spray. Only the 2 gallon diluted application in the upper canopy produced more droplets than either of the undiluted treatments.

More total volume was recovered with the one and two gallon treatments. However, this does not mean there is more Bt there than with the lesser amount of undiluted spray. It should be noted that there was little difference between the diluted applications.

Droplet sizes that were selected for the program were achieved. Droplets from the standard Tee Jet, flat fan tips were smaller than one would expect with 8004 and 8008 tips. There was little difference between sizes in the upper and lower canopy.

There was little difference in washoff data except between one and two gallons diluted treatments in the lower canopy.

Analysis based on pre- and post-egg mass counts show no statistical differences between the treatments. There were statistical differences between all treatments and the control.

Based on defoliation, there were no statistical differences between treatments, but significant differences between each treatment and the control.

This study demonstrates no statistical differences between any of the treatments but significant difference between each treatment and the control. Therefore, there is no advantage in using 2 gallons of spray volume per acre. Results also point out the disadvantage of using one gallon per acre when 40 ounces will do the job as well. Certainly, more emphasis should be put on low volume undiluted applications of Bt.

A large scale pilot test (5,000-10,000 acres) should be conducted with Dipel 8AF in 1989 using Micronair AU-5000 atomizers. If successful, undiluted applications should be used operationally in 1990. In 1990, extensive small plot (50-100 acres) testing should be conducted to compare Tee Jet nozzles with flat fan tips to Micronair AU-5000 atomizers using undiluted Bt at low volume (32-64 oz/acre).

Project Number: GM 85.2.3
Project Title: Sterile F_1 Demonstration Project - Bellingham, Washington.
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leaders: V. C. Mastro, C. P. Schwalbe and A. Pellegrini-Toole
Cooperators: Eric LaGasa

This project was initiated in 1985. Release of F_1 egg masses were made in 1985-1986. Details of treatment can be found in Laboratory Reports 1985 through 1987. The site was monitored in 1987 with pheromone-baited traps and no males were trapped. In 1988, the site was again monitored with traps at the same density and, again, no males were trapped. Following the two years of negative survey, the population is considered eradicated and successfully completes the first eradication use of the F_1 egg mass release technique.

Project Number: GM 85.2.4
Project Title: Sterile F_1 Demonstration Project - Darke Co., Ohio.
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: V. C. Mastro, C. P. Schwalbe and A. Pellegrini-Toole

Details of this project were presented in Annual Reports 1985 through 1987. A single release of F_1 egg masses was made at the Darke County site in 1985. Monitoring the release site in 1986 and 1987 with pheromone-baited traps resulted in capture of 2 and 1 males, respectively. It was unknown if these captures represented a remnant population or if these were incidental captures related to the nature of the release site (truck stop and road side rest). In 1988, 20 acres which included all of the 1985 F_1 egg mass release were trapped at 9 traps/acre and the surrounding square mile was trapped at 36 traps/sq. mi. In 1988, no males were captured in any trap. Considering the trap density used the conclusion is that there is no residual wild population.

Project Number: GM 86.2.1
Project Title: Sterile F_1 Demonstration Project - Southern Shores, Dare Co., North Carolina.
Report Period: October 1, 1987 - September 30,1988
Report Type: Final
Project Leaders: V. C. Mastro, A. Pellegrini-Toole and C. P. Schwalbe
Cooperators: W. Wescott, H. Singletary, K. Wilson

Releases of F_1 egg masses were made at the Southern Shores site in 1986 and 1987. Preliminary data from those years were reported in the last Annual Report. The following presents the remainder of the 1987 data and the results of monitoring the site with pheromone baited traps in 1988.

In 1987, the only ratio not reported was that derived from mating evaluation of females collected as immatures. These data determined the sterile:fertile wild female ratio to be 8.1:1 (n=227). This ratio should be approximately half of observed male ratios because of the skewed 2:1 male:female ratio displayed by F_1s. The female ratio is roughly 25% of the male mating evaluation ratio found.

If the post-season egg mass sample is representative of the entire post-season egg mass population, then it should provide the best indication of impact of the F_1 egg mass release. The ratio for sterile:fertile post-season egg masses presented in Table 1 indicates a high degree of sterility in field egg mass population. However, when the various mating types are separated, the sterile:fertile male mating ratio was very low, $23F_1$:34 wild (0.7:1). This same low sterile:fertile male ratio has been noted in other sterile release sites. An explanation for this low ratio is the F_1 males are at a competitive disadvantage because of behavior (see GM 7.2.3) or asynchrony of adult emergence. Another explanation is the post-season egg mass samples do not represent the true field mating population.

In 1988, the entire release area was trapped to determine if a remnant wild population existed. Because there was a high probability of a small residual population in the core area (based on 1987 overflooding estimates), approximately 20 acres were trapped at 9 traps/acre. The remainder of the treatment area and surrounding area was trapped at approximately 36 traps/sq. mi.

On June 16, one male was captured in the core area. By June 27 a total of 349 had been captured in the core area (20 acres) and an additional 171 were captured in the area survey at 36 traps/sq. mi. Within the mass trapping 20 acre area, the majority of males were trapped in the periphery traps. In Figure 1, the pattern of capture indicates that the greatest number of males were originating from outside of the core area with some small pockets inside of it. Within the area trapped at 36 traps/sq. mi., male captures were scattered and clusters of males were captured in trap numbers 49, 50, 51; 26, 27, 28; and 30 & 34, all of which are outside of the area treated with sterile egg masses in either 1986 or 1987. Subsequent egg mass surveys have found wild egg masses in one of these locations outside of the treatment areas. In all traps for the entire 1988 season, 1904 males were captured.

Although it is impossible to determine the precise cause of the outcome of this test, some conclusions may be drawn. Expected overflooding ratios were

not achieved within the release area. A known reason is the poor egg hatch of F_1 eggs in both years, but particularly in 1987. Possible contributing reasons are: 1) a lack of competitiveness on the part of F_1 males either for behavioral or asynchrony of development reasons, 2) poor estimates of the density of the native population and 3) biased sampling techniques. Also, clearly contributing to the outcome was a failure to recognize that the wild population covered a larger geographic area than was treated.

Table 1. A comparison of evaluation techniques used for estimating overflooding ratios in Southern Shores, Dare Co., North Carolina in 1986 and 1987.

	1986		1987	
Techniques	F_1:wild ratio	Percent fertile	F_1:wild ratio	Percent fertile
Observed male ratios:				
Chromosome analysis	30.2:1(n=156)	3.21	158:1(n=159)	0.63
Mating evaluation	14:1(n=449)	6.68	34.8:1(n=672)	2.88
Monitor females	6.1:1(n=256)	14.06	2.3:1(n=69)1/	30.30
Observed female ratios:				
Mating evaluation	3.5:1(n=239)	28.6	8.1:1(n=227)	11.0
Field collected post season egg masses	78·0(n=83)	0.0	45:1(n=92)3/	2.17
Number of males trapped	4,621 (4,097)2/		2,382 2/	

1/ A large majority of monitor females (n=209) failed to oviposit for unknown reasons.

2/ Number in brackets is the number of males trapped before an influx of migrant males. In 1987 there were negligible numbers of migrant males.

3/ The ratio presented is the ratio of egg masses produced by pairs where at least one parent was an F_1 vs where both parents were wild. In all, a parent could be identified from 100 egg masses. The F_1:wild male ratio was 23:34 and the F_1:wild female ratio was 67:2.

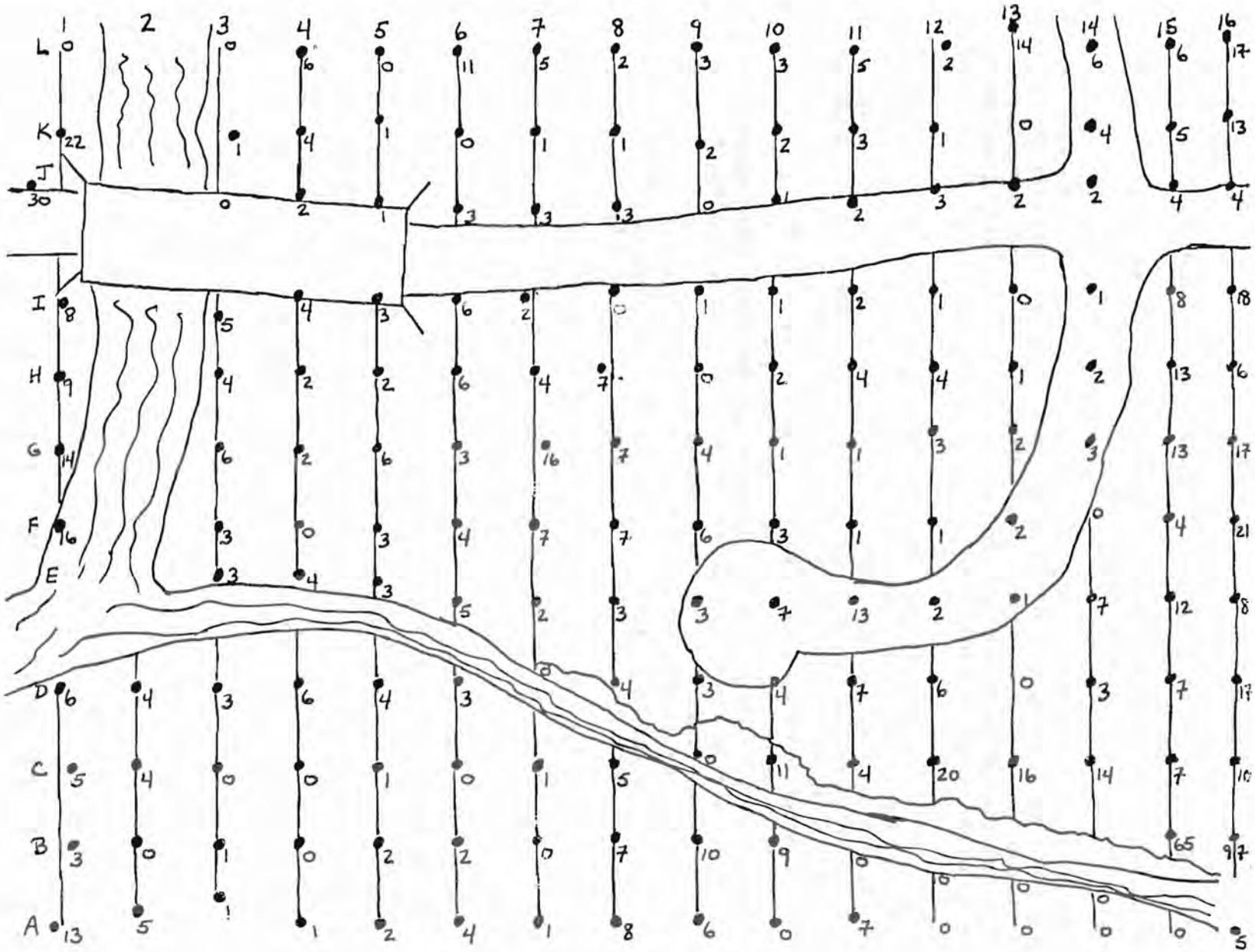


Figure 1. 1988 Southern Shores, N. C. core area trapping grid

Project Number:	GM 86.2.2
Project Title:	Sterile Male Demonstration Project - Allen Co., Ohio
Report Period:	October 1, 1987 - September 30, 1988
Report Type:	Interim
Project Leaders:	V. C. Mastro, C. P. Schwalbe, A. Pellegrini-Toole and D. Gerold
Cooperators:	D. Barth, K. Roach, Ohio Department of Agriculture, B. Smith, USDA, APHIS, PPQ

Releases of F_1 sterile egg masses were made in Allen County in 1986 and 1987. Descriptions of the site, of release activities and partial results of evaluation are present in previous Annual Reports. This report completes summation of the 1987 data and reports results of monitoring the site with pheromone baited traps in 1988.

The sterile F_1:wild female ratio calculated from mating evaluation of field collected larvae and pupae was much lower than expected. Also, the male ratios reported earlier were much lower than expected (Table 1). Reasons for these low F_1:wild ratios included poor F_1 egg hatch in 1987 (mean = 18.2%) and possibly poor survival because of host quality (i.e., predominately black locust). The post season egg mass evaluation, however, even indicates that the actual male mating ratio in the field was much lower (i.e., 1 F_1:13 wild males). This is even lower than the mating ratio estimated using monitor female data. The total data may reflect a combination of suboptimal F_1 performance: poor hatch, < 1/2 of the expected hatch rate; poor survival because of poor host quality or the inherent problems noted with the NJS strain of the past several years; and a lack of competitiveness in the adult stage because of behavioral differences of asynchrony between F_1 and wild adult emergence.

Table 1. Results of monitoring releases of F_1 sterile egg masses in Allen Co., Ohio in 1986 and 1987.

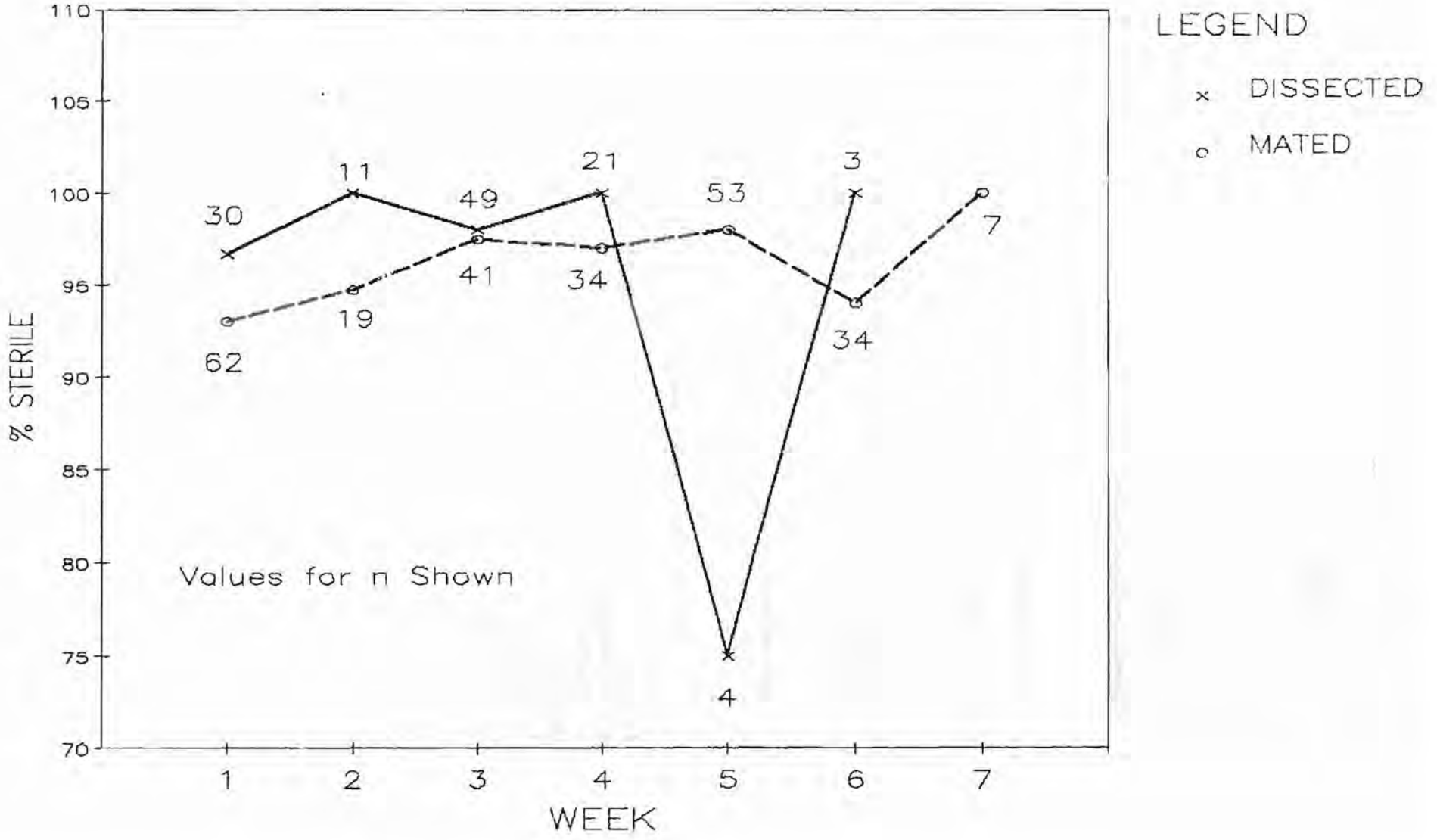
Monitoring technique	1986	1987	1988
Number of males trapped	389	233	32
Number of F_1 egg masses released	19,525	19,737	
Observed male ratios:			
Chromosome analysis	34:1(n=70)	38.3:1(n=118)	
Mating evaluation	3.2:1(n=137)	24:1(n=250)	
Monitor female fertilization	3.1:1(n=88)	4.7:1(n=63)	
Observed female ratios:			
Mating evaluation	2.0:1(n=93)	4.1:1(n=92)	
Field collected post season egg masses	4:1(n=5)	3.5:1(n=9) F_1:Wild =1:13 F_1:wild =6:3	

In 1988, the release area was monitored using the same trapping density as in previous years (i.e., approximately 36 traps/sq. mi.). In all, 32 males were captured in 1988, 10 in two traps within the release area, and the others in traps close to it. A search of the release area in October of 1988 succeeded in recovering 4 whole and one partial egg masses. Obviously, the objective of eradication was not achieved. The population has apparently not changed in density greatly since 1985, when 20 males were recovered with a less intense trapping effort. It is possible that if the F_1 strain was performing optimally, an F_1 release would not be successful in a situation wher a strain has been naturally selected to survive on a poor host.

Figure 1.

A Comparison of Two Evaluation Techniques
For Monitoring Sterile:Fertile Mating Ratios

ALLEN CO. 1987



Project Number: GM 86.2.3
Project Title: Sterile F_1 Demonstration Project, Manor, Washington
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leader: V. C. Mastro, A. Pellegrini-Toole and C. P. Schwalbe
Cooperators: E. Lagasa, Washington State Department of Agriculture

This project was initiated in 1986 and releases of F_1 egg masses were made in both 1986 and 1987. A discussion of the wild population and preliminary 1986 and 1987 results can be found in the last Annual Report (1987). In 1988, no further F_1 egg mass release was done and the area was trapped to determine if eradication had been achieved. Traps were placed to approximate a rate of 36/sq. mile. In all, 86 males were captured in 1988. Two traps which accounted for a large proportion of the capture (40 males) are located in what was considered the core area and 2 additional traps on the edge of the treatment area captured a total of 18 moths. Thirteen traps surrounding the treatment area captured between 1 and 4 males. It appears that the treatment did cover most or all of the infested area. Obviously, eradication was not achieved even though most evaluation techniques in both years indicate good overflooding ratios. Post-season egg mass evaluation in 1986, however, indicated poor interaction with the native population and even though explanations can be offered for this discrepancy (see 1987 Annual Report), male trapping data for both years indicates very low F_1 survival rates. In part, low F_1 egg hatch rates can be noted as contributing to poor F_1 survival.

However, this does not explain the discrepancy of the relatively high overflooding ratios derived from sampling and the poor degree of population suppression.

This site will not be retreated with sterile insects in 1989; alternate control strategies will be used.

Table 1. A summary of treating an isolated gypsy moth population in Manor, Washington, with releases of F_1-10k egg masses.

	1986			1987		
Number of F_1 egg masses released		47,876			43,705	
Monitoring technique	n	F_1:wild	% fertile	n	F_1:wild	%fertile
Observed male ratios						
Male chromosome[1/]	86	86:0	0.0	34	34:0	0.0
Male mating[1/]	96	31:1	3.1	168	167:1	0.6
Monitor females	199	199:0	0.0	48	5.9:1	14.6
Observed female ratios						
Female mating[1/]	49	23.5:1	4.1	41	40:1	2.4
Post-season egg mass evaluation overall	7	0.8:1	57:1	None available for evaluation		
Male ratio	5	1:4	80.0			
Female ratio	5	1:4	80.0			
Number of males trapped	528[2/]			205[2/]		

1/ Insects collected from the field as immatures and evaluated at the Otis Methods Development Center.

2/ Trapping density in 1986 ca. 240 trap/sq. mi., 1987 ca. 36 trap/sq. mi.

Project Number: GM 86.2.5a
Project Title: Behavioral Competitiveness of F_1-sterile Gypsy Moth Larvae Under Field Conditions
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INTRODUCTION

Major obstacles in any sterile insect release program include potential differences in the behavior and physiological performance of released (sterile) and target (feral) insects. Gypsy moth larval behaviors are particularly important in influencing generational survival, primarily in their interactions with natural enemies during later stadia and as pupae. Previous experiments have compared F_1-sterile, nonirradiated lab-strain, and wild larval behaviors but have concentrated on single stadia and employed insects reared under controlled conditions. The following experiment attempts to compare location and activity patterns of F_1-sterile, lab-strain, and wild gypsy moths under field conditions, from first instar establishment to pupation.

METHODS

Three large screen cages (ca 7.2 X 7.2 X 5.5 m) were erected in an oak forest within the Massachusetts Military Reservation (Cape Cod, MA). Two cages were supported by an internal framework of PVC tubing, while the third was suspended from four 6 m corner posts. The fine screen mesh (ca 13 X 13 strands/cm^2) prevented ingress of predators and parasitoids, and cage bottoms were secured to the ground to limit entrance by ground-dwelling natural enemies. Each cage enclosed two ca 5 m tall "red" oak (Quercus sp.) trees and surrounding understory vegetation and forest litter. Enclosed trees were surrounded by a scaffolding system that permitted access to nearly all leaves and branches.

The ground surface within each cage was divided into a 1-m grid, yielding 49 1-m^2 grid-squares per cage. A wooden shelter, consisting of a 15 X 20 cm plywood rectangle raised ca 5 cm off the ground at one end, was placed in the center of each grid-square.

F_1-sterile (laboratory strain, male parent irradiated), standard laboratory strain ("New Jersey"), and feral (collected in West Virginia, winter 1988) gypsy moth egg masses were released into separate cages in late May, 1988. These treatments are hereafter referred to as F_1, NJ, and WV, respectively. Egg masses were laid on a paper substrate or loosely contained within a coarse mesh envelope, and were affixed to tree boles at ca 2 m height. I attempted to establish a population of at least 1000 first instars per cage. Thus, eight NJ, 15 WV, and 20 F_1 egg masses were released into their respective cages.

In each cage, all oak branches longer than 1 m were numbered. A single branch was randomly-selected for each diurnal or nocturnal observation period. A 1-m portion of bole surface, centered at the intersection of the selected branch, was also monitored. Prior to the onset of the fourth stadium, burlap bands (ca 20 cm wide) were attached to each oak bole at ground level and at 1 m

(ca 20 cm wide) were attached to each oak bole at ground level and at 1 m height. Bands on the tree from which a branch was selected were also examined during a given observation period. Finally, ground grid-squares were randomly-selected for examination. Squares were classified as "proximal" (within 2 m of oak trees) or "distant" (more than 2 m from a tree), roughly dividing each set of grid-squares in half. Four proximal and two distant grid-squares were selected for each observation period; later, eight and four squares, respectively, were selected.

Larval activity and location patterns were monitored from first-instar establishment until pupation. During each observation period, the selected branch, bole surface, burlap bands, and understory plants, ground litter, and wooden shelters within each grid-square were carefully examined in each cage. All larvae found were classified, by stadium, into various location and categories (Table 1). In addition, special conditions were noted (e.g. sun exposure, utilization of dead leaves, molting, etc.)

Table 1. Location and activity categories used to classify gypsy moth larvae.

Location	Activity
I. On trees	
A. Leaves	A. Inactive
1. Upper surface	B. Active
2. Lower surface	1. Crawling
B. Branches (>1 cm diameter)	2. Feeding
C. Twigs (<1 cm diameter)	C. Disturbed
D. Bole	
1. Bole surface	
2. Burlap bands	
II. Off trees	
A. Proximal (within 2 m of bole)	
1. Leaf litter	
2. Woody debris	
3. Shelters	
4. Understory plants	
B. Distant (>2 m from bole)	
1. Leaf litter	
2. Woody debris	
3. Shelters	
4. Understory plants	
C. Cage or scaffolding	

Diurnal observations (n=62) were conducted between 0500 and 2100 EDT at irregular intervals. A maximum of two diurnal observations was made in a single day, one in the morning and one in the afternoon and separated by at

least four hours. Nocturnal observations (n=13) occurred between 2200 and 0430 EDT, with no more than one per 24-hr period. Night observations employed a headlamp and dim flashlight.

An electronic hygrothermograph recorded hourly temperature and humidity data throughout the study. Weather conditions (temperature, relative humidity, cloud cover, wind speed and direction) were also noted at the start of each observation period.

At about the time cage-released eggs began to hatch, additional NJ, F_1- and WV first instars were established on red oak foliage in an outdoor insectary. These insects were used to supplement cage populations when few or no larvae were detected during observation periods. Released larvae were in about the same stadia as those in the cages. About 2000 WV, 1450 F_1- and 100 NJ first, second, and third instars were so released (Table 2).

Table 2. Supplemental larval releases in field cages.

Julian date	Treatment	Stadium	N (approximate)
155	WV	I	700
157	F_1	I	200
159	WV	I	200
160	F_1	I	200
161	WV	I	250
165	F_1	I,II	250
165	WV	I,II	250
167	WV	I,II	300
168	WV	II	150
168	NJ	II	60
169	NJ	II	60
172	F_1	II,III	300
172	WV	II,III	90
176	F_1	II,III	125
180	F_1	III	150
181	WV	II,III	70
183	F_1	III	120
187	F_1	III	100

As larvae completed development, gypsy moth prepupae ("spins") and pupae were collected daily from each cage. Entire trees and the enclosed ground surface were carefully examined. Collected pupae were sexed and their locations recorded (Table 1).

The data collected in this study are qualitative, or nominal, data and were analyzed using contingency table ("Chi-square") procedures. The null hypothesis examined was that larval location and activity patterns, or pupal location patterns, do not differ between treatments (NJ, F_1, and WV). For each stadium, observed frequencies (numbers of insects recorded in each

activity or location category) were compared to frequencies expected if the null hypothesis is true, at $\alpha=0.05$. Adjacent categories were pooled prior to analysis if more than 20% of the expected frequencies were less than 5.0, or if one expected frequency was less than 1.0. Data for each stadium were analyzed separately, but data for two or more stadia were pooled if numbers were small. Pooling was performed if contingency tables for individual stadia were not heterogeneous. Diurnal and nocturnal larval data were analyzed separately.

RESULTS

Figure 1 shows trends in mean larval stadium over time. In general, larval development in the F_1 and WV cages was comparable, but lagged 7-10 days behind that in the NJ cage.

Regardless of treatment, nearly all first and second instars were found on oak foliage during the day (Fig. 2) and most were situated on leaf undersurfaces (Fig. 5). First instar distribution on upper or lower leaf surfaces did not differ among treatments ($X^2=5.73$, df=2, $P>0.05$), nor did frequencies occurring on or off leaves ($X^2=6.00$, df=2, $P>0.05$). In the second stadium (Fig. 2), there were no among-treatment differences in diurnal location on or off leaves ($X^2=2.58$, df=2, $P>0.25$), but utilization of upper and lower leaf surfaces (Fig. 5) differed among treatments ($X^2=9.16$, df=2, $P<0.02$). Much of this difference can be attributed to the greater than expected occurrence of NJ second instars on upper leaf surfaces.

In all cages, most third instars were also found on foliage (Fig. 2). Location patterns on oak leaves, in other on-tree sites, and off host trees did not differ among treatments ($X^2=6.83$, df=4, $P>0.10$). Utilization of upper and lower foliar surfaces was similar in the three cages (Fig. 5; $X^2=5.39$, df=2, $P>0.05$).

Young larvae were difficult to locate at night. Because of smaller numbers for individual stadia, nocturnal location data for the first to third stadia were pooled (Fig. 2). Most young larvae were also found on foliage, and location patterns did not differ among treatments. At night, early-instar larvae were more often found on the undersides of oak leaves (Fig. 5). Utilization of upper or lower leaf surfaces did not differ among treatments ($X^2=3.26$, df=2, $P>0.10$).

In all cages, a majority of fourth-stadium larvae was still found on oak foliage during the day (Fig. 3). Fourth instar location patterns differed significantly among treatments ($X^2=34.09$, df=8, $P<0.001$). This result can largely be attributed to NJ larval location patterns, since F_1 and WV fourth-instar patterns were not different ($X^2=7.70$, df=4, $P>0.10$; only nonsignificant pairwise comparison). A lower than expected diurnal utilization of burlap bands by NJ fourth instars accounts for much of the discrepancy. For those larvae found on oak foliage, utilization of upper and lower leaf surfaces differed among treatments (Fig. 5; $X^2=15.83$, df=2, $P<0.01$). WV larvae were found predominantly on lower leaf surfaces, while NJ and F_1 fourth instars were more common on upper surfaces.

Nocturnally, most fourth instars were also found on oak foliage in all cages (Fig. 3), and location patterns did not differ between treatments ($X^2=2.58$,

df=2, P>0.25). Like younger larvae, fourth instars were most frequently found on leaf undersurfaces at night; treatment differences were not significant (Fig. 5; X^2=1.90, df=2, P>0.30).

Diurnal location data for fifth (N=977) and sixth (N=97) instars were pooled (Fig. 4). Location patterns differed significantly among treatments (X^2=120.80, df=10, P<0.001), but subdivision of the contingency table data reveals no definite trends. It appears that location patterns exhibited by F_1 fifth and sixth instars, particularly in the use of foliage and burlap bands, differed considerably from NJ or WV location patterns. For larvae found on foliage, utilization of upper or lower leaf surfaces did not differ between treatments (X^2=4.49, df=2, P>0.10). A majority of NJ and F_1 fifth and sixth instars were found on upper surfaces, while more WV larvae were observed on leaf undersides (Fig. 5).

Nocturnal location data for fifth and sixth instars were also pooled (Fig. 4). Location patterns did not differ among treatments (X^2=11.55, df=6, P>0.05). At night, most fifth and sixth instars found on foliage occurred on leaf undersurfaces (Fig. 5); treatment differences were not significant (X^2=0.09, df=2, P>0.90).

I recorded the incidence of direct larval exposure to sunlight, and Figure 6 summarizes exposure patterns of larvae on upper leaf surfaces. Generally, most NJ and F_1 larvae were not directly exposed to the sun. However, treatment effects were significant during stadia II (X^2=9.64, df=2, P<0.01), III (X^2=33.04, df=2, P<0.001) and IV (X^2=9.33, df=2, P<0.01), due to the preponderance of WV larvae in direct sunlight. No first instars of any treatment were observed in direct sunlight. A few fourth, fifth, and sixth instars were observed in direct sun exposure in other on-tree or off-tree locations.

Some dead oak leaves, typically brown and somewhat curled, remained attached to twigs throughout the study period. First instars were not found on these dead leaves, and no larvae of any stadium utilized dead leaves at night. However, older larvae utilized dead leaves during the day (Fig. 7). The relative use of dead leaves varied among treatments for third (X^2=15.69, df=2, P<0.01) and fourth (X^2=45.80, df=2, P<0.001) instars. This result is attributable to the greater than expected occurrence of WV larvae on dead foliage.

Most larvae recorded during diurnal observation periods were inactive, regardless of stadium (Table 3). Treatment differences were not significant during the first (X^2=6.81, df=4, P>0.10), second (X^2=5.13, df=2, P>0.05), third (X^2=3.88, df=4, P>0.40), and fourth (X^2=3.60, df=2, P>0.20) stadia. Fifth- and sixth-instar activity patterns differed among treatments (X^2=9.95, df=4, P<0.05), primarily due to the relative infrequency of active F_1 larvae.

Most larvae observed at night were also inactive (Table 4). Treatment differences were not significant during the first three stadia (X^2=3.40, df=2, P>0.10). F_1 fourth instars were more frequently inactive during nocturnal observations (X^2=6.80, df=2, P<0.05). Treatment differences were also significant during the fifth and sixth stadia (X^2=6.04, df=2, P<0.05). However, the probability of obtaining this Chi-square value was just slightly less than α(0.049 vs 0.050), and specific treatment effects cannot be

isolated. In any event, diurnal and nocturnal activity data undoubtedly reflect disturbance during observation, and treatment effects should be viewed with caution.

I recovered 497 NJ, 187 F_1, and 293 WV pupae from the field cages (Fig. 8). Treatment differences in pupal location were significant for males (X^2=74.33, df=10, P<0.001) and females (X^2=67.89, df=10, P<0.001). For both sexes, a considerable disparity between observed and expected frequencies occurred in most treatment and location category combinations. In general, more F_1 pupae were collected under burlap bands than were NJ or WV pupae. Conversely, fewer F_1 pupae were found in other on-tree locations, particularly in oak foliage. For a given treatment, pupal location patterns did not differ between sexes.

DISCUSSION

Larval development in the F_1 and WV cages lagged behind that in the NJ cage (Fig. 1). Microclimatic differences were unlikely; the three cages were set up on adjacent sites and enclosed similar vegetative structure. F_1-sterile gypsy moth development is somewhat longer than development in nonirradiated strains under laboratory conditions (Lance et al. 1984, Montgomery, unpubl. data). Inherent strain differences in larval developmental rates were probably accentuated by supplemental larval releases in the F_1 and WV cages (Table 2). Released insects were in approximately the same developmental stage as those established in the cages. However, physiological and behavioral "costs" associated with such dietary and habitat transfers could have slowed the development of released insects, thus reducing overall cage performance (as measured by mean stadium).

Supplemental releases were necessary for several reasons. F_1 egg hatch was extremely low; some of the released egg masses produced no first instars, while the remainder generally produced less than 20 per mass. Normally, F_1 egg hatch is about 40% of that exhibited by nonirradiated strains, yielding 150-350 first instars per egg mass. Reasons for this much lower than expected egg hatch are not known.

In the WV cage, an irradiated strain was inadvertently released. Once this error was discovered, all egg masses were removed from the cage and any established larvae killed with a pyrethrum spray, applied from a mistblower (Pyrenone^R, ca. 780 ppm in distilled water). Feral West Virginia egg masses were released about 24 hrs after the spray. By the time these eggs hatched, oak leaves may have become less favorable for first instar establishment. Additionally, the spray appeared to have a moderate phytotoxic effect, perhaps further reducing foliar suitability. Foliage killed or damaged in this way largely accounts for the more frequent occurrence of WV larvae in dead leaves (Fig. 7).

I have no evidence that F_1 or WV first instars experienced greater dispersal or other types of differential mortality (e.g. natural enemies). In any event, first instar establishment in the F_1 and WV cages was inadequate, requiring supplemental releases of insectary-reared insects.

More pupae were collected from the NJ cage than from the F_1 or WV cages (Fig. 8). I have no information whether this difference represents differential larval or pupal survival, or a combination of both. No gypsy moth parasitoids

were observed in any of the cages, and none were recovered from collected pupae. Birds and small mammals were apparently excluded. Resident *invertebrate predators were found in all cages, however, and were occasionally* seen consuming larvae and pupae. These included reduviid and pentatomid bugs, ground beetles, carpenter ants, spiders, and harvestmen. A few virus-killed larvae were observed in each cage. Undoubtedly, differences in pupal numbers reflect, at least in part, the aforementioned difficulties in establishing F_1 and WV first instars.

Of interest in late larval location patterns (Fig. 4) are the relatively frequent utilization of foliar resting sites during the day, especially by NJ and WV larvae, and the scarcity of larvae in off-tree locations. Diurnally, late-stage larvae are not usually found on leaves in low-density populations. However, older larvae often remain on leaves when populations are high, presumably in response to defoliation-mediated changes in foliar food quality (Campbell 1978, Lance et al. 1986). The largest larval population was maintained in the NJ cage; defoliation there was noticeable and estimated at about 35-40% by the end of feeding. Defoliation was less pronounced in the WV cage (ca. 20%) but many leaves suffered an apparent phytotoxic response (necrotic spots, curling, death of some leaves) to the pyrethrum spray. Thus, leaf damage in the NJ and WV cages may have been sufficient to induce larval utilization of oak leaves during the day. This "behavioral shift" has been noted at defoliation levels much below the complete defoliation often associated with gypsy moth outbreaks (Campbell 1978, Lance et al. 1987, Leonard 1970). However, very few larvae were feeding during diurnal observation periods (Table 3).

Defoliation in the F_1 cage was negligible, due to smaller larval populations and larger foliar biomass. However, a smaller proportion of F_1 fifth and sixth instars did remain on foliage during the day.

Relatively few older larvae (generally <10%) were observed in ground litter or in other off-tree locations (Figs. 3 and 4). Generally, larval occurrence in off-tree locations is inversely related to the availability of suitable on-tree resting sites (Campbell et al. 1977). Undoubtedly, some larvae resting under burlap bands would have moved off trees if these artificial resting sites were not present. Liebhold et al. (1986) showed that the presence of burlap bands did reduce off-tree movement.

F_1 fifth and sixth instars were more frequently observed under burlap than were NJ or WV larvae. In 1987, released F_1 fifth instars were more often found off a white oak tree than were NJ or feral larvae (Hansen 1987); this result was not observed on an adjacent red oak. Mastro and Schwalbe (1986) reported a similar result on white oak trees. Greater utilization of burlap bands and increased off-tree movement may represent a common phenomenon in F_1 larvae. Selection of diurnal resting sites may involve various tactile or light stimuli (Campbell et al. 1975b, Wallis 1959). Perhaps F_1 larvae exhibit aberrant thigmotropic or phototropic responses as a consequence of parental irradiation. Alternatively, F_1 larvae may exhibit greater irritability or increased crawling tendencies. In any event, these phenomena warrant further study.

Burlap bands are employed to estimate gypsy moth density (Weseloh 1985a) or to assess various population characteristics, and are employed to quantify the

abundance and distribution of released F_1-sterile and native insects. Differential utilization of burlap bands by fifth- and sixth-stadium F_1 larvae could lead to erroneous overflooding ratio calculations.

The relative impact of gypsy moth parasitoids is influenced by larval and pupal location and activity patterns. Reardon (1976) reported that larvae and pupae occurring on tree boles generally suffered higher parasitism rates than did those in foliage or in ground litter. Weseloh (1985a) also showed higher parasitism on tree boles than on branches or foliage. Specifically, the tachinid Parasetigena silvestris concentrates attacks on late-stadium gypsy moth larvae exposed on tree boles, rather than larvae hidden under artificial bark flaps or in foliage (Odell and Godwin 1979, Weseloh 1974). Moving caterpillars may be preferentially attacked by P. silvestris (Weseloh 1976). Females of another tachinid, Blepharipa pratensis, deposit microtype eggs on leaf undersurfaces (Godwin and Odell 1984), so larvae utilizing lower leaf surfaces may suffer greater parasitism.

The tachinid Compsilura concinnata attacks several gypsy moth stadia; larvae situated on foliage close to the ground appear to be preferentially parasitized (Weseloh 1982). Females of the chalcid Brachymeria intermedia, a pupal parasitoid, favor warm and sunny conditions for oviposition (Barbosa and Frongillo 1979, Barbosa et al. 1978). Gypsy moth pupae occurring in exposed situations (e.g. crown periphery, exposed branches) may suffer more B. intermedia attacks.

The effect of gypsy moth predators is also influenced by larval behavior. Larval and adult Calosoma sycophanta (Coleoptera: Carabidae) preferentially consume gypsy moth larvae and pupae found on tree boles, near ground level; gypsy moths on foliage or branches are rarely attacked (Weseloh 1985a,b). Birds seem to feed most heavily on larvae or pupae situated in the crown or on boles (Campbell and Sloan 1976). Small predatory mammals (especially mice and shrews) forage extensively in forest litter, and the white-footed mouse (Peromyscus leucopus) concentrates feeding searches near tree trunks (Campbell and Sloan 1976). In low-density populations, resting gypsy moth larvae and pupae occurring in the litter suffer greater mortality, primarily because they are more likely to be eaten by small mammals than those on trees (Campbell and Sloan 1976).

The relationship between F_1-sterile gypsy moth behavior and natural enemy impacts will depend on the location and nature of the release site. Sites distant from areas generally infested by the gypsy moth are unlikely to support populations of introduced predators (C. sycophanta) or parasitoids (P. silvestris, B. pratensis, B. intermedia). The polyphagous C. concinnata has been collected from released F_1-sterile gypsy moths in Oregon, North Carolina, and Washington. However, in isolated infestations occurring in forest or park situations, I believe that the greatest impact would be exerted by ground-dwelling small mammals. If a characteristic of F_1-sterile gypsy moths, increased off-tree movement could increase predation by small mammals, thus causing a relative reduction in F_1 populations and reducing the efficacy of an F_1 release.

CONCLUSIONS

Under field conditions, diurnal and nocturnal activity patterns exhibited by

F_1-sterile, untreated lab-strain, and feral gypsy moth larvae were generally similar in all stadia. Diurnal location patterns exhibited by young F_1-sterile gypsy moth larvae (L_1 - L_4) were similar to those exhibited by wild and untreated lab-strain larvae. Nocturnal location patterns displayed by all stadia were also similar among treatments. However, this study supports previous experiments in describing "aberrant" diurnal location patterns in F_1-sterile fifth (and sixth) instars. Specifically, F_1 larvae were more frequently found under burlap bands (at 0 and 1 m heights) than were feral and untreated larvae during the day. This result, and earlier documentation of more frequent off-tree movement by F_1 fifth instars, could indicate increased tendencies for downward movement and/or inadequate responses to on-tree resting sites as a consequence of paternal irradiation. Practical consequences may include higher relative mortality rates among F_1-sterile fifth instars and pupae, and erroneous population estimates from burlap-band sampling. We have no evidence, however, that "aberrant" late-instar behaviors occur in F_1-sterile release sites or under any field conditions (tree conditions, site and weather factors, etc.) different from those encountered in our experiments.

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Table 3. Diurnal activity patterns of gypsy moth larvae in field cages.

Stadium	Treatment	Number of larvae[a]: Inactive	Crawling	Feeding	Disturbed	N
I	NJ	94 (0.83)	9 (0.08)	6 (0.05)	4 (0.04)	113
	F_1	31 (0.79)	2 (0.05)	2 (0.05)	4 (0.09)	39
	WV	42 (0.71)	13 (0.22)	1 (0.02)	3 (0.05)	59
II	NJ	83 (0.84)	9 (0.09)	6 (0.06)	1 (0.01)	99
	F_1	44 (0.76)	6 (0.10)	6 (0.10)	2 (0.03)	58
	WV	86 (0.90)	2 (0.02)	4 (0.04)	4 (0.04)	96
III	NJ	82 (0.84)	5 (0.05)	8 (0.08)	2 (0.02)	97
	F_1	75 (0.83)	8 (0.09)	5 (0.06)	2 (0.02)	90
	WV	131 (0.88)	5 (0.03)	10 (0.07)	2 (0.01)	148
IV	NJ	147 (0.87)	12 (0.07)	8 (0.05)	1 (0.01)	168
	F_1	82 (0.94)	4 (0.05)	1 (0.01)	0	87
	WV	114 (0.90)	8 (0.06)	3 (0.02)	2 (0.02)	127
V and VI	NJ	416 (0.94)	18 (0.04)	7 (0.02)	0	441
	F_1	339 (0.98)	4 (0.01)	1 (0.00)	0	344
	WV	276 (0.96)	8 (0.03)	2 (0.01)	0	286

a Numbers in parentheses are proportions of treatment totals (N).

Table 4. Nocturnal activity patterns of gypsy moth larvae in field cages.

Stadium	Treatment	Number of larvae[a]: Inactive	Crawling	Feeding	Disturbed	N
I to III	NJ	25 (0.78)	1 (0.03)	4 (0.13)	2 (0.06)	32
	F_1	31 (0.94)	1 (0.03)	1 (0.03)	0	33
	WV	31 (0.86)	1 (0.03)	2 (0.05)	2 (0.05)	36
IV	NJ	17 (0.61)	4 (0.14)	7 (0.25)	0	28
	F_1	17 (0.94)	0	1 (0.06)	0	18
	WV	10 (0.62)	0	6 (0.38)	0	16
V and VI	NJ	75 (0.83)	2 (0.02)	13 (0.14)	0	90
	F_1	13 (0.62)	3 (0.14)	5 (0.24)	0	21
	WV	37 (0.86)	3 (0.07)	3 (0.07)	0	43

a Numbers in parentheses are proportions of treatment totals (N).

FIGURE 1. Relative larval development recorded during diurnal and nocturnal observation periods (only observations with N at least 5 included).

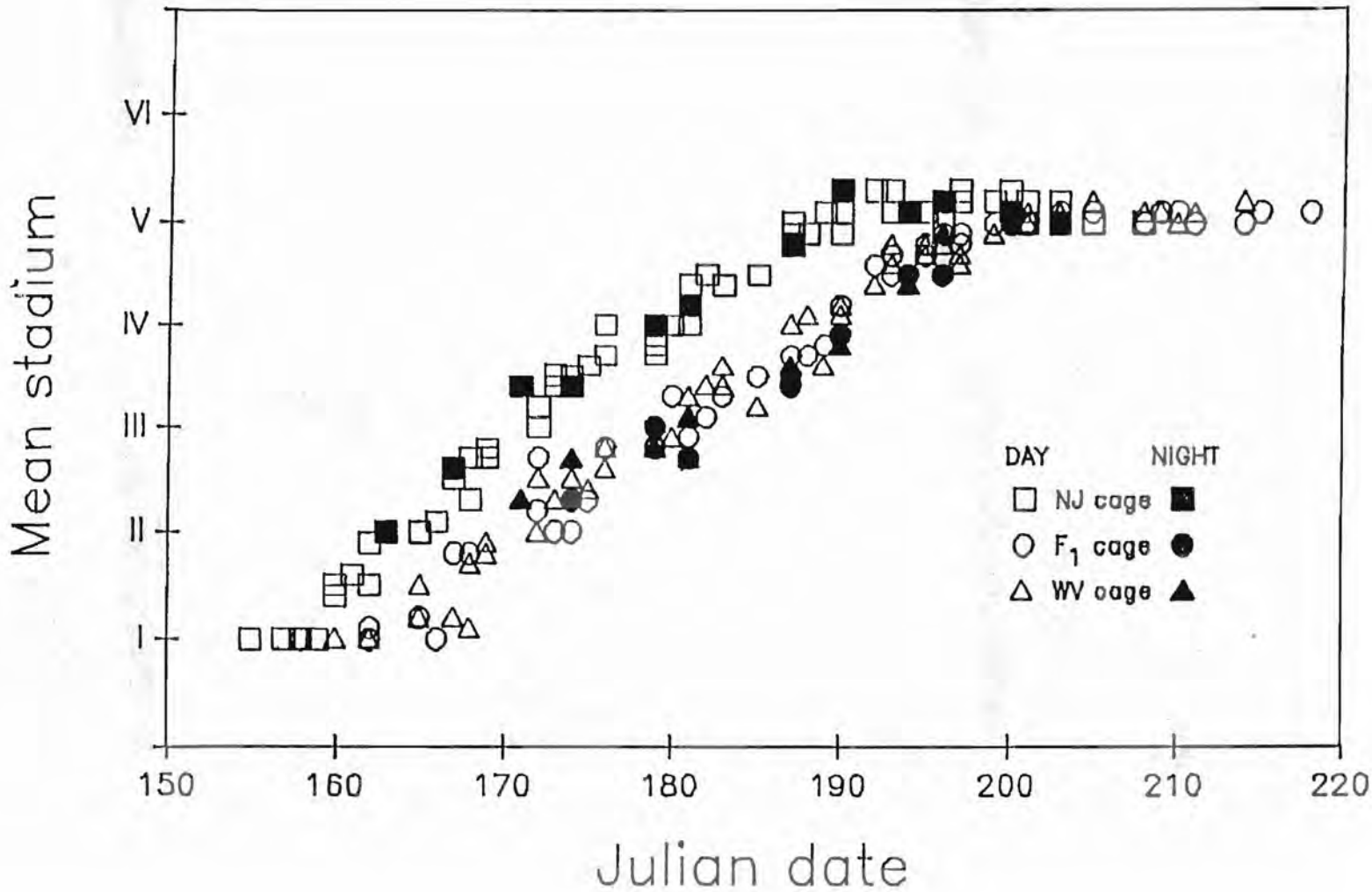


FIGURE 2. Diurnal location patterns of first (A), second (B) and third (C) instars, and nocturnal location patterns of first. second, and third instars (pooled data) (D).

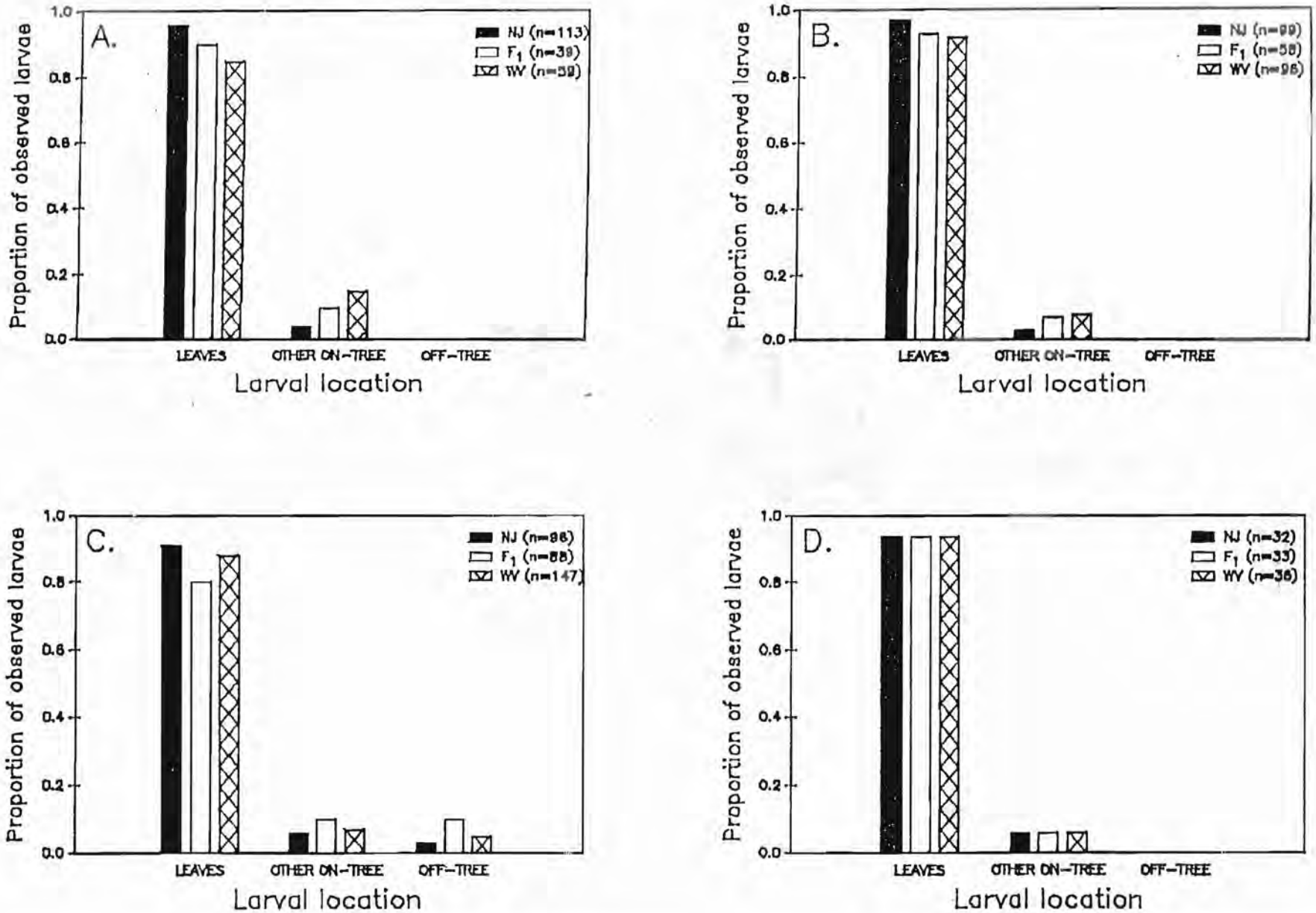


FIGURE 3. Diurnal (A) and nocturnal (B) location patterns of fourth instars.

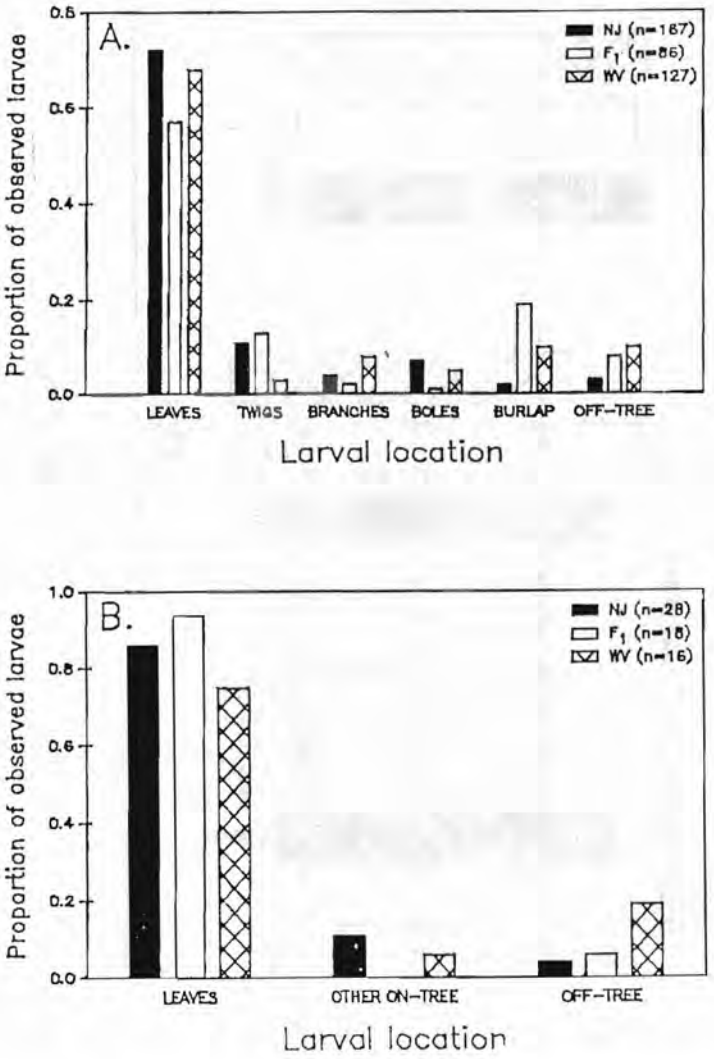


FIGURE 4. Diurnal (A) and nocturnal (B) location patterns of fifth and sixth instars.

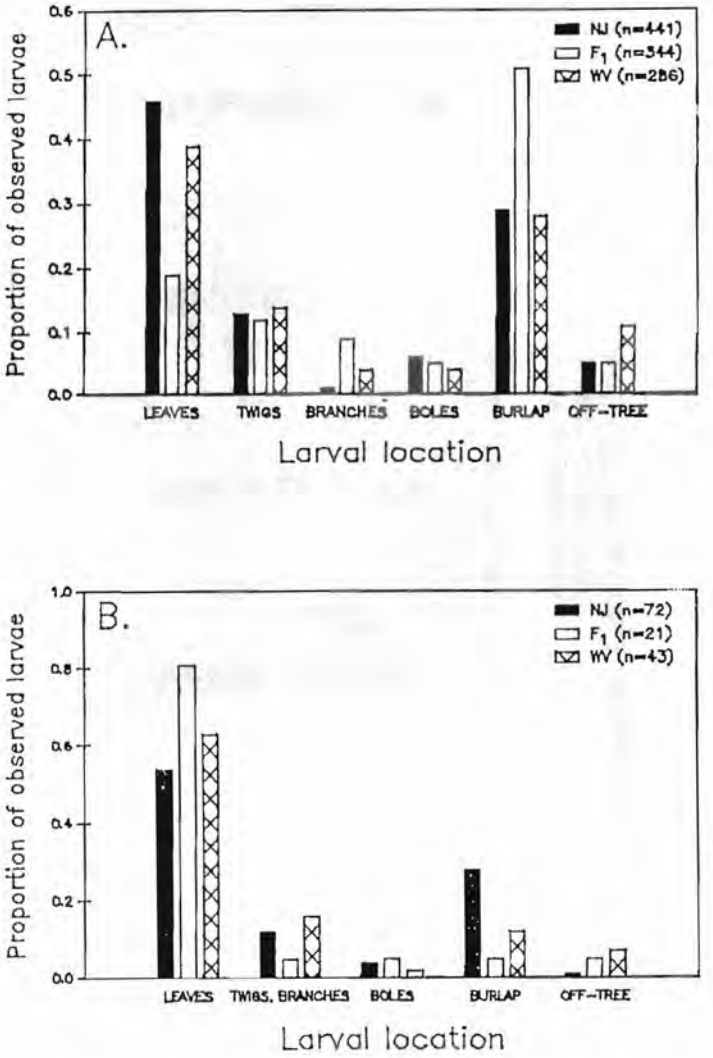
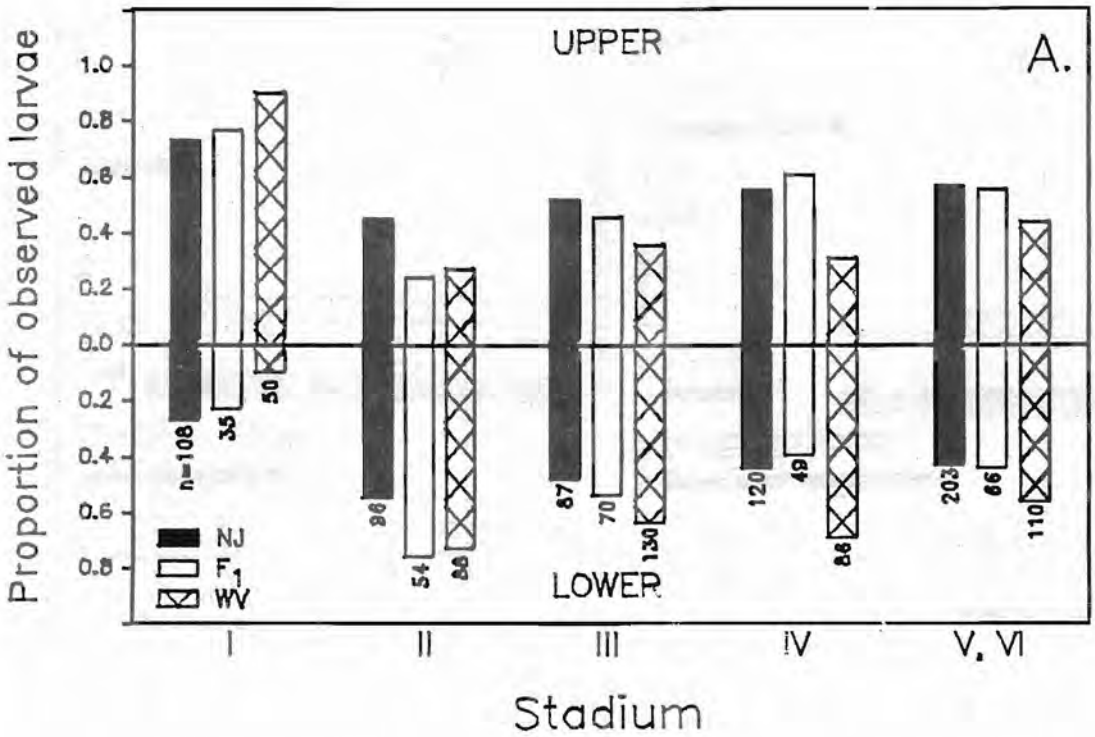


FIGURE 5. Diurnal (A) and nocturnal (B) utilization of upper and lower oak leaf surfaces.



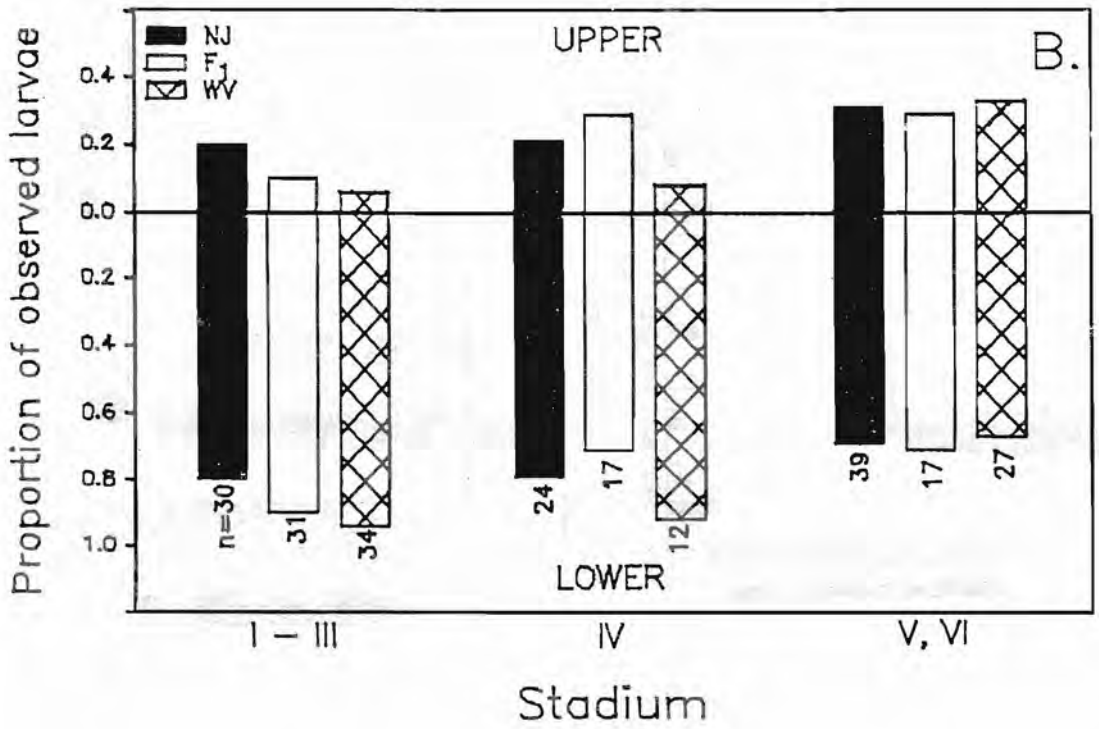


FIGURE 6. Sun exposure of larvae on oak leaves.

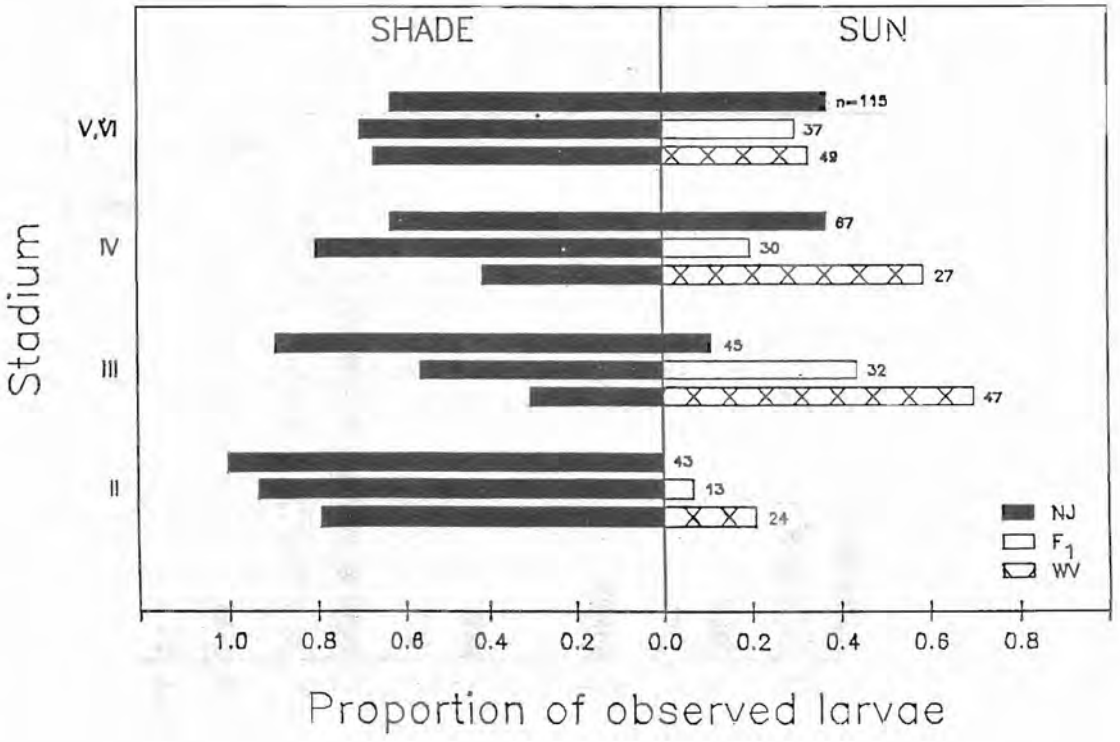


FIGURE 7. Diurnal location patterns on live or dead oak foliage.

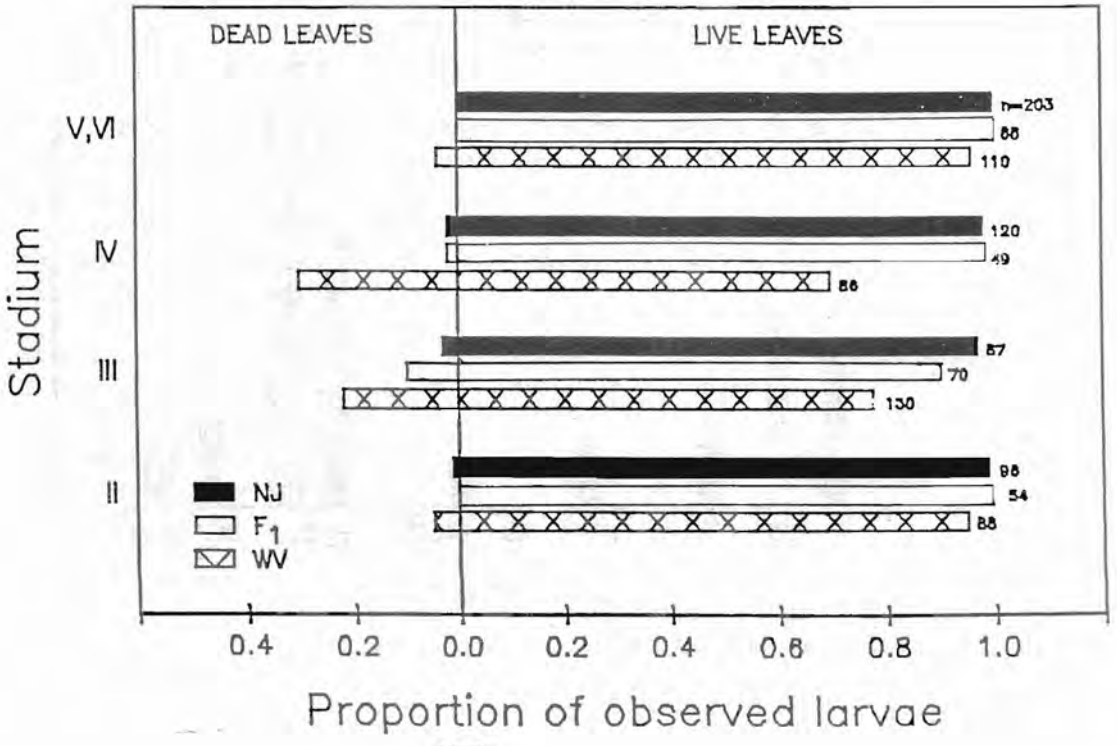
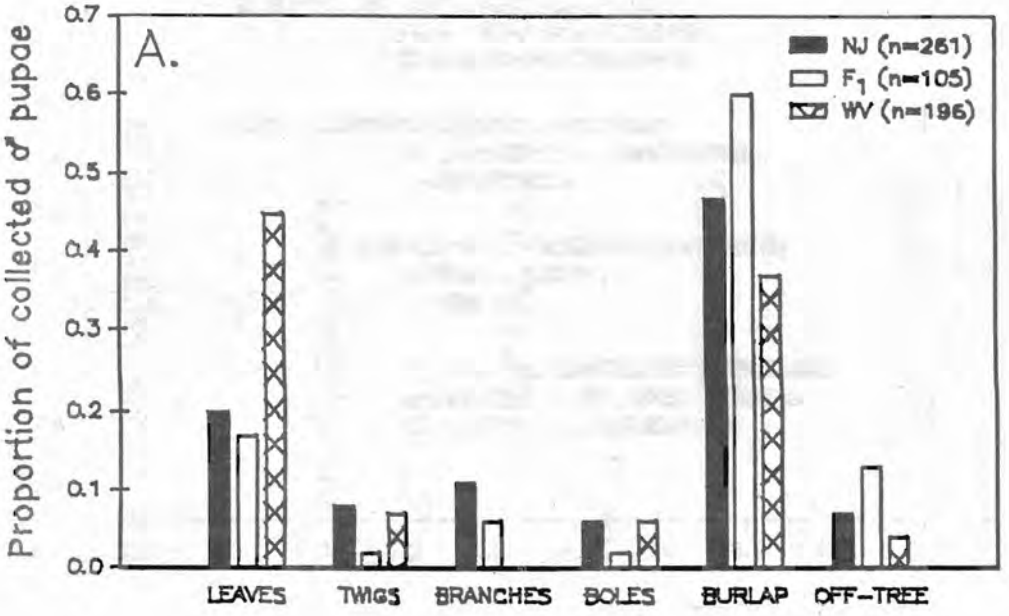
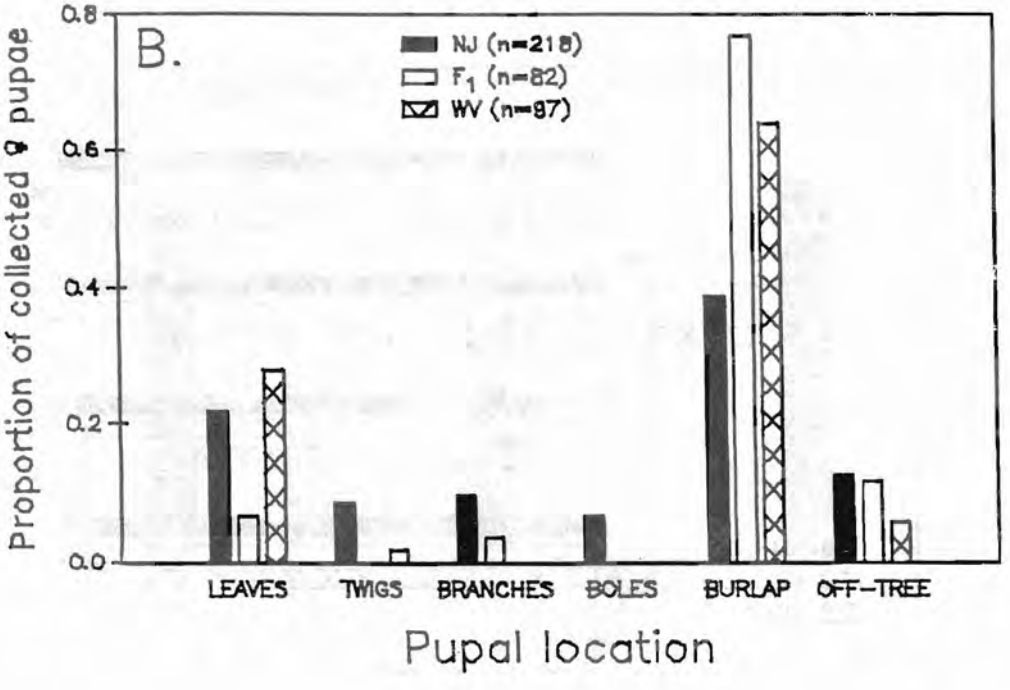


FIGURE 8 Location patterns of male (A) and female (B) pupae recovered from field cages.





Project Number: GM 86.2.5b
Project Title: Behavioral Competitiveness of F_1-sterile Gypsy Moth Adults Under Field Conditions
Report Period: May 1, 1988 - September 30, 1988
Report Type: Interim
Project Leader: R.W. Hansen

INTRODUCTION. F_1-sterile gypsy moths have a male-biased sex ratio, and adult males are considered the "agents" for successful implementation of an F_1-sterile release program. However, F_1 females will outnumber feral females in field release situations, and may have an important role if their presence "disrupts" the interaction between feral (fertile) females and F_1-sterile males. Such a situation might occur if F_1 males preferentially mate with F_1 females, thus "diluting" their availability for feral female matings. In any event, mating and other behaviors of adult F_1-sterile females have not been studied.

Adult F_1-sterile males appear "generally competitive" with feral males in their ability to locate and mate with females (Odell, unpubl.). However, we have little detailed information on the relative mating abilities of F_1 males under field conditions.

This study attempts to generally describe and compare location and activity patterns of F_1-sterile, "New Jersey", and wild (WV) female moths under field conditions. Further, "mating patterns" exhibited by combinations of F_1, NJ, and WV females and males will be examined.

METHODS. Adults were released into two of the large field cages (described in preceding report) in August, 1988. Adults employed in these experiments eclosed from pupae collected in the cages or reared on red oak foliage in an insectary. For each treatment (NJ, F_1, WV), about 10 male or female pupae were placed in 480 ml paper cups containing a layer of excelsior. Cups were held in the insectary until adults emerged. For a given release, moths less than 24 hr old were collected; occasionally, 24-48 hr old males were used.

Treatments and numbers of moths involved in each release depended on their availability and thus daily eclosion patterns. A total of 10 adult releases were made in the NJ and WV cages (Table 1). Release 2-N compared activity patterns of NJ moths reared on artificial diet in the laboratory, and NJ moths reared on foliage in the insectary; this release will not be considered further in this report.

Collected male and female moths were individually numbered on a forewing with a permanent ink pen. Different colors were used to identify NJ, F_1, or WV moths. Any resident females were removed from the cage, and resident males captured in a pheromone trap, prior to release. Females were gently placed on oak boles at 2 m above ground level. Half of the marked females were released on each of the two caged trees. Marked males were then scattered about the periphery of the cage. Releases were made between 0900 and 1000 hrs EDT.

Cages were visited at 30-min intervals following release, until ca 1700 hrs (about 6 hrs total observation time). Each marked female was categorized as occurring on boles, branches, or off the tree, and its upward or downward

occurring on boles, branches, or off the tree, and its upward or downward movement from the release location noted (in 0.5-m increments). Marked females were also classified into various activity categories: inactive, calling, crawling, wing-fanning, associated with male (no mating), mating, and ovipositing, as well as combinations thereof. Male and female pairs were considered to be mating only if we observed genital coupling. The type and number of the male involved in each mating were recorded.

RESULTS. Location and activity data for F_1, NJ, and WV females were pooled over all releases. Over time, released females of all treatments moved downward on oak trees (Fig. 1). However, treatment differences are unlikely because of the high variability at each observation interval. Most females remained on boles throughout observation periods (Fig. 2). NJ moths seemed most likely to move to oak branches over time, but the difference is probably not significant. For all treatments, a small proportion of females moved off trees and into litter or on understory plants, beginning about 3 hours after release.

Behaviors exhibited by F_1-sterile female moths were generally similar to those displayed by NJ or WV females (Fig. 3). Females of all treatments began calling soon after release; mating peaked about 1.5 to 3.5 hours, and oviposition around 5 hours, after release. However, the largest proportion (40% or more) of released females were inactive at a given interval, regardless of treatment.

MATING PATTERNS

Most released females mated once, but a small proportion were not observed mating and others mated at least twice (Fig. 4). Mating frequencies differed among treatments (X^2=20.50, df=4, P<0.001), as F_1 females were most likely to remain unmated. For all treatments, most released males did not mate (Fig. 4), but a small proportion mated two or more times. Male mating frequencies did not differ among treatments (X^2=2.82, df=4, P>0.50).

The treatment of the female and male involved did not affect the relative start (F=1.14, P>0.30) or duration (F=1.41, P>0.20) of a female's initial mating (Table 2). Male and female treatment probably did not affect start or duration of a female's second mating, though small numbers precluded statistical analysis. When releases involving only F_1 and WV, or NJ and WV, adults are considered, between-treatment matings occurred about as frequently as within-treatment matings (Table 3; initial female matings only). The treatment of the male involved in an initial mating also had no apparent effect on the likelihood of, or type of male(s) involved in, subsequent matings; again, small samples prevent statistical comparisons.

A few unmarked ("wild") males mated with released females in early releases. It is highly unlikely that these males were truly "feral; they undoubtedly originated with larval introductions in the cages (see previous report), and represent pupae that escaped collection. Matings with unmarked males were included in female mating frequencies (Fig. 4) but not in other summaries (Tables 2-4).

The contributions of F_1, NJ, and WV males to initial and total female matings are described for individual releases in Table 4. When released with WV

males, NJ males account for an increasing proportion of matings as the overall male:female ratio increased (relative proportions of NJ and WV males held nearly constant). Trends are not clear for F_1 males released with WV males.

DISCUSSION. The generally downward displacement of female moths in this study agrees with data of Odell and Mastro (1980). Previous experiments (Hansen, unpubl.) suggested that most downward movement is relatively short-range (less than 1 m) and that mated females move farther than virgin females. Females of all treatments exhibited generally similar sequences of calling, mating, and oviposition on a "background" of inactivity. In any event, F_1-sterile female location and activity patterns appear similar to those of untreated lab-strain and wild females.

For all treatments, multiple mating was observed in both sexes. Multiple mating has been demonstrated among gypsy moth males (Odell, unpubl.) but observed with females only in high-density populations (Cardé and Hagaman 1984). Multiple mating by gypsy moth females could affect F_1 sterile releases if fertile females mating with F_1 males are more likely to mate again, as in Heliothis zea (Carpenter et al. 1987), and if a subsequent mating with a feral male yields viable eggs. Sperm deposited last (i.e. closest to oviposition) appears to achieve fertilization precedence in some Lepidoptera (Wantanabe 1988), though this awaits confirmation for the gypsy moth. Alternatively, multiple mating by F_1 females might "soak up" feral males, reducing their chances of mating with a feral female. My data show no obvious among-treatment differences in mate selection, and provide no evidence that females mated with F_1-sterile males are more likely to mate again.

In their general "mating capabilities", F_1 males, and lab-strain (NJ) males in general, appear reasonably competitive with their wild counterparts. This seems especially true for F_1 males as male:female and overflooding ratios approach those expected in operational F_1-sterile releases (e.g. Table 4, release 5-W). However, this study was less than ideal for examining mating behaviors: sample sizes were small, females were not continuously monitored (brief matings may have escaped detection), unmarked males were not completely eliminated, and egg masses were not collected to detect the impact of multiple matings.

LITERATURE CITED

Carde', R.T. and T.E. Hagaman. 1984. Mate location strategies of gypsy moths in dense populations. J. Chem. Ecol. 10:25-31.

Odell, T.M. and V.C. Mastro. 1980. Crepuscular activity of gypsy moth adults. Environ. Entomol. 9: 613-617.

Wantanabe, M. 1988. Multiple matings increase the fecundity of the yellow swallowtail butterfly, Papilio xuthus L., in summer generations. J. Ins. Behav. 1:17-29.

Table 1. Summary of adult releases.

Release	Date	Females: Treatment	Females: n	Males: Treatment	Males: n
2-N	8/4	NJ	20	NJ	40
3-N	8/8	NJ	20	NJ	15
				WV	15
3-W	8/8	NJ	8	F_1	15
		WV	12	WV	15
4-N	8/10	NJ	10	NJ	15
		WV	10	WV	15
4-W	8/10	F_1	6	F_1	15
		WV	6	WV	15
5-N	8/12	NJ	12	NJ	10
				WV	10
5-W	8/12	F_1	6	F_1	30
		WV	12	WV	15
6-N	8/15	NJ	10	NJ	8
		WV	10	WV	9
6-W	8/15	F_1	10	F_1	20
		WV	10	WV	10
7-W	8/17	F_1	10	F_1	15
		WV	10	WV	15

Table 2. Mean start and duration of first and second matings.

Female treatment	Male treatment	First mating n	First mating Start[a]	First mating Duration[a]	Second mating n	Second mating Start	Second mating Duration
F_1	F_1	10	2.5	2.5	2	5.2	1.0
	NJ	0	---	---	0	---	---
	WV	13	2.5	2.8	3	5.5	2.0
NJ	F_1	2	1.0	1.0	0	---	---
	NJ	23	2.1	2.3	2	3.2	1.5
	WV	16	2.0	2.2	2	4.0	2.5
WV	F_1	28	2.1	2.0	5	3.7	2.4
	NJ	9	2.4	2.8	1	2.0	2.0
	WV	25	2.7	3.0	5	3.5	1.2

[a] Units are "observation periods" (i.e. no. of 30-min intervals)

Table 3. Relative participation of released F_1, NJ, and WV males in initial matings of F_1, NJ, and WV females (data pooled from releases involving F_1-WV or NJ-WV treatment pairs).

Female treatment	Male treatment	No. of initial matings	%
F_1	F_1	10	43
	WV	13	57
NJ	NJ	22	49
	WV	23	51
WV	WV	9	50
	NJ	9	50

Table 4. Relative participation of released F_1, NJ, and WV males in initial and total matings (individual releases).

Release	Overall M:F ratio	Total no. of matings	Male treatment	n	Male involvement (%): Total matings	Male involvement (%): First matings
			NJ and WV males			
6-N	0.9:1	20	NJ	8	37	35
			WV	9	63	65
3-N	1.5:1	20	NJ	15	47	47
			WV	15	53	53
4-N	1.5:1	20	NJ	15	50	50
			WV	15	50	50
5-N	1.7:1	13	NJ	10	77	77
			WV	10	23	23
			F_1 and WV males			
7-W	1:1	20	F_1	15	50	53
			WV	15	50	47
3-W	1.5:1	27	F_1	15	40	50
			WV	15	60	50
4-W	2.5:1	10	F_1	15	44	38
			WV	15	56	62
6-W	1.5:1	21	F_1	20	40	41
			WV	10	60	59
5-W	2.5:1	21	F_1	30	81	88
			WV	15	19	12

FIGURE 1. Movement of released female moths on oak trees.

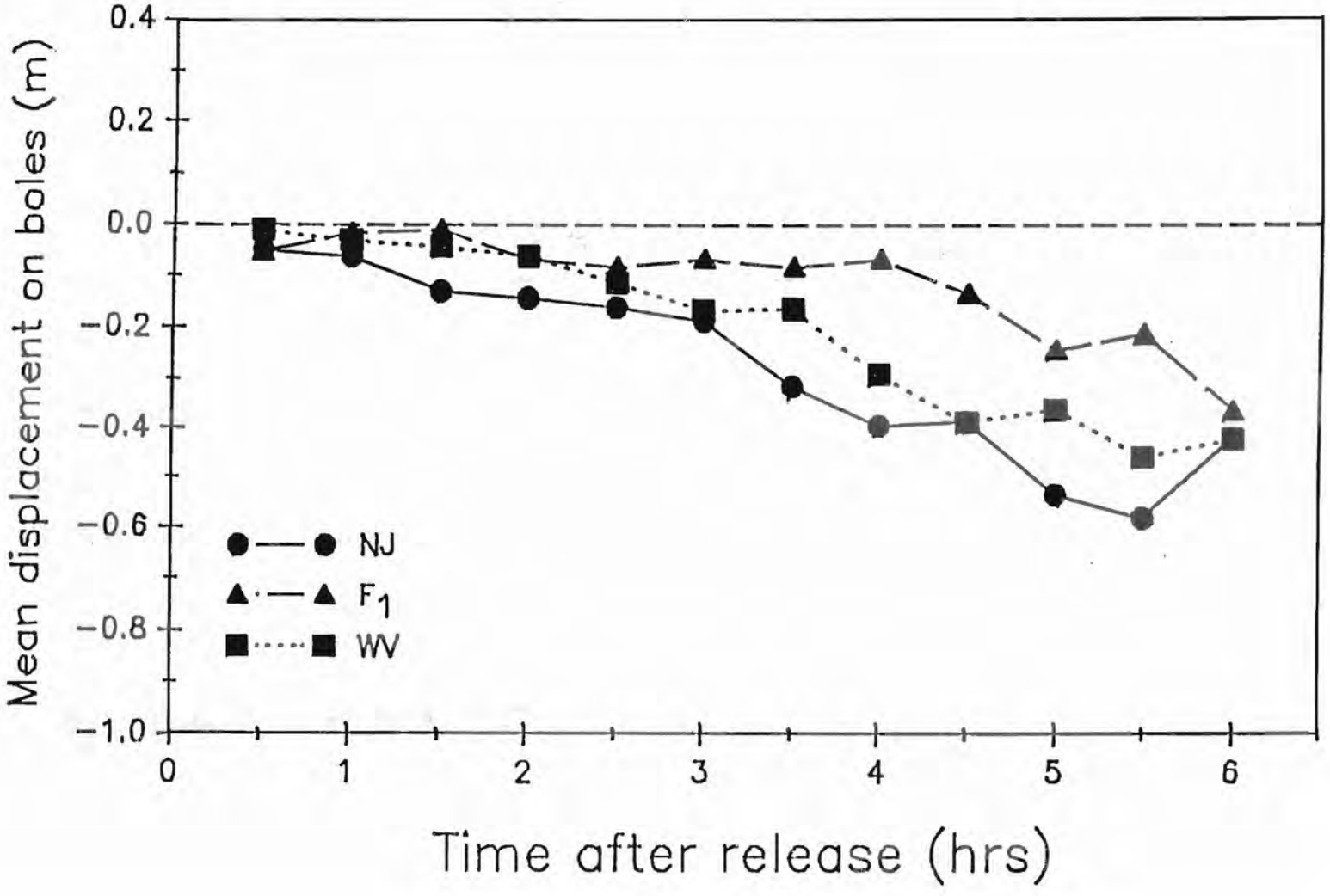


FIGURE 2. Proportion of released female moths located on oak boles (A) and branches (B), or off oak trees (C).

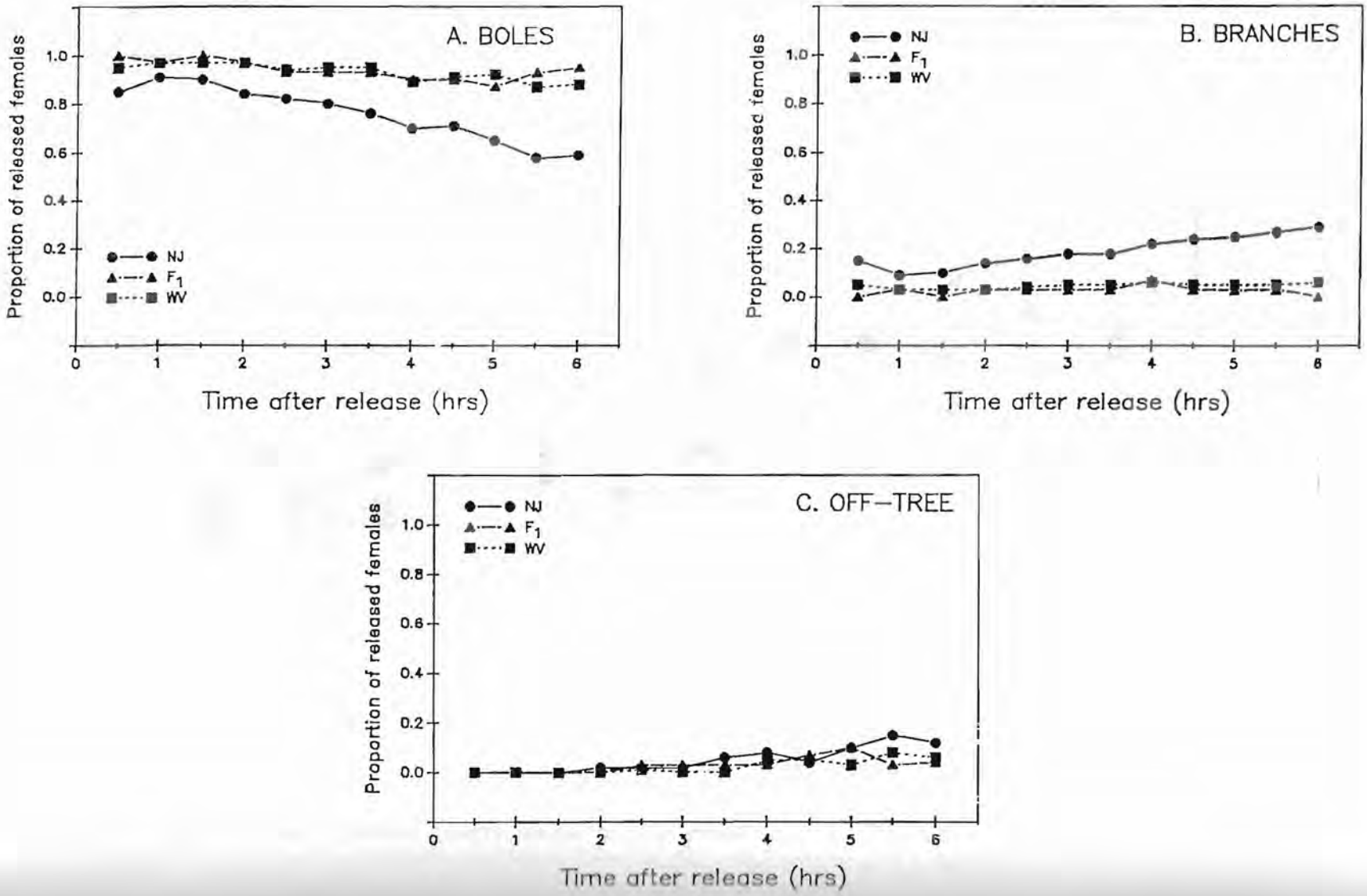


FIGURE 1. Proportion of released female moths calling (A), mating (B), ovipositing (C), or inactive (D).

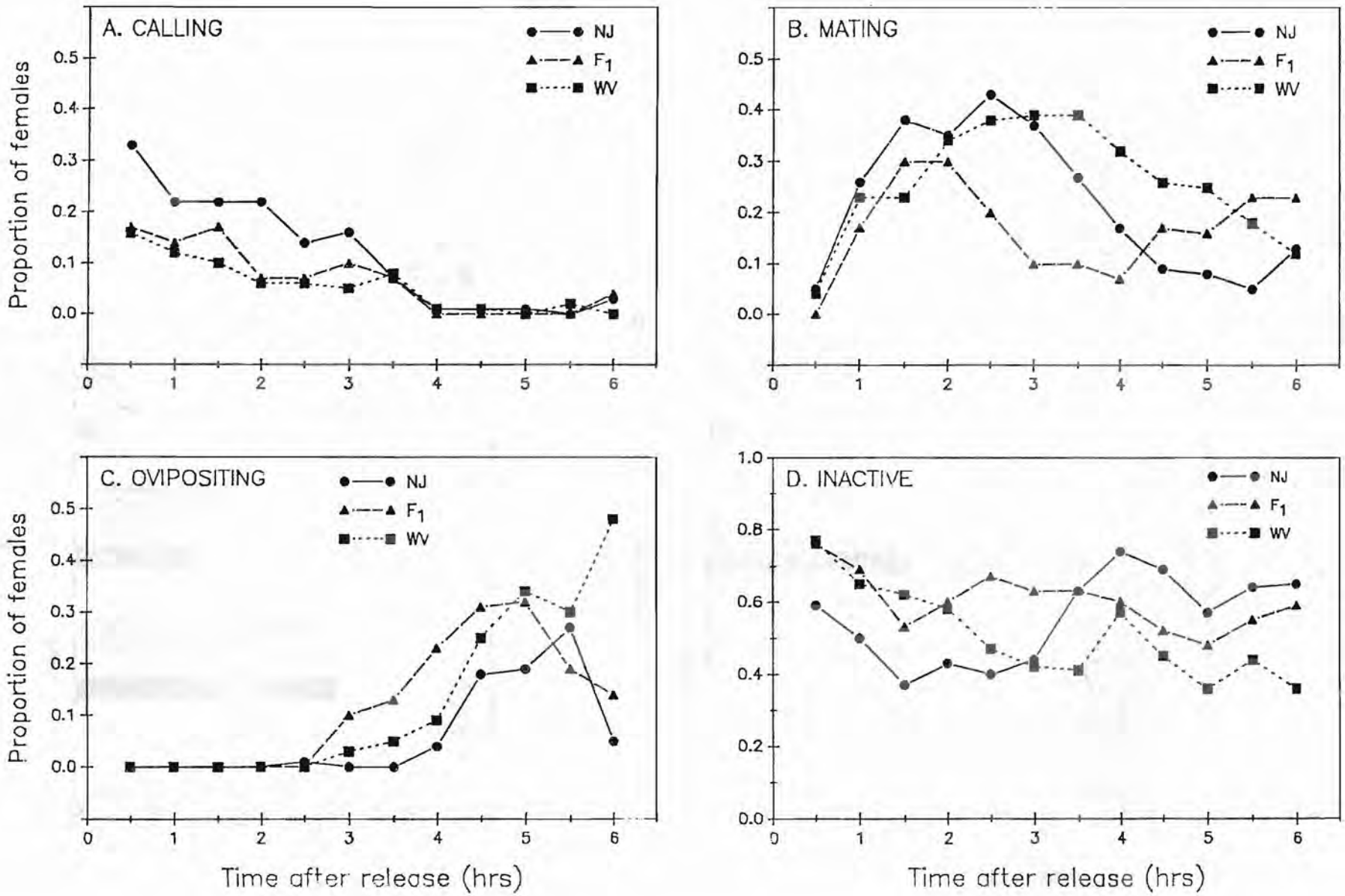
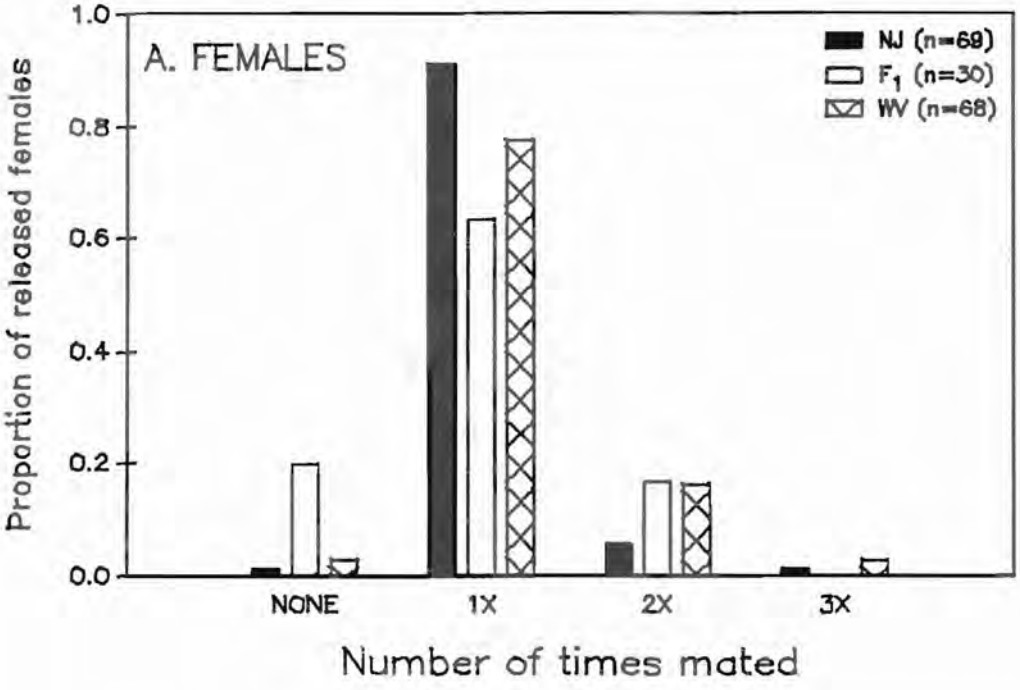
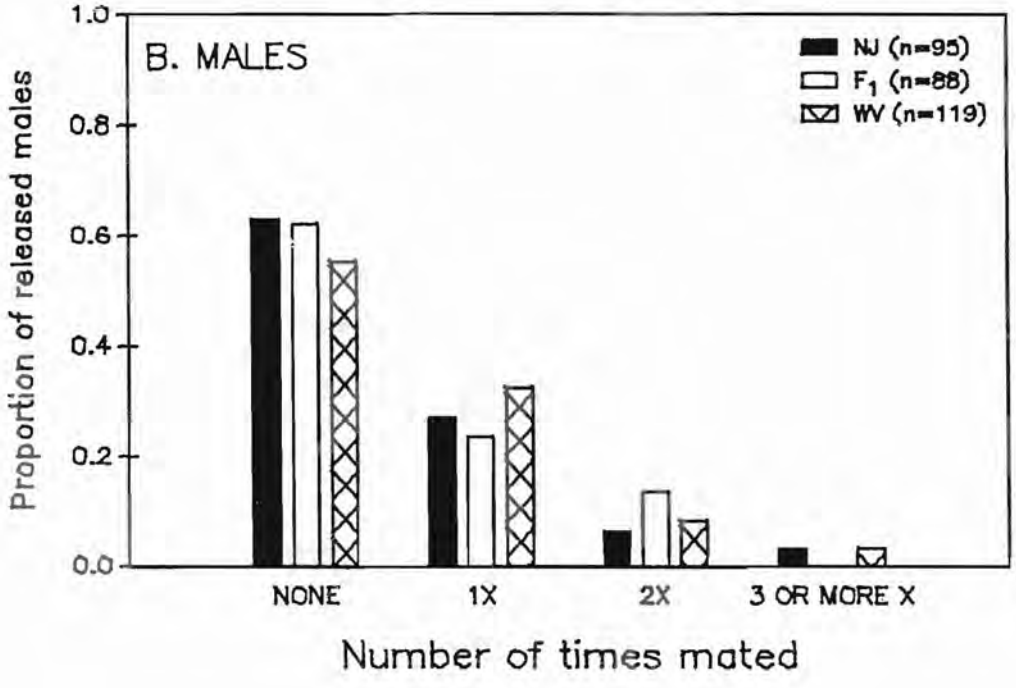


FIGURE 4. Mating frequencies of released female (A) and male (B) moths.





Project Number:	GM 87.2.2
Project Title:	Sterile Male Demonstration Project, Gates Co., North Carolina
Report Period:	October 1, 1987 - September 30, 1988
Report Type:	Interim
Project Leaders:	V C. Mastro, A. Pellegrini-Toole, C. P. Schwalbe
Cooperators:	H. Singletary, K. Wilson, W. Wescott

This project was initiated in 1987 with a release totaling approximately *100,000 egg masses in a 12-15 acre area. At the time of the first release, a* good estimate of the native insect density could not be made nor could the geographic limits of the population be defined (see GM 7.2.2, 1987). Preliminary results of the 1987 release were presented in the last laboratory report. This completes reporting of 1987 results and presents preliminary 1988 results in which an additional release of F_1 egg masses was made.

In preliminary analysis of 1987 data, wide difference was noted between sterile:fertile ratios projected by the different evaluation techniques (Table 1). The male chromosome analysis technique can only be used for insects collected before the 5th instar whereas the male mating evaluation technique can be used for any male collected as a larva or pupa. If there was a large amount of F_1 male mortality in the 5th instar or pupal stage, the chromosome evaluation technique would overestimate the sterile:fertile male ratio in the adult stage. Figure 1 plots data from two evaluation techniques over all sampling weeks and no abrupt loss of steriles is noted.

Because of the large differences in estimated overflooding ratios in 1987 projecting an overflooding rate for the 1988 release was equally difficult (Table 1). In 1988, an additional 100,000 egg masses were released. Hatch of wild and F_1 egg masses in 1988 was nearly synchronous (Fig. 5). However, the proportion of F_1 eggs hatching was poor (mean of 16.6%, low of 5.7%, high of 35.2%). To date, data are available for calculating three estimates of the male overflooding ratio. As opposed to data in 1987, all three estimates are approximately the same (Table 1). All three estimates are for greater than 95% of the adult male population to be sterile. Results from 1988 (Fig. 2) display a gradual drop in the proportion of males determined to be sterile from week 6 until the final week of sampling. However, this was only approximately a 6% decrease.

Table 1. Results of monitoring sterile F_1:wild overflooding ratios in Gates Co., North Carolina.

	1987			1988		
Number F_1 egg masses released	100,300			100,000		
Expected S:F male ratio	None			58:1 - 2666:1 4/		
Monitoring technique	No. samples	Ratio S:F	% fertile	No. samples	Ratio S:F	% fertile
Male chromosome analysis 1/	147	147:0	0.0	73	22.3:1	4.3
Male mating evaluation 1/	623	28.7:1	3.4	770	23.8:1	4.0
F_1 monitor female egg mass evaluation	58 2/	3.3:1	23.3	32	31:1 3/	3.1
Female mating evaluation 1/	484	14.6:1	6.4	NOT COMPLETE		
Post season egg mass evaluation	1196	12:1	7.7	NOT COMPLETE		
Male parent	880	1:2.4				
Female parent	966	6.8:1				
Number of males trapped	2534			2966		

1/ Insects collected as immatures (larvae or pupae) and evaluated at the Otis Methods Development Center.

2/ An additional 278 monitor females placed could not be used for establishing a mating ratio: 233 failed to oviposit; 48 produced egg masses with all unembryonated eggs and; 7 produced egg masses that had degrees of embryonation that statistically could not be separated from the two possible mating types.

3/ An additional 339 monitor females placed could not be used for establishing a mating ratio: 311 failed to oviposit; 27 produced egg masses with all unembryonated eggs and; 1 produced an egg mass that had a degree of embryonation that could not be statistically separated from the two possible mating types.

4/ Based on high and low ratios observed and male trap catches = 20% of male population in 1987 and projected number of wild egg masses in the spring of 1988.

Post season egg mass evaluation (when complete) should provide the best estimate of F_1 impact on the native population. Trapping in 1988 resulted in approximately 19% more males than in 1987. Pattern of trap catches in 1988

(Fig. 4) was similar to 1987. However, additional traps added in 1988 to the periphery of the treatment area did capture some moths.

The sterile male treatment area is now considered part of a larger surrounding population. In 1989, no further sterile male releases are planned. However, the area will be aerially treated with Bt.

When the remaining data are complete for this project, a final report will be prepared. Generally, the results of this project have demonstrated that F_1 sterile insects released as eggs into a wild population grow, survive and interact with it. However, the expected result, eradication, was not achieved. The variability in the results of using various sampling techniques also demonstrates a need to refine or develop techniques with reliable indicators of the impact of an F_1 release.

Figure 1. A Comparison of Two Evaluation Techniques For Determining the Proportion of a Field Population That is Sterile. GATES COUNTY 1987

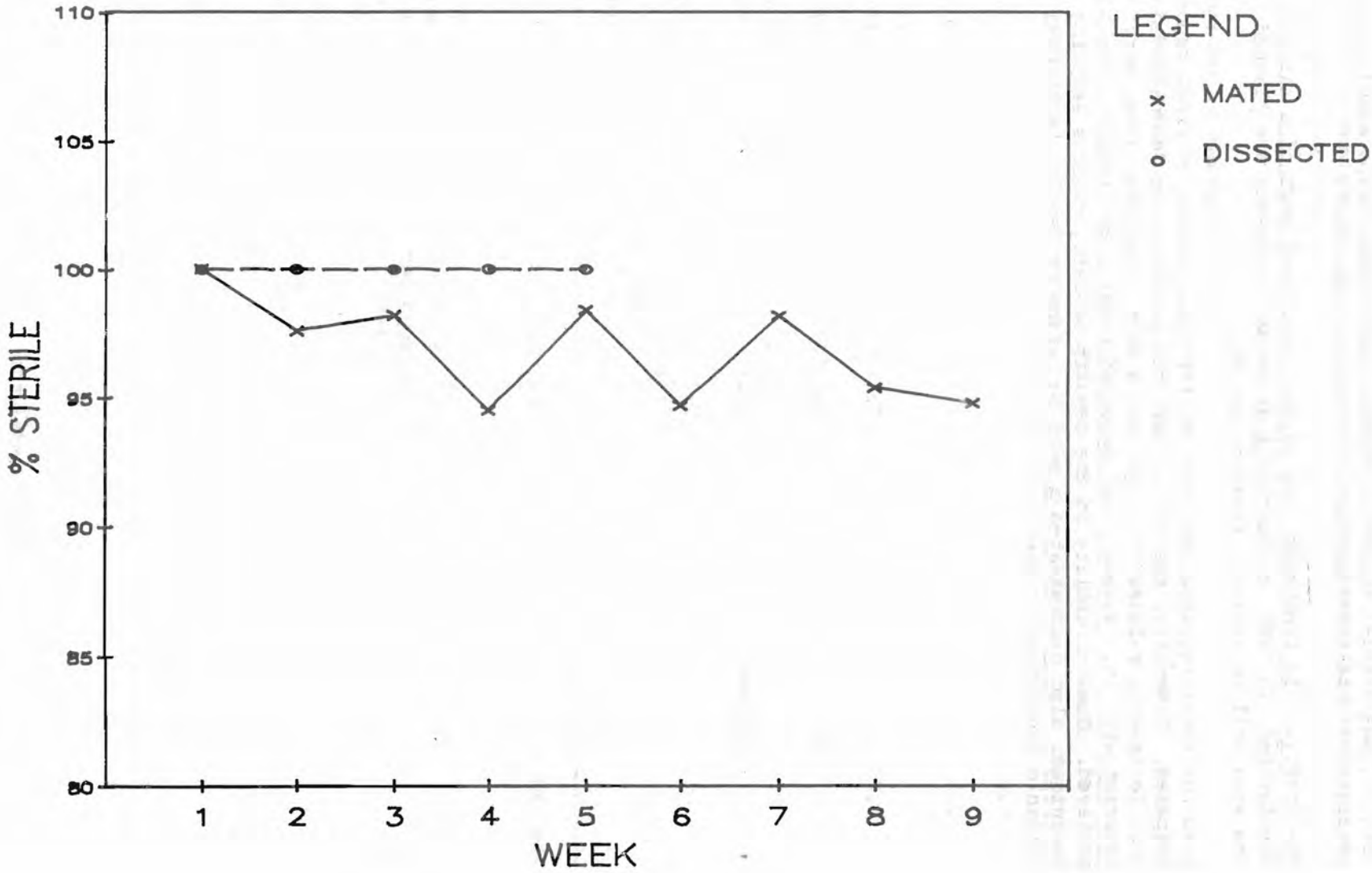


Figure 2. A Comparison of Two Evaluation Techniques For Determining the Proportion of a Field Population That is Sterile. GATES COUNTY 1988

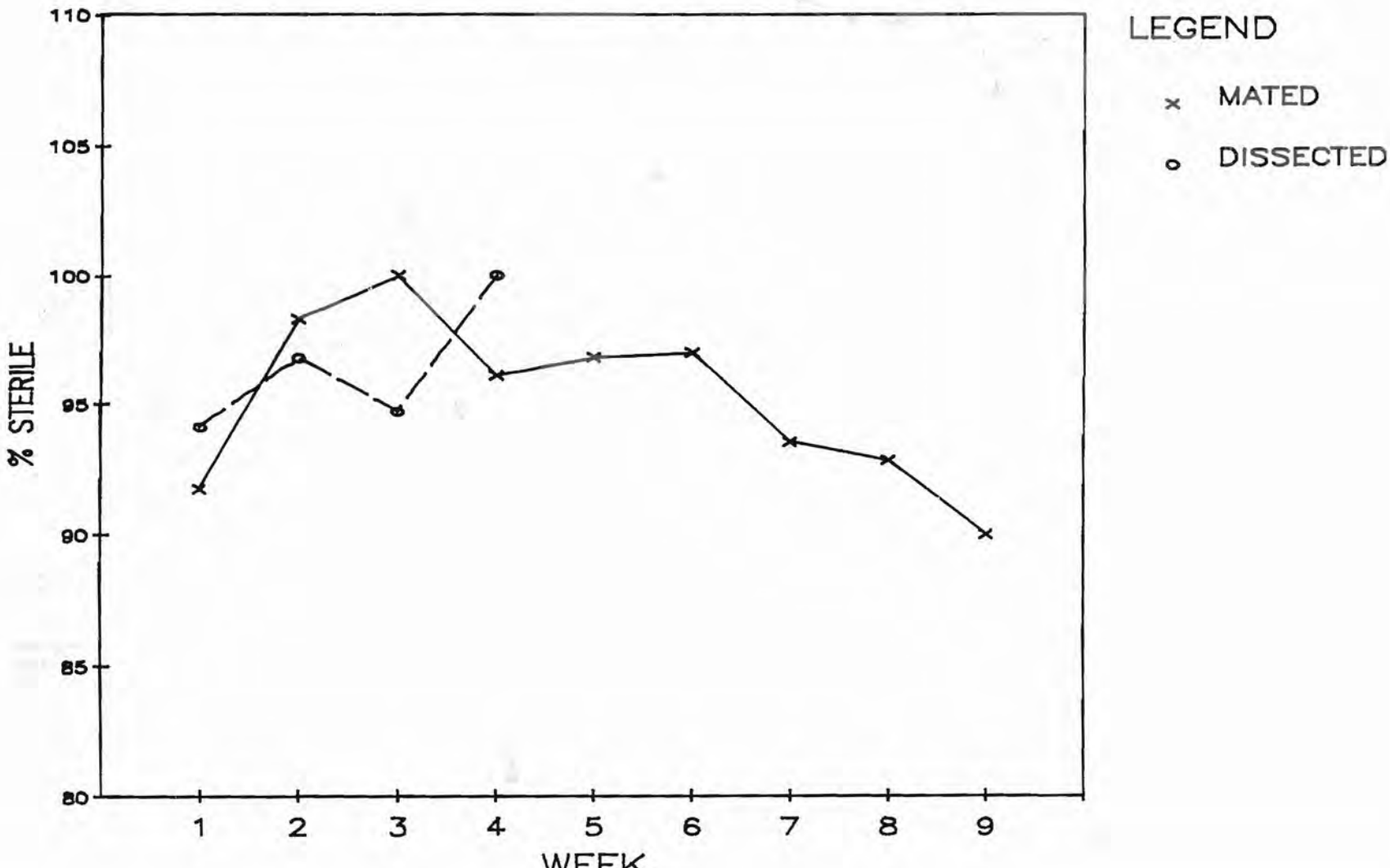


FIGURE 3. 1988 GATES CO. TRAP SURVEY

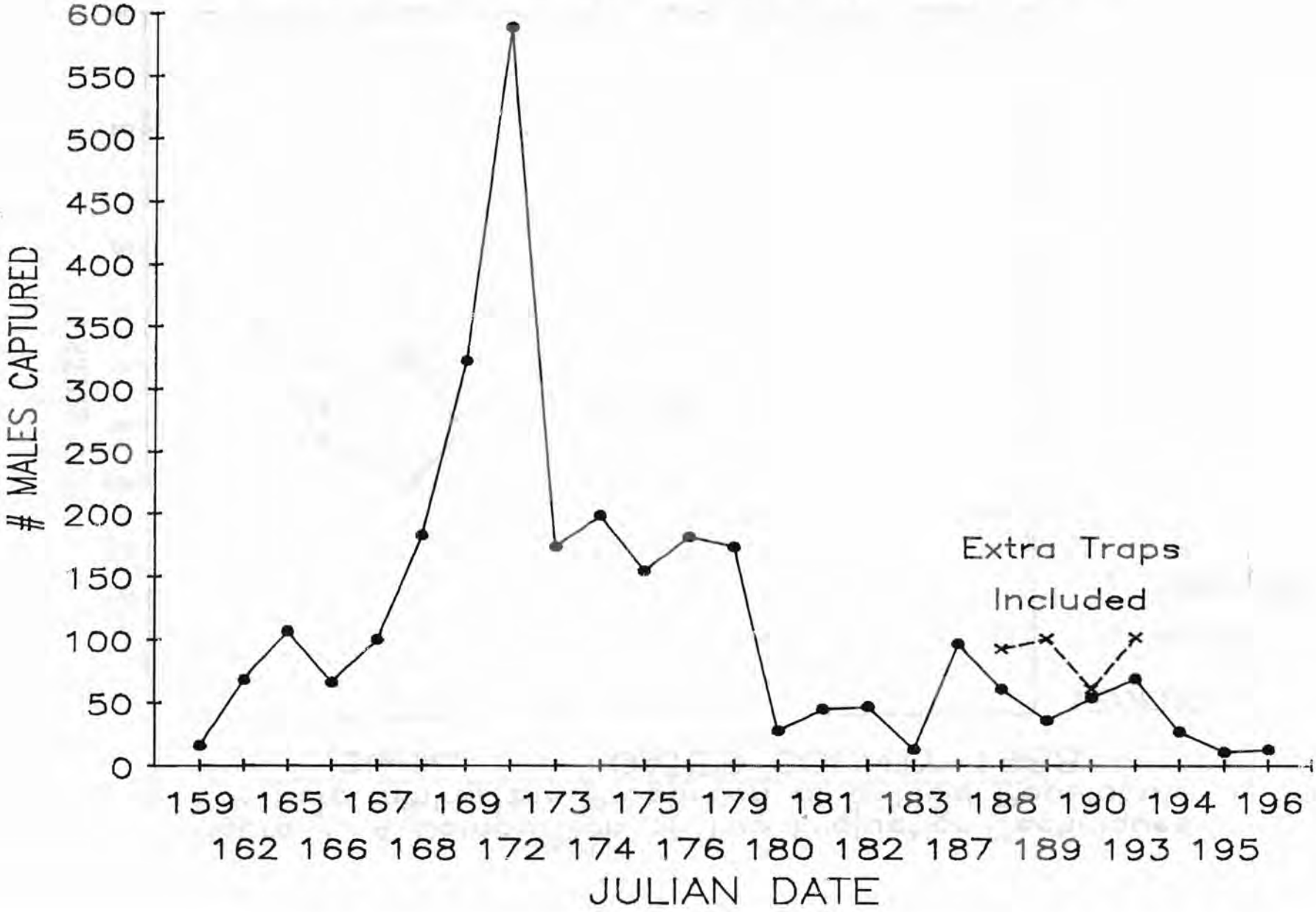


Figure 4.

GATES CO., MALE TRAPPING RESULTS 1988

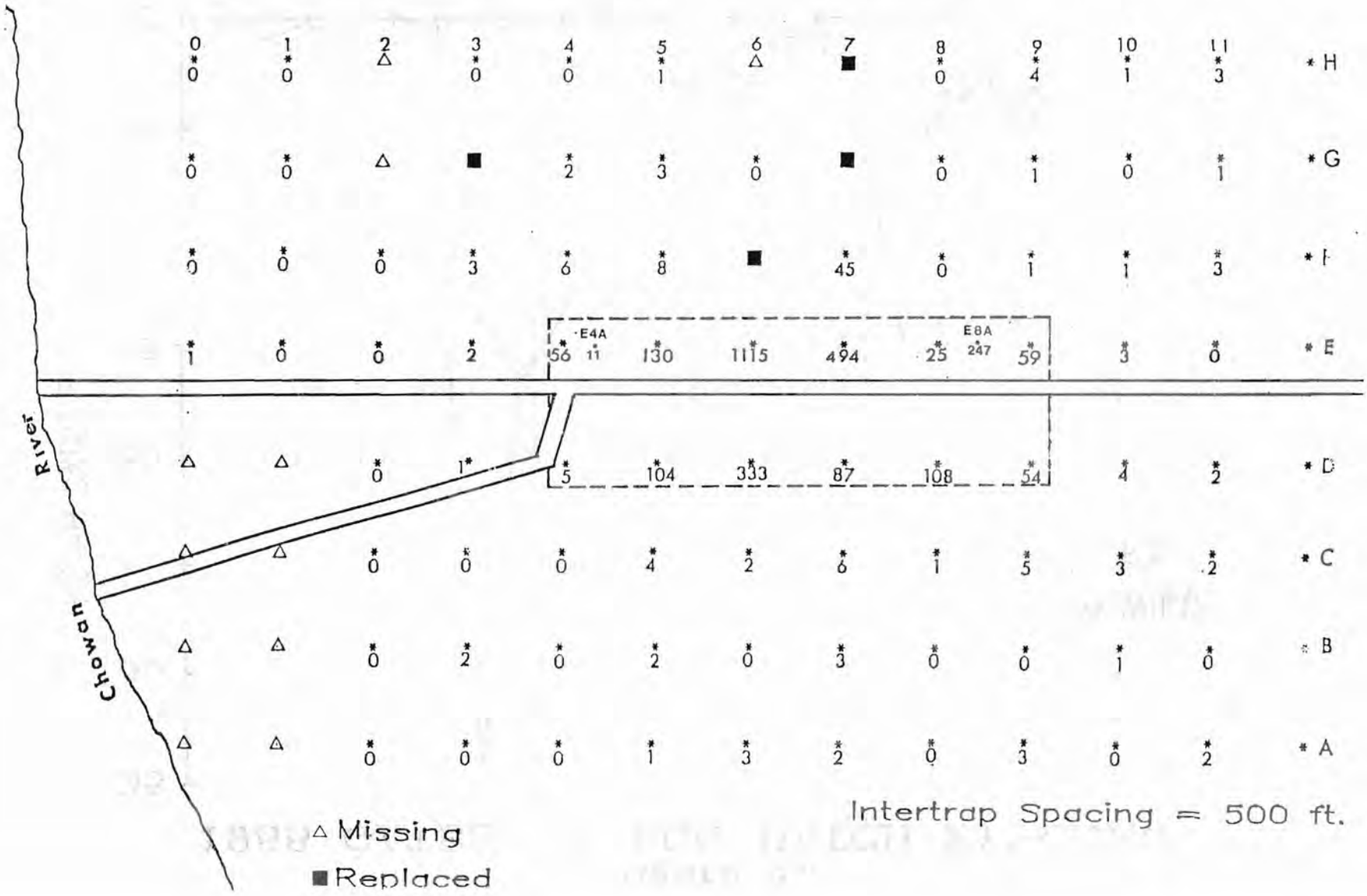
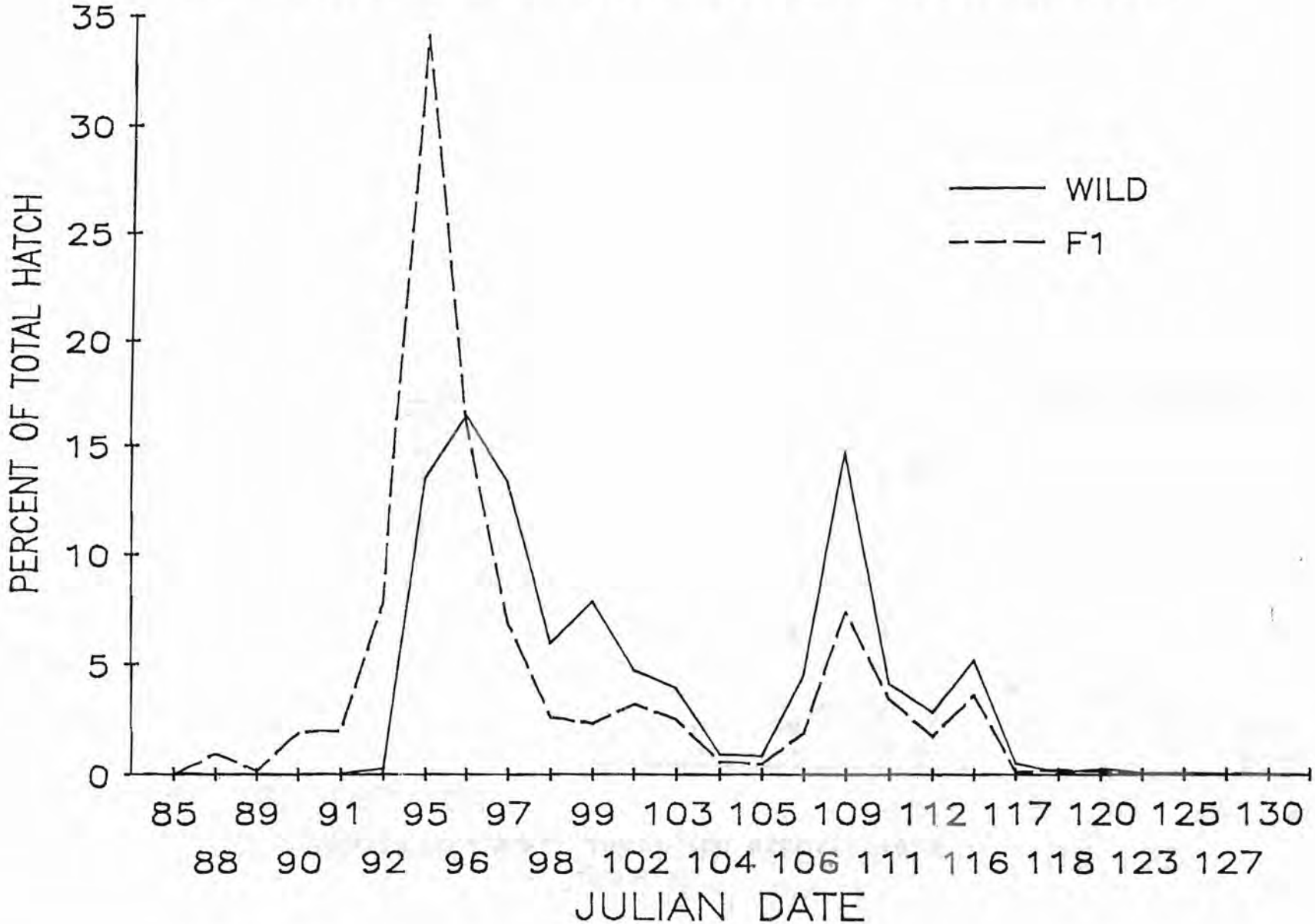


Figure 5.

1988 GATES CO. EGG HATCH SYNCHRONY



Project Number: GM 87.2.3
Project Title: Competitiveness of Gypsy Moth Adults
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: V. C. Mastro, A. Pellegrini-Toole, R. Hansen and C. P. Schwalbe

The last Annual Report summarized tests in which the attractiveness of various types of female gypsy moths were compared in the field. Generally, F_1 females (6, 8 and 10 krad) were as successful in attracting males as laboratory reared or wild females. Also, periodicity of mating and elapsed pre-mating times were similar in most cases. We concluded that F_1 females (10k) could be used to assess mating success of F_1 and wild males in a field situation. F_1 females, however, produce a larger proportion of egg masses (23%) from which male parental type cannot be determined compared to the normal laboratory strain (9%). The major advantage of using F_1 females to evaluate sterile male releases, mating disruption tests or mass trapping projects is that if they escape there are no additional fertile insects added to the wild population.

In 1987, we began to investigate the incidence of multiple mating by female gypsy moths of various strains. Although earlier studies had demonstrated that male gypsy moths are capable of fertilizing several females on the same day, the incidence of multiple mating by females was largely unexplored. To clarify the impact of an F_1 release, the incidence and frequency of multiple mating by both sexes must be understood.

The objectives of the following studies were to 1) determine if females that mate with males of various types (i.e., wild, laboratory, F_1) are more or less likely to remate, 2) characterize egg masses oviposited by multiply-mated females and 3) determine with what frequency multiple mating occurs under controlled population conditions.

LABORATORY STUDIES

METHODS AND MATERIALS

To address the first two objectives, a laboratory experiment was run where females of various strains (e.g., NJSS or wild) were placed in large petri dishes (15 cm dia x 4 cm) with a 4 cm hole cut in the center of the lid. The hole was covered with 26 mesh saran screen and provided a resting site for females as well as air circulation. Petri dishes were placed vertically in a rack to facilitate observation of females' calling behavior and mating. In testing, females were placed in petri dishes, allowed to begin calling, and either a normal laboratory or F_1 male was introduced initially. Time to initiate mating and length of mating was recorded. Once mating was completed, the first male was removed and a second was introduced. Four different mating sequences were tested:

	Initial Mating	Second Mating
1)	Normal (NJSS, wild)	F_1 10k
2)	F_1 10k	Normal (NJSS, wild)
3)	F_1 10k	F_1 10k
4)	Normal (NJSS, wild)	Normal (NJSS, wild)

Females were allowed to oviposit and the egg masses were collected and held under standard conditions for embryonation and diapause. When the diapause period is complete, all egg masses will be evaluated for hatch and embryonation.

RESULTS AND DISCUSSION

Initial results indicate that F_1 males (progeny of males treated with 10 krads) are less likely to mate with a virgin female than either NJSS laboratory reared or wild males (Tables 1 and 2). In addition, normal males (NJSS and wild) are more likely to mate with a female that already has mated once. These results differ from past studies which concluded that virgin F_1 males had as great a propensity to mate as normal males.

Females, however, that initially mated with an F_1 male did not significantly remate more often than females that initially mated with a normal male (Tables 1, 2 and 3). It appears that at least in this small test arena nearly 30 percent of the females will remate when paired with an "aggressive" normal male (Table 3). However, only approximately 4 percent remate when the second male partner is an F_1.

Clearly, if these results are applicable to a field situation, F_1s are at a competitive disadvantage. The magnitude of the disadvantage, however, will be determined in part by the degree of sterility displayed by egg masses produced by females initially mating with an F_1 male and subsequently with a normal male and vice-versa.

Tables 1 and 2 list the results of this test, respectively, when using NJSS laboratory reared females and wild (i.e., field collected as pupae - Pennsylvania).

Table 1. Incidence of NJSS females remating when an initial mating involved various types of males.

	Initial mating			Second mating		
Number pairs	Male type	No. of pairs mating	Percent mating[1/]	Male type	No. of pairs mating	Percent mating[1/]
48	NJSS	39	81.3 a	F_1-10k	2	5.1 a
48	F_1-10k	31	64.6 b	NJSS	9	29.0 b
48	F_1-10k	33	68.8 b	F_1-10k	2	6.1 a
48	NJSS	38	79.2 a	NJSS	11	22.9 b

1/ Vertically, percentages followed by the same letter are not significantly different according to Chi-square of the 5% level of significance.

Table 2. Incidence of Pennsylvania wild females remating when an initial mating involved various types of males.

Number pairs	Initial mating: Male type	Initial mating: No. of pairs mating	Initial mating: Percent mating[1/]	Second mating: Male type	Second mating: No. of pairs mating	Second mating: Percent mating[1/]
12	wild	12	100.0 a	F_1-10k	0	0.0 a
12	F_1-10k	12	100.0 a	F_1-10k	0	0.0 a
7	F_1-10k	6	85.7 a	wild	1	16.7 a
7	wild	7	100.0 a	wild	2	28.7 a

1/ Vertically, percentages followed by the same letter are not significantly different according to Chi-square at the 5% level of significance.

Table 3. Incidence of remating females (NJSS + wild) when an initial mating involved various types of males (Tables 1 and 2 combined).

Number pairs	Initial mating: Male type	Initial mating: No. of pairs mating	Initial mating: Percent mating[1/]	Second mating: Male type	Second mating: No. of pairs mating	Second mating: Percent mating[1/]
60	Normal	51	85.0 a	F_1-10k	2	3.9 b
55	F_1-10k	37	67.7 b	Normal	10	27.0 a
60	F_1-10K	45	75.0 b	F_1-10k	2	4.4 b
55	Normal	45	81.8 ab	Normal	13	28.9 a

1/ Vertically, percentages followed by the same letter are not significantly different according to Chi-square at the 5% level of significance.

FIELD CAGE STUDIES

METHODS AND MATERIALS

In separate studies, we investigated the frequency of female multiple mating in an arena closer to natural field conditions. Field cages 12' x 12' x 6'h were used to establish populations of male and female adult gypsy moths of different densities. Two female densities were used in these tests, 4 or 40 females per cage, while the male density was held constant at 40 males per cage. In all tests two types of males (20 of each type) were introduced into a cage at a time. Males were marked to distinguish between strains. Following is a list of the various combinations tested. All insects were one day old when tested (the day after eclosion).

Male to female ratio 40:4

Female type	Male combinations tested
F_1-10k	(PA wild - F_1-10k) (NJSS-F_1-10k)
NJSS	(PA wild - NJSS) (NJSS - F_1-10k) (NJSS-Ashumet)
PA-wild	(PA wild - F_1-10k) (PA wild - NJSS)
Ashumet	(NJSS - Ashumet)

Male to female ratio 40:40

Female type	Male types
F_1-10k	NJSS F_1-10k
NJSS	NJSS F_1-10k

Females in field cages were observed constantly (9 a.m. - 4:00 p.m.) and when a mating occurred, the time was recorded and the male was uniquely marked on the wing. A mating was only noted when the pair was observed to be in copula. At the conclusion of a day's testing, females were recovered and allowed to oviposit. The resulting egg masses were held under the standard laboratory embryonation and diapause conditions.

RESULTS AND DISCUSSION

Figures 1 and 2 compare the proportions of females mating various numbers of times when the female density was altered. The data were pooled for all male types tested. A higher proportion of females mated (once and multiply) when the female density was lower (i.e., the male to female ratio was 40:4). Apparently, male mating pressure at skewed, higher male densities, results in larger number of matings per females. Because numbers of females tested, particularly in the 40:4 male:female ratio, were small, proportions based on these numbers may be misleading. For example, in Figure 1, at the 40:4 sex ratio although 33% of the F_1 females that mated twice mated a third time, this percentage is based only on 2 females mating three times and 6 females mating twice. Similarly, the percentage of Ashumet females mating is based on only 4 females.

Table 4. Proportions of females mating various numbers of times when simulated male:female density was 40:4 and 40:40. All male mating types were pooled.

Male:female ratios	Female type	Number tested	No. mating once	%	No. mating twice	%	No. mating thrice	%
40:4	PA wild	6	6	100	4	66.6	0	-
	NJSS	16	16	100	3	18.8	0	-
	Ashumet	4	3	75	1	33.3	0	-
	F_1-10k	8	8	100	6	75.0	2	33.3
40:40	NJSS	80	52	65.0	4	11.5	0	-
	F_1-10k	40	27	67.5	2	7.4	0	-

The proportion of females which remate does not appear to be different when the first male partner was a PA-wild or an F_1-10k male (Figure 3). However, at both male:female ratios tested, females mated to laboratory strain males (NJSS & Ashumet pooled) at 40:4 and to NJSS males at 40:40 tend to remate less often.

In field cage tests where the male:female populations were established at 40:40, NJSS males mated more often initially and also accounted for larger proportions of rematings than F_1 males (Figure 4). In these tests, a larger portion of the F_1 male population did not mate initially. However, those that do mate tend to remate as aggressively as NJSS males. For example, a significantly smaller proportion (24 of 80) F_1 (30%) males mated once vs 39 of 80 (48.8%) NJSS. However, of the 24 F_1 males mating once, 9 or 37.5% mated a second time, whereas 15 of 39 (38.5%) NJSS males mating once mated a second time. In other words, there appear to be differences in the proportions of the male populations that participate in mating.

Figure 5 summarizes the time spent mating by F_1-10k and NJSS males with NJSS virgin females. Mating times are similar for both strains of males and also for males with various mating histories. Mating times appear to decrease when males are mating for the fourth time. However, the number of matings involved are too small for statistical analysis. Similar amounts of time are spent mating with virgin F_1-10k females (Fig. 6). Again, the time spent mating for both types of males are similar. When mating times are combined for NJSS and F_1-10k virgin females, the mating times are similar for both strains of males (Fig. 7).

Egg masses from all females ovipositing are now being evaluated for degree of embryonation and hatch and results will be reported in the next Annual Report.

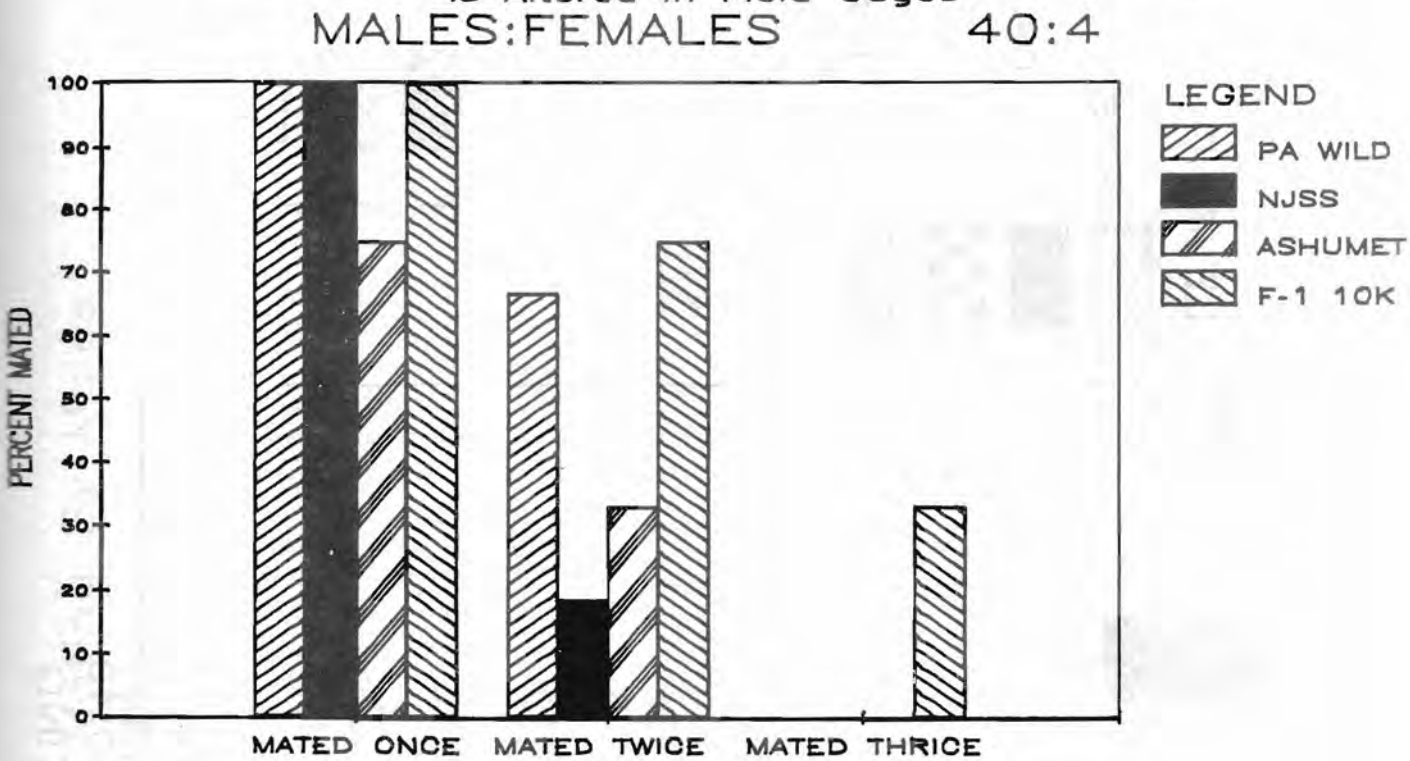
CONCLUSIONS

Female multiple mating has been confirmed in the laboratory and in field cage studies. The frequency of female multiple matings appears to be related to mating pressure. In the laboratory, females would mate and remate when paired with normal males more often than when paired with F_1 males. Remating frequency appears to be independent of the first type of mating (e.g., type of male partner) and is influenced by the type of the second male. When male:female ratios are manipulated in field cages, the incidence of multiple mating by females is greater when males are present in greater numbers (i.e., 40:4 male:female). Also, in field cages a larger proportion of F_1 males failed to mate initially indicating some lack of competitiveness when compared to the normal NJSS. Those F_1s who mated initially, however, displayed similar multiple mating patterns both in frequency and duration as the NJSS males. Although data are limited, there may be an equal lack of competitiveness in NJSS normal males. In two field cage tests (40:4 male: female ratios) where NJSS males were compared to wild males, the latter male type accounted for a majority of the mating. In these two tests, of the eight females mating the first time, 7 mated with wild males while one mated with a NJSS male.

This, as to now unrecognized, lack of competitiveness (if it is real and not an artifact of test design) is an important portion of the F_1 release model that will influence release strategy. The differences must also be further

quantified so that it can accurately be accounted for. Data from evaluation of egg masses of multiple-mated females are yet needed to indicate if F_1 males are at a further competitive disadvantage because of sperm precedence.

Figure 1 & 2 Proportions of Females Mating Initially and Remating When the Female Density is Altered in Field Cages



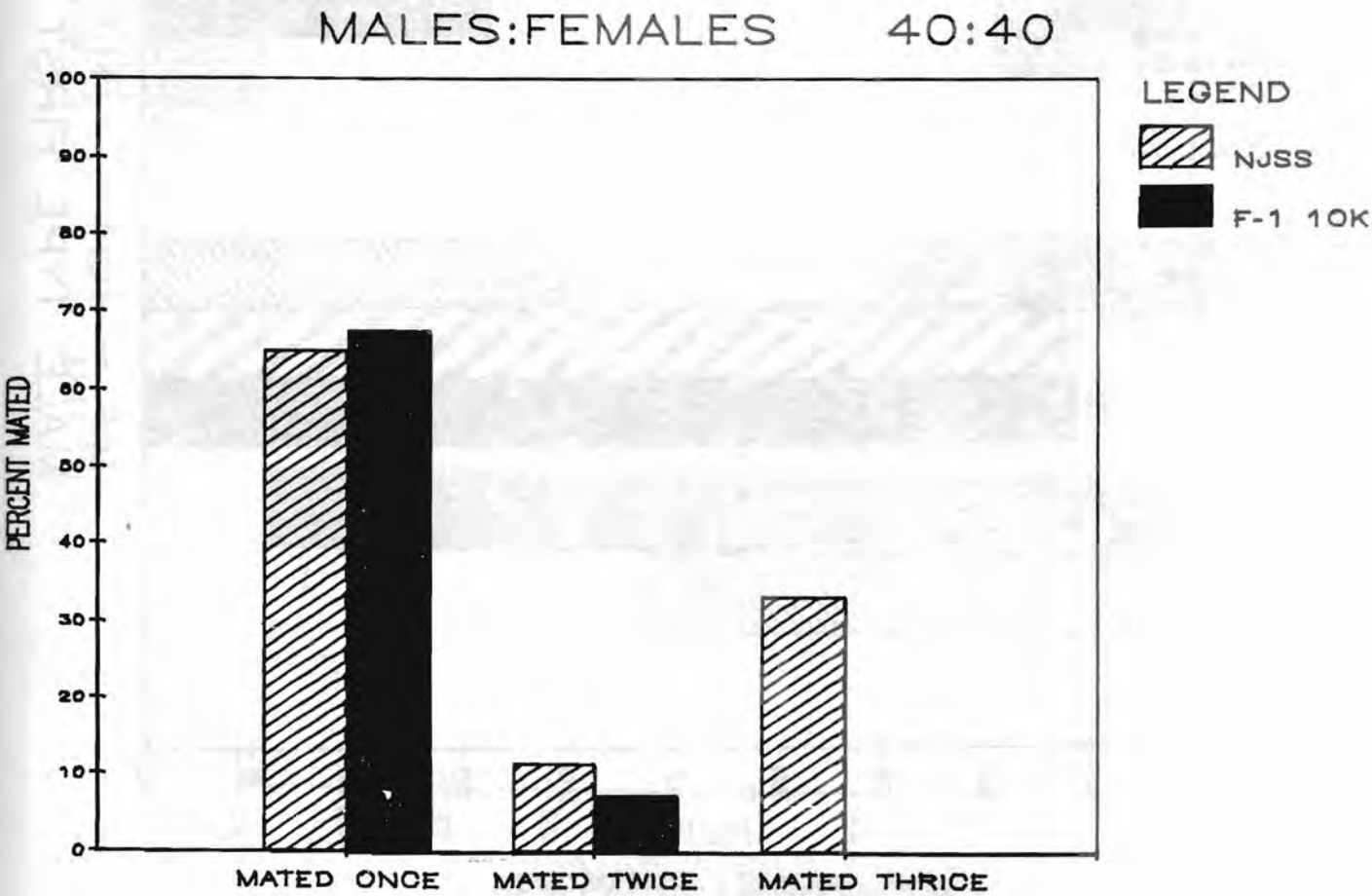


Figure 3. Proportions of Females Remating When the Initial Mating Was With Males of Various Types.

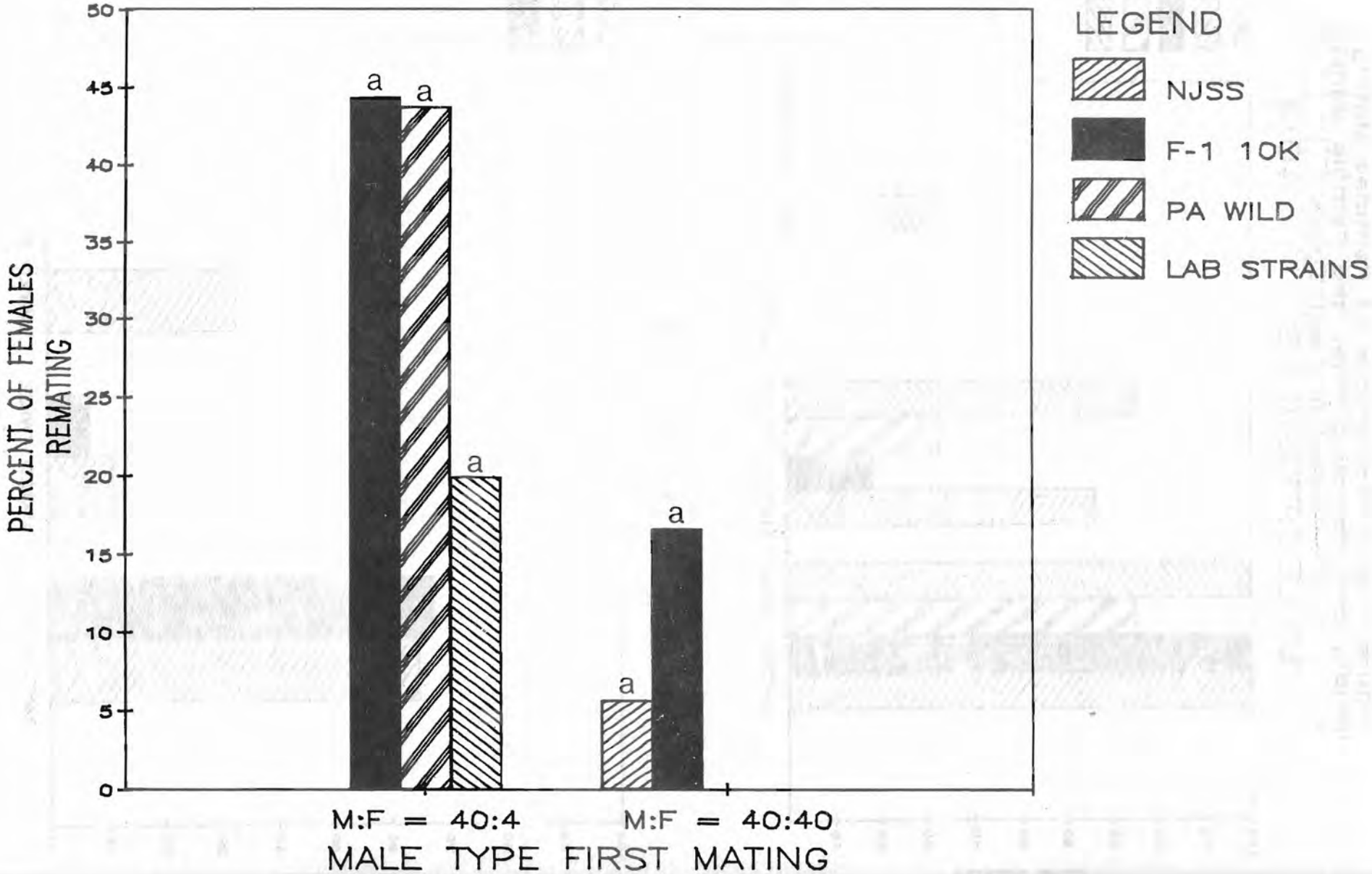


Figure 4. Incidence of Mating By Males in Field Cages.

MALE:FEMALE DENSITY 40:40

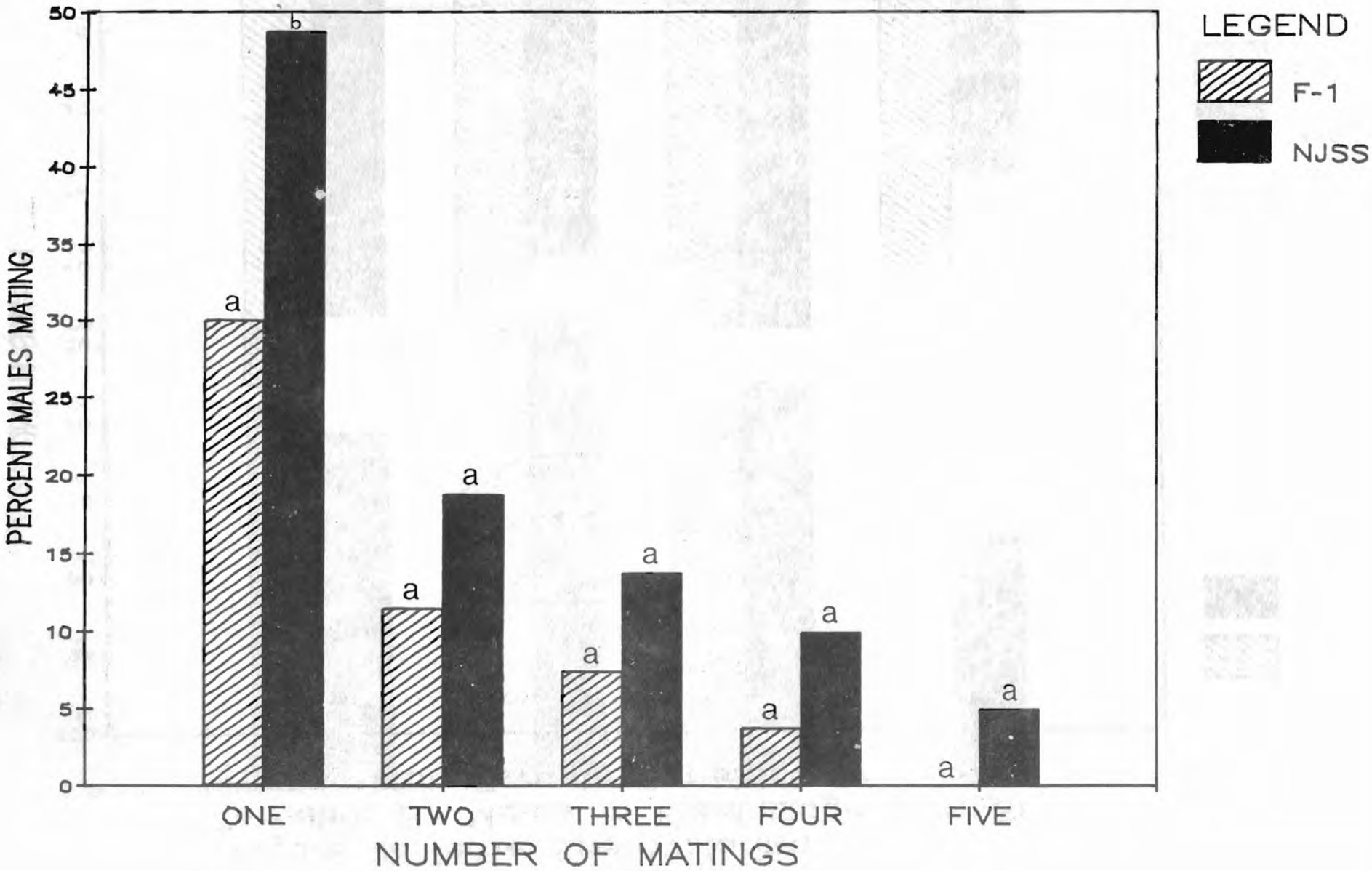


Figure 5. Time Spent Mating With NJSS Virgin Females By Males in Field Cage With Simulated Male:Female Densities of 40:40.

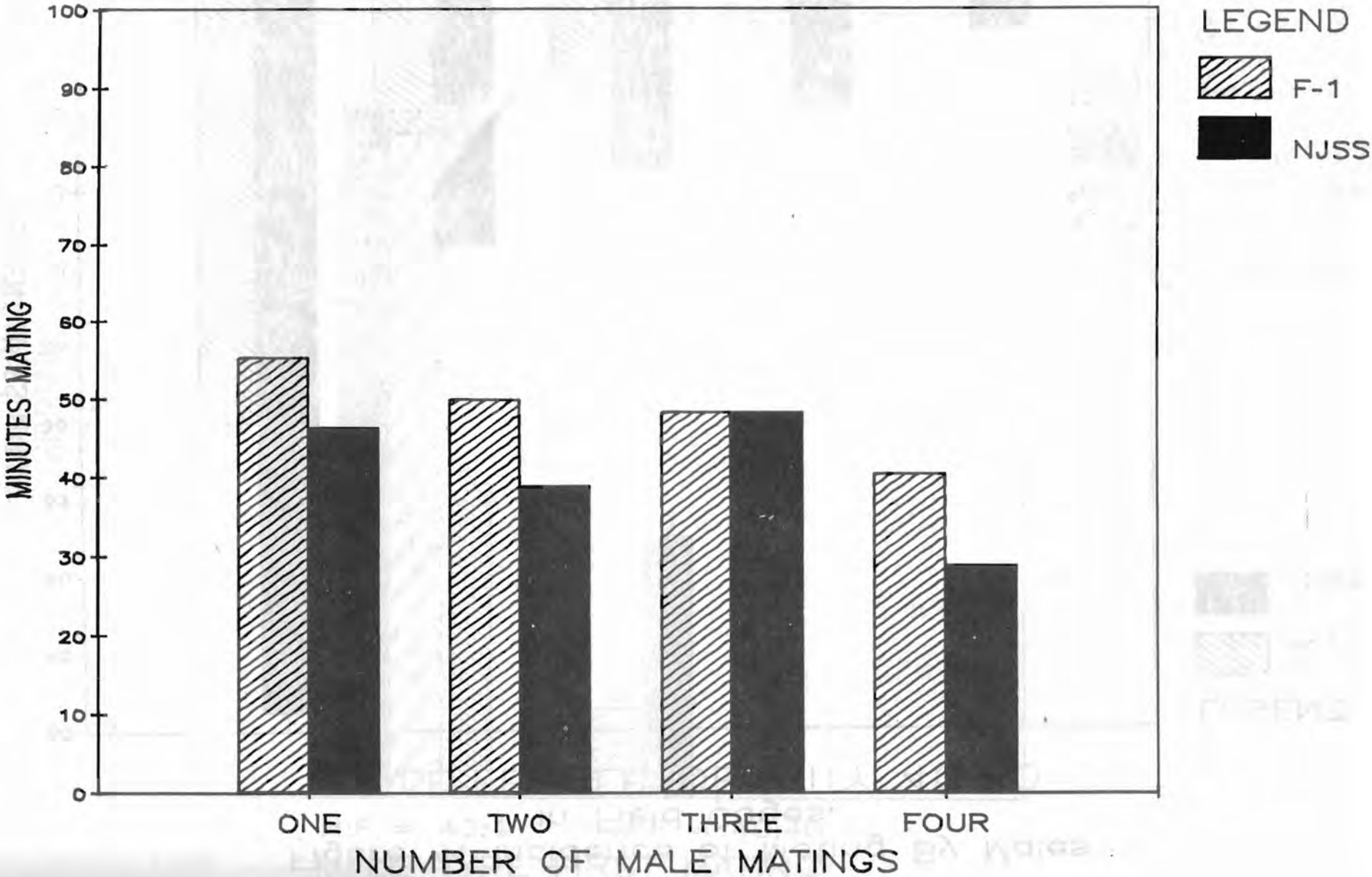


Figure 6. Time Spent Mating with F-1 10K Virgin Females By Males in Field Cages With Simulated Male:Female Densities of 40:40.

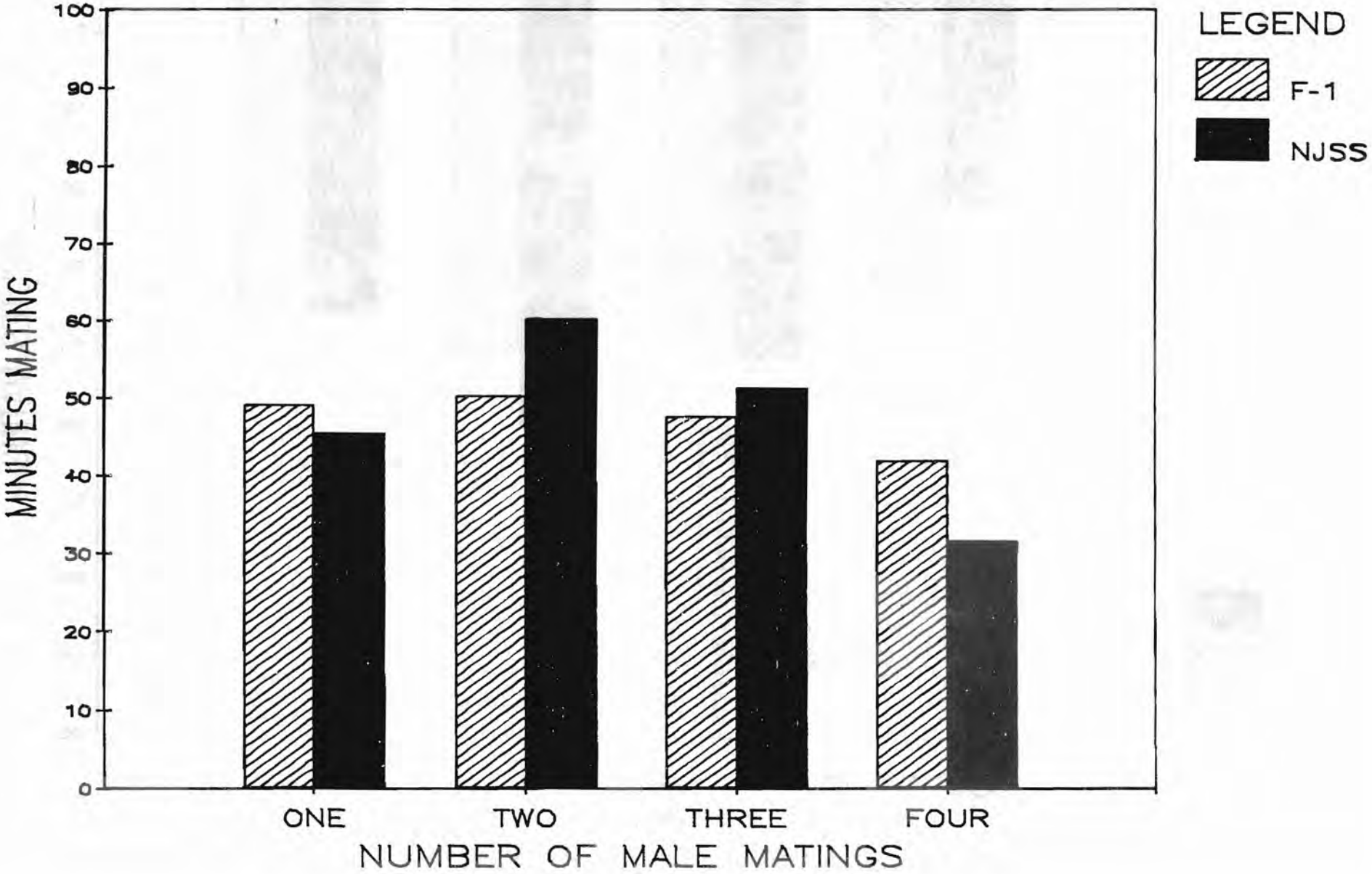
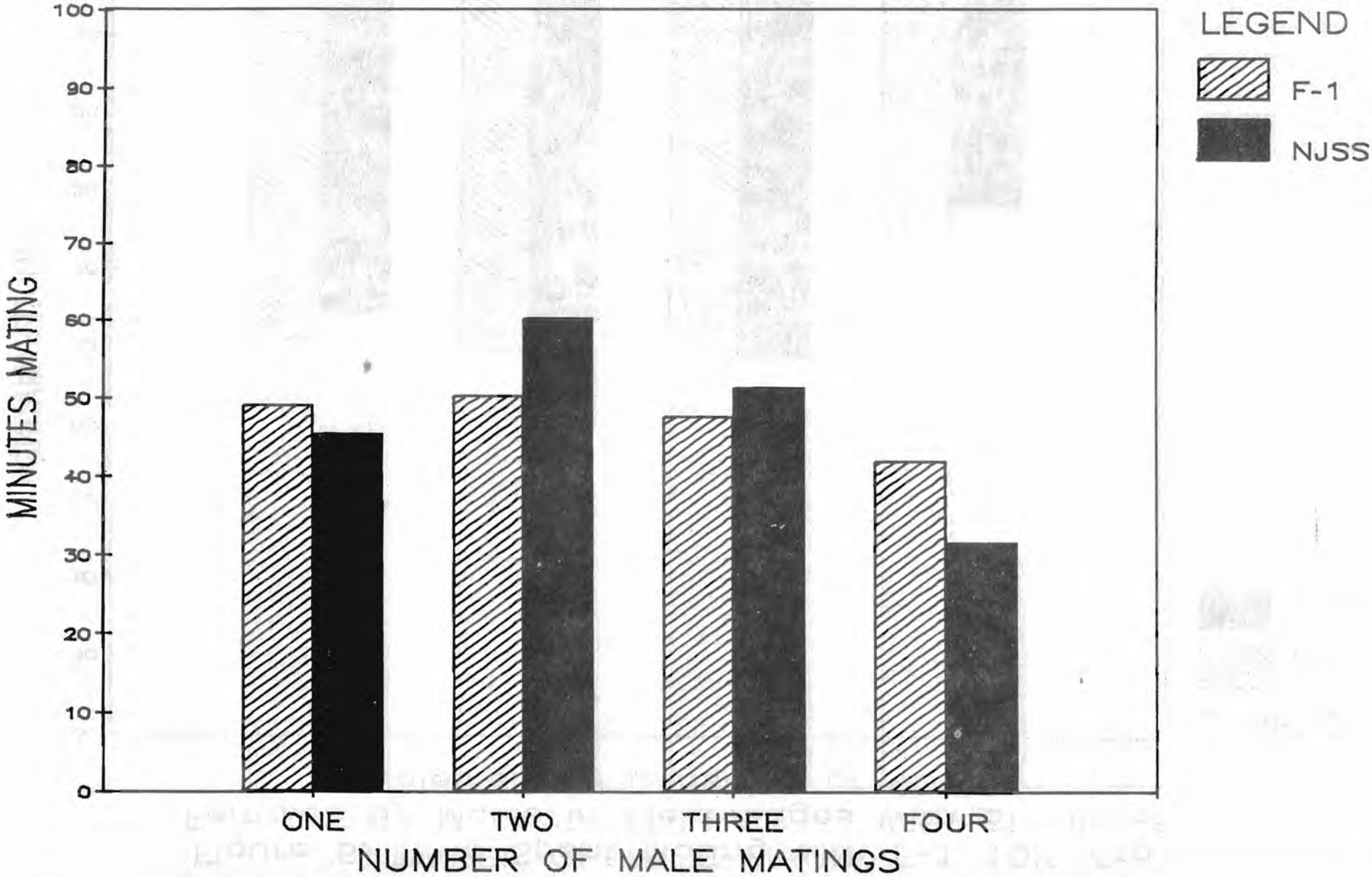


Figure 7. Time Spent Mating With NJSS & F-1 10K Females By Males in field Cages With Simulated Male:Female Densities of 40:40.



Project Number: GM 87.2.6
Project Title: Large Capacity Design Trap Test
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leader: V. C. Mastro, E. Paszek

To determine how 4 different trap characteristics affected male capture, traps of various design were arrayed randomly in a grid (16 x 16-100 meter spacing) in Freetown State Forest in 1981.

The four characteristics studied were: trap size (pint, quart, 1/2 gallon and gallon); number of entry ports (4, 8, 12 and 16); size of hood (no hood, 1/3 hood, 2/3 hood and full hood); and entry port size (width 3/16", 4/16", 5/16" and 6/16"). Trap designs employing all combinations of the four characteristics were constructed (256 designs) and deployed. Every 4 days, traps were emptied and the data recorded for a total of six readings. All traps were baited with laminated (Hercon) pheromone dispenser and contained the same killing agent (DDVP).

Table 1 presents a summary of the four design characteristics and their impact on trap catch. Apparently, in this test only two characteristics significantly affect trap efficiency. The designs employing quart and gallon containers captured significantly more males than the 1/2 gallon trap "standard". Also, entry ports 3/16" and 3/8" wide significantly reduced trap catch when compared to the standard (5/16"). The two other trap characteristics (hood type and number of entry ports) did not significantly affect trap capture. Since traps were not rerandomized, positional effects are probably responsible for the inconsistent results. It is of interest, however, to note that entry port number and hood did not dramatically affect catch.

Table 1. Effect of various design factors on males captured.

Trap Size	Mean no. males		S. D.
1/2 gallon	45.03	a	36.7628
pint	39.96	a	19.2791
quart	78.95	b	38.9543
gallon	86.61	b	46.0415
No. of entry ports			
8	59.53	a	38.7405
4	60.73	a	42.4037
12	62.63	a	40.7195
16	67.66	a	45.3424
Size of hood			
full hood	54.81	a	41.2852
no hood	70.99	a	43.8921
1/3 hood	60.98	a	41.9487
2/3 hood	64.66	a	38.7160
Entry port size			
5/16	69.81	a	43.4924
3/16	49.45	b	38.5070
4/16	75.70	a	47.8040
6/16	55.69	b	30.5017

1/ Within a trap characteristic means followed by the same letter are not significantly different than the standard (i.e., 1/2 gal, 8 entry ports, full hood and 5/16" width entry ports) according to contrast (SAS) at the 5% level of significance.

Project Number: GM 88.2.2
Project Title: 1988 Detection Dispenser Study
Report Type: Final
Report Period: October 1, 1987 - September 30, 1988
Project Leader: V. C. Mastro and E. C. Paszek

Pheromone dispensers are used in traps to survey large areas for incipient gypsy moth infestations. Hercon Laboratories Corporation, a subsidiary of Health-Chem Corporation, has been the sole supplier of (+) disparlure dispensers. This year, Consep Membranes, Inc. and Atomergic, Inc. provided dispensers for evaluation. The following dispensers were aged in the greenhouse for 4, 8 and 16 week intervals: Hercon orange 8 mil polylaminate, the standard 1987 and 1988 Hercon laminates, Consep-Membrane Biolure and BBL-PVC coated 11 mm string. The Atomergic dispensers were aged for 5 weeks and the slab-oak PVC dispensers were not aged. The dispensers were bioassayed 7/22/88 - 7/25/88 in milk carton traps in State Game Lands Forest north of DuBois, Pennsylvania. They were replicated 5 times and a total of 4 readings were made.

Table 1. 1988 Detection Dispenser Study, mean numbers 1/ 2/ of males captured per day per trap (standard error in parenthesis).

Dispenser type lure content	Dispenser age in weeks 0	4	8	16
1987 Hercon (D0117-500ug)	32.10 a (4.81)	28.85 abc (4.67)	23.89 abcd (3.07)	13.15 efgh (2.11)
1988 Hercon (D0048-500ug)	26.30 abc (3.00)	23.30 abcde (3.87)	23.65 abcde (3.75)	15.15 defgh (3.61)
1988 OR/LAM (2.09)	29.00 ab (3.09)	23.70 abcde (3.30)	29.85 ab (4.19)	22.65 abcde (3.11)
1988 BBL/PVC (2 mg)	7.85 gh (1.61)	18.55 bcdef (3.03)	19.05 bcdef (3.77)	18.00 cdefg (3.62)
1988 Consep (0.5 mg)	8.40 fgh (1.21)	11.30 fgh (1.84)	9.85 fgh (1.48)	10.60 fgh (1.75)
1988 Slab-Oak PVC (2 mg)	7.05 h (1.67)			
Meyer-Schwarz Cotton - 100 ug	21.80 abcde (3.07)			
1988 Atomergic	5.80 h (1.02)	11.00 fgh (5.80)		
No Bait	0.00 (0.00)			

1/ For analysis data that were transformed to $x' = x + 0.5$, actual means are presented. Data from blank traps were not used in the analysis.

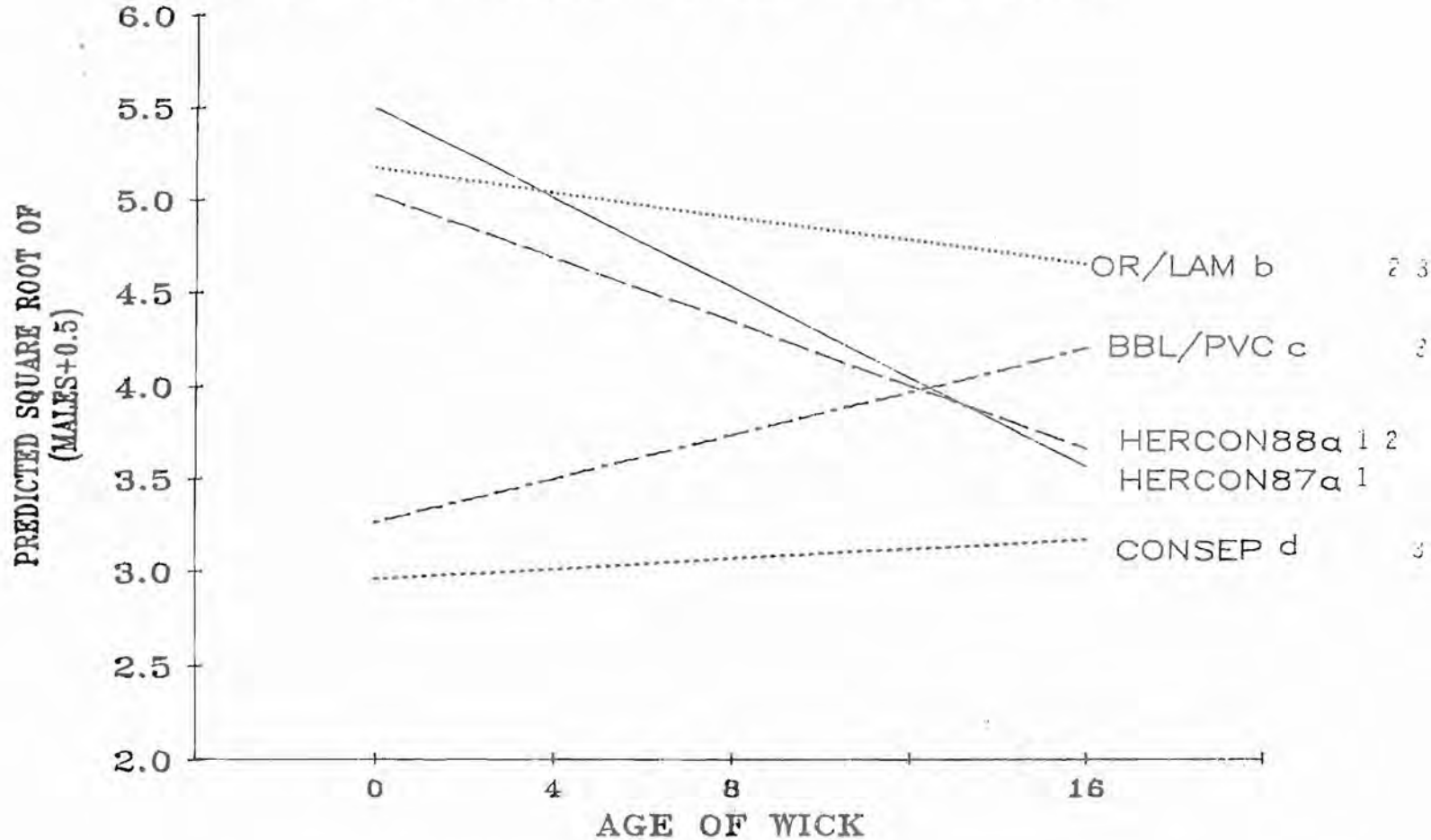
2/ Means followed by the same letter are not significantly different at the 5% level according to Tukey's Studentized Range Test.

The standard survey dispensers, 1987 Hercon and 1988 Hercon, captured the most moths followed by Hercon's orange 8 mill polylaminate. The catch for the three laminates began to diminish at the 16 week aging interval. The BBL/PVC coated 11 mm string captured fewer moths at 0-4 week aging interval and increased its catch at the 8 and 16 week intervals.

Consep Biolure captured a small number of moths at the four aging intervals followed by slab oak PVC and Atomergic.

In this study Hercon's orange 8 mill polylaminate is the only lure dispenser that compares favorably with the two standard survey dispensers (1987 and 1988 Hercon).

EFFECT OF WICK AGE ON GYPSY MOTH MALE CAPTURE



Treatments followed by the same letter are not significantly different according to regression at the 5% level F(-95, 2, 156). The slope of treatment regression lines followed by the same number are not significantly different at the 5% level.

Project Number: GM 88.2.1
Project Title: A New Large-Capacity Gypsy Moth Trap Design
Report Period: October 1, 1987 - September 30, 1988
Report Type: Preliminary
Project Leader: E. C. Paszek

The milk carton trap is cumbersome for assembling and storing. A trap which can be nested and assembled readily in the field would be useful in survey programs where large capacity is needed.

The following trap designs using Cono Cups [1/] were tested in Truro, Massachusetts on August 9 through August 19, 1988.

1. Cono cup with 8 entrance ports (1x5/16") and the standard 9.5"[2] pagoda overhang, which is used on the MC trap. This pagoda overhang extends 3" from the body of the cono cup trap.

2. Cono cup trap as described in (1) above but with the bottom cut out and an 11.5x13" clear plastic Baggie[R] attached.

3. Cono cup with 8 entrance ports (1x5/16") with a 6.75" square pagoda overhang. This pagoda overhang extends 1.5" from the trap body.

4. Cono cup (no pagoda overhang) with one circular 1" dia. entrance port cut in the apex of the pour spout. Spout in closed position forms a 1.5" gable above recessed entrance port.

5. Cono cup with 8 entrance ports (1x5/16") without pagoda overhang.

6. Standard 1/2 gallon milk carton trap.

1/ Cono cup, 33.8 oz., one liter Continental Bondware, 1701 Golf Rd., Tower 2, Rolling Meadows, IL 60008 (Division of Continental Can Company, Inc.)

Table 1. Cono cup trap designs tested in 1988.

Trap Type	Number of moths captured in 5 traps 8/10 24 hr.	8/15 96 hr.	8/19 96 hr.	Total
Cono cup 9.5"^{2} pagoda 8 entrance ports	49	28	1	78
Cono cup 9.5"^{2} pagoda + bag 8 entrance ports	46	52	6	104
Cono cup 6.75"^{2} pagoda 8 entrance ports	51	39	6	96
Cono cups - no pagoda 1-1" circular port in gable of trap	4	10	2	16
Cono cups - no pagoda[1/] 8 entrance ports	-	-	2	
Standard milk carton traps	59	34	6	99

1/ Traps placed in field 8/16/88

Results

In this study, the Cono cups with the pagoda overhangs compared favorably to the standard milk carton trap (Table 1). The 8.5" tall cono cup is 3.5" in diameter at its widest point, tapering down to 3" diameter at the bottom. This feature allows it to be nested for storage. Three hundred cono cup containers fit into a 23x16.5" box. The container is readily assembled with a 1/2 overlap closure fastened with a 3/4" plastic clip, which easily slides off and on. This overlap closure prevents rain from entering the trap.

The pagoda overhang, which was added to traps 1 and 2 is the standard size used on milk carton traps. It's size was reduced in Design No. 3 by 1.5". These pagoda overhangs aid in guiding moths to the entry ports. In traps without overhangs, moths tend to hover around entry ports with the majority flying away.

Trap design No. 3, with the pagoda overhang reduced by 1.5" was as effective in capturing moths as the standard milk carton trap and trap designs No. 1 and No. 2, which had 3" overhangs.

Trap design Nos. 4 and 5 had no pagoda overhangs. However, in trap No. 4, the recessed one-inch diameter circular entry port has a 1.5" gable extending over it. This gable aids moths to enter the trap. Design No. 5, with 8 entry ports was placed in the trap lines when the moth flight was diminishing rapidly. Two moths were captured in a 96 hour period as compared to 6 in the

standard milk carton trap. These traps without pagoda overhangs could be used for monitoring populations in moderate populations. Trap design No. 3, with 1.5" overhang should be bioassayed with a clear plastic bag. The plastic bag may increase the efficiency of the trap by increasing its capacity from 1 liter to approximately 1.9 liters, the capacity of the 2 quart milk carton trap. The bag has to be punctured as it accumulated rain water. This design study should be done on a larger scale in 1989. The traps will be weathered outdoors this fall to check on their exposure to moisture and sunlight.

Weathering of the cono cup traps outdoors indicated that the plasticized outside of the cup resists water, however, the top of the cup and entrance ports absorbed water and became water-logged and sagged. A plastic pagoda type hood placed over the cup would prevent the top of the trap from absorbing water.

A model of this trap has been designed with this feature. It should be bioassayed this coming field season.

Project Number: GM 88.2.3
project Title: Arizona-Washington Long Life Dispenser Aging Study
Report Period: October 1, 1987 - September 30, 1988
Report Type: Final
Project Leaders: E. C. Paszek, V. C. Mastro, B. A. Leonhardt

In operational gypsy moth survey programs, traps often are placed in the field a month or more prior to moth flight. Past studies have demonstrated that Hercon dispensers aged for as long as 2.5 months in a greenhouse prior to testing retained activity. This study was designed to determine the longevity of dispensers aged under different field conditions. Two extreme climates, hot and dry (Phoenix, Arizona) and cool and wet (Enumclaw, Washington) were the areas selected for this aging study. All dispensers were held at these sites in USDA milk carton traps. The following 4 Hercon dispensers, H67-1 (2 mil PVC, 7.7mg/in^2), H67-2 (8 mil PVC, 8.2mg/in^2), H67-3 (8 mil poly, 8.65mg/in^2), and the 1987 standard 500ug laminate (H87) were aged at bi-monthly intervals for 12, 10, 8, 6, 4, 2 months from 8/22/87 through 7/14/88 (i.e., 52 dispenser type x age combinations including unaged dispenser of each type). The following 3 dispensers, H74-1 (Hercon laminate, 7mg/in^2), 2 Agron Poly trap dispensers, Recon C (7mm cut length, 2mg) and Recon D (13.4mm cut length, 2mg) were aged for only two periods of 12, 6 months. Also, unaged dispensers were tested resulting in an additional 15 dispenser type x age combinations. Eric LaGasa of the Washington State Department of Agriculture, Kent, Washington, and Ernie Miller, USDA, APHIS, SCI & TECH of Pink Bollworm and Range Test Station, Phoenix, Arizona, were cooperators who aged these dispensers.

Traps (USDA milk carton) baited with the 67 dispenser x age combinations, previously described, were evaluated in field bioassay. A cotton wick impregnated with 100ug of (+) disparlure (Meyer Schwarz) provided a standard for comparison. In addition, unbaited traps served as a control. Testing was done north of Du Bois, Pennsylvania, in a moderate gypsy moth infestation. The test design was a randomized complete block with four complete blocks which were read and randomized three times.

Similarly aged lures of all dispenser types for the 12, 6 and unaged periods will be chemically analyzed for residual lure content and emission rates by B. A. Leonhardt (Chemical Ecology Laboratory, Beltsville, MD, ARS).

RESULTS AND DISCUSSION

Table 1 lists the 69 treatments showing the mean number of males captured and standard deviations. The H87 Hercon standard wick dispenser (H87), regardless of wick age, captured the largest number of moths (Fig. 1). Statistical comparison of the regressions for a wick type between the two aging locations (Fig. 1) did not reveal differences at the 5% level of significance. Therefore, the data were pooled for a wick type and a comparison of the pooled data for each wick type was carried out. The standard dispenser used in all USDA detection programs and manufactured by Hercon (H87) performed significantly better (5% level) than any other dispenser design (Fig. 2). Performance the other four candidate dispensers produced by Hercon (H67-1, H67-2, H67-3, H74-1) was not significantly different from each other but all did capture significantly fewer moths than the standard detection dispenser (H87). Male captures in traps baited with the two formulations produced by

Recon (Rec-C and Rec-D) were significantly less (5% level) than any other dispenser type tested. The increased capture noted for Rec-C with increased aging may indicate that initially this dispenser is releasing too much lure and as the reservoir is depleted, the emission rate slows to approximate the optimum capture range. Chemical analysis of residual content and measured emission rates in the laboratory will reveal the shortcomings of this and other dispensers.

The average monthly temperature for Arizona for the 12 month period of August 1988 - July 1989 was 75.2°F with a 12 month total precipitation of 7.43", compared with Washington which had an average temperature of 51.7°F and a total amount of precipitation of 84.42" (Fig. 1a and 1b). Although these appear to be large differences, they did not appreciably affect the field performance of wicks aged in the two locations.

The H87 Hercon laminate remained active under two extreme different sets of field conditions for up to twelve months. There is a deterioration in the numbers of males captured as the wick is aged for greater lengths of time (i.e., negative slope (Fig. 2)). In other words, a dispenser placed in the field 11 months prior to flight will not perform as well as a fresh dispenser. However, traps may be placed several months prior to flight without great losses in male captures.

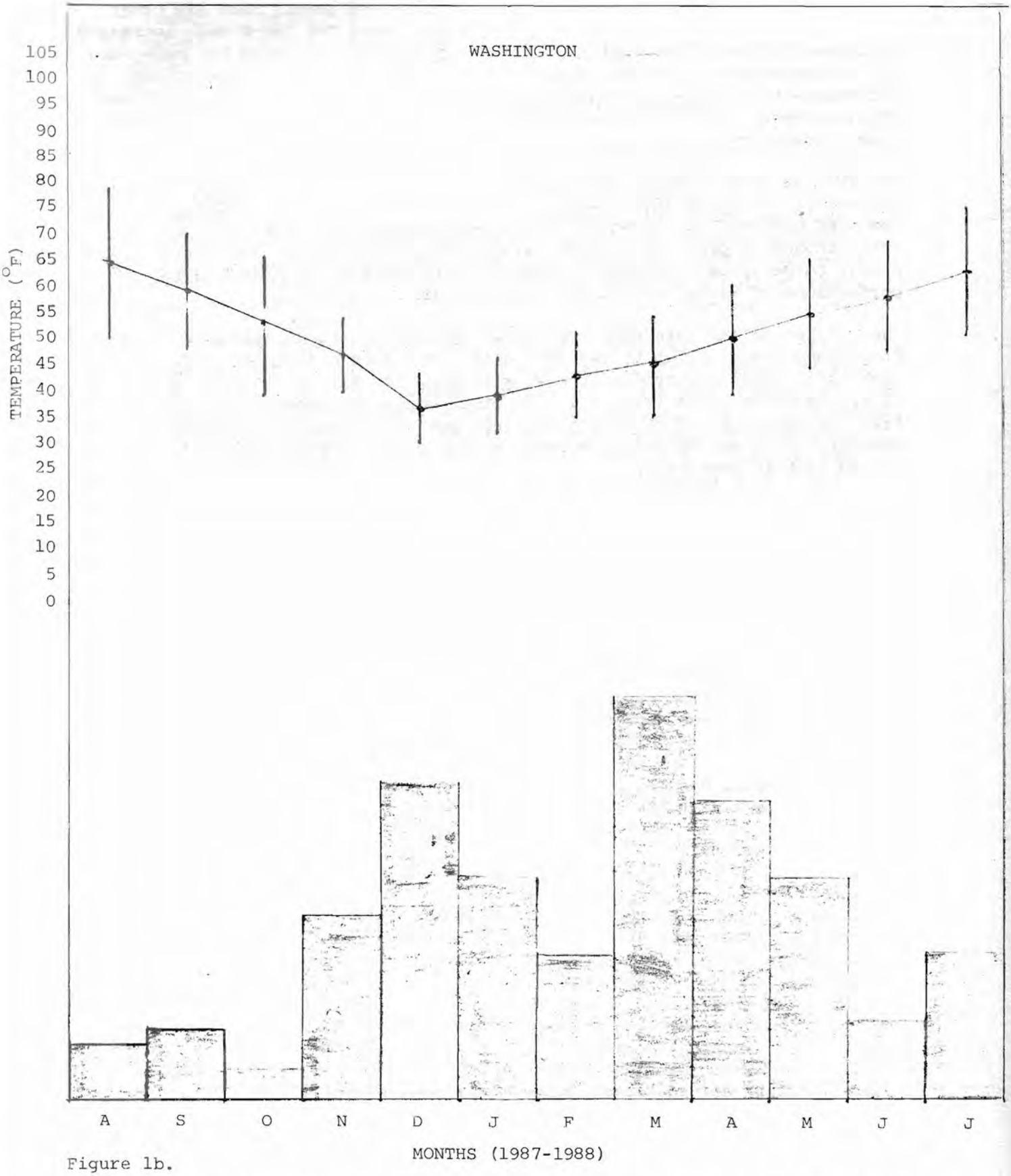


Figure 1b.

Average monthly maximum and minimum air temperature and total monthly rainfall at Enumclaw, Washington, August 1987 through July 1988. Total rainfall for 12 month period was 84.42 inches.

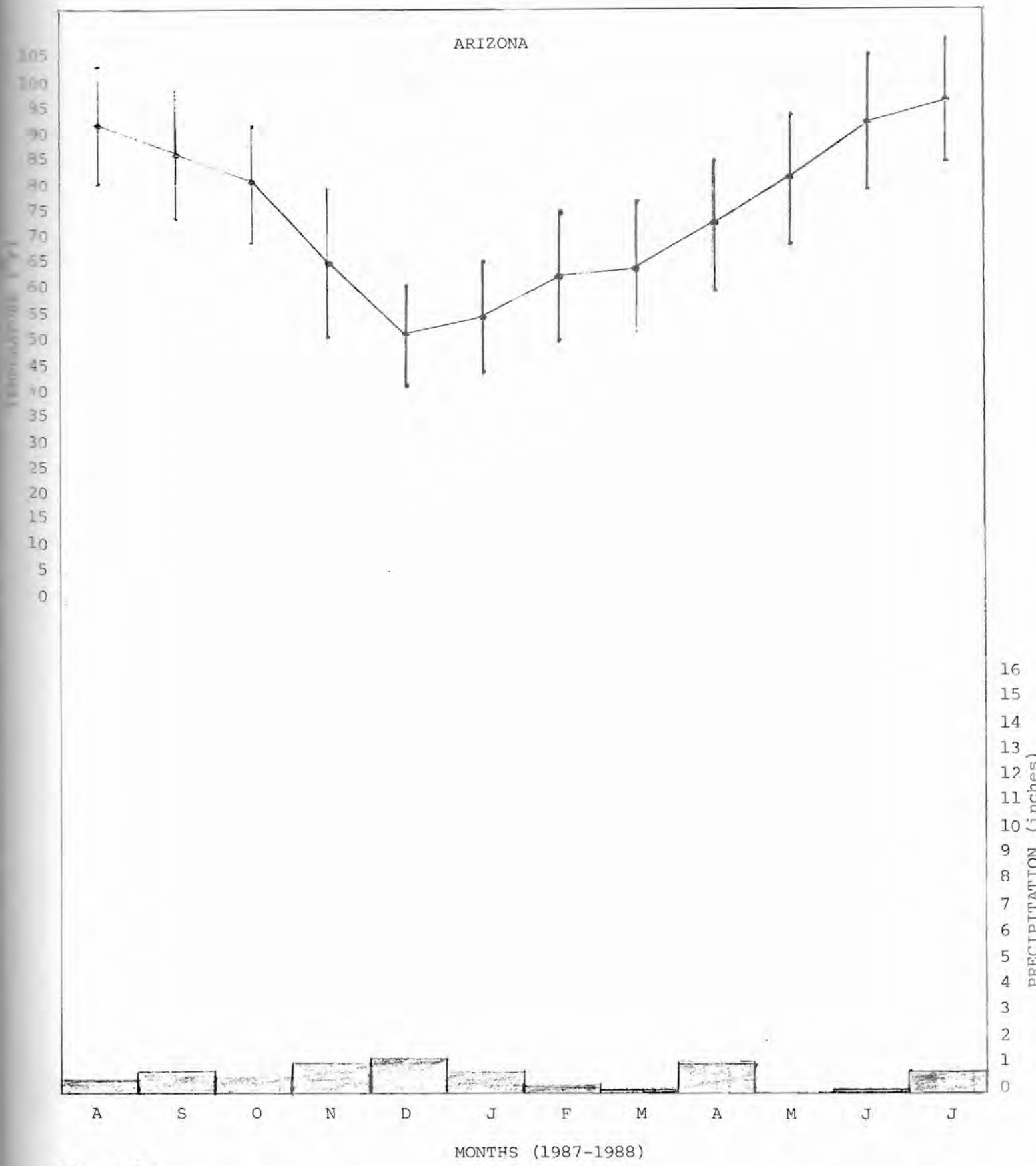


Figure 1a.

Average monthly maximum and minimum air temperature and total monthly rainfall at Phoenix, Arizona, August 1987 through July 1988. Total rainfall for 12 month period was 7.43 inches.

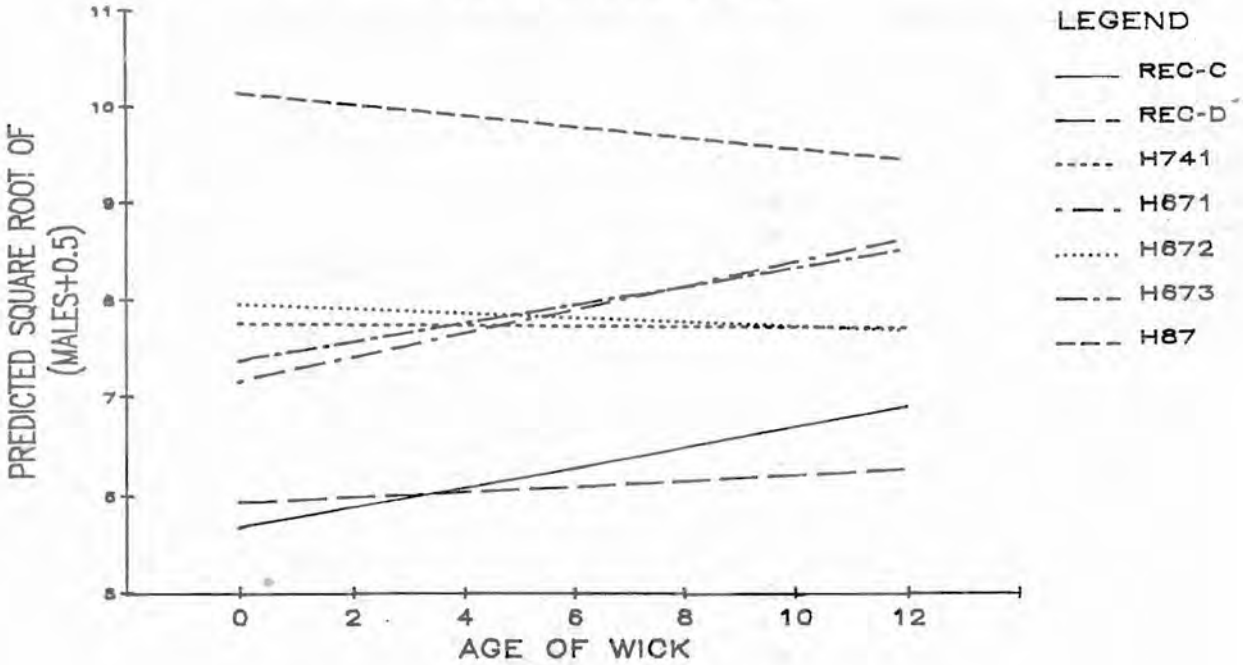
Table 1. Arizona - Washington cooperative long life dispenser study.

Dispenser type	Pre-aging in months	Aging location	Mean number males captured	Standard deviation	Number of observations
Recon C	0		33.33	19.91	12
Recon C	6	AZ	52.08	37.64	12
Recon C	12	AZ	52.92	37.38	12
Recon C	6	WA	44.27	24.86	11
Recon C	12	WA	52.08	42.32	12
Recon D	0		36.42	34.92	12
Recon D	6	AZ	42.67	38.94	12
Recon D	12	AZ	37.83	27.93	12
Recon D	6	WA	49.64	23.80	11
Recon D	12	WA	40.42	32.11	12
Hercon 74-1	0		60.50	32.90	12
Hercon 74-1	6	AZ	55.17	39.51	12
Hercon 74-1	12	AZ	63.17	37.24	12
Hercon 74-1	6	WA	70.58	40.20	12
Hercon 74-1	12	WA	60.50	41.01	12
Hercon 67-1	0		58.64	51.10	11
Hercon 67-1	2	AZ	65.25	37.75	12
Hercon 67-1	4	AZ	56.00	20.48	12
Hercon 67-1	6	AZ	55.83	29.30	12
Hercon 67-1	8	AZ	62.83	41.38	12
Hercon 67-1	10	AZ	62.75	38.26	12
Hercon 67-1	12	AZ	71.58	62.42	12
Hercon 67-1	2	WA	49.33	49.18	12
Hercon 67-1	4	WA	65.83	31.95	12
Hercon 67-1	6	WA	78.83	46.59	12
Hercon 67-1	8	WA	92.83	36.40	12
Hercon 67-1	10	WA	64.27	30.75	11
Hercon 67-1	12	WA	70.83	57.41	12
Hercon 67-2	0		88.75	52.30	12
Hercon 67-2	2	AZ	40.17	14.69	12
Hercon 67-2	4	AZ	76.25	34.97	12
Hercon 67-2	6	AZ	57.75	32.74	12
Hercon 67-2	8	AZ	54.25	37.88	12
Hercon 67-2	10	AZ	57.00	32.88	12
Hercon 67-2	12	AZ	58.67	38.01	12
Hercon 67-2	2	WA	52.17	24.31	12
Hercon 67-2	4	WA	64.18	48.67	11
Hercon 67-2	6	WA	58.18	47.98	11
Hercon 67-2	8	WA	68.92	37.49	12
Hercon 67-2	10	WA	58.58	28.05	12
Hercon 67-2	12	WA	71.25	42.13	12
Hercon 67-3	0		58.92	34.64	12
Hercon 67-3	2	AZ	73.92	35.61	12
Hercon 67-3	4	AZ	55.16	39.44	12

Table 1. Arizona - Washington cooperative long life dispenser study. (Continued)

Dispenser type	Pre-aging in months	Aging location	Mean number males captured	Standard deviation	Number of observations
Hercon 67-3	6	AZ	72.91	56.29	11
Hercon 67-3	8	AZ	56.75	33.36	12
Hercon 67-3	10	AZ	68.58	65.34	12
Hercon 67-3	12	AZ	65.50	32.95	12
Hercon 67-3	2	WA	57.09	64.28	11
Hercon 67-3	4	WA	80.50	57.16	12
Hercon 67-3	6	WA	55.92	34.34	12
Hercon 67-3	8	WA	93.67	39.25	12
Hercon 67-3	10	WA	59.75	25.41	12
Hercon 67-3	12	WA	73.92	28.46	12
Hercon 87	0		94.42	47.44	12
Hercon 87	2	AZ	112.42	58.20	12
Hercon 87	4	AZ	99.42	71.16	12
Hercon 87	6	AZ	91.83	58.98	12
Hercon 87	8	AZ	107.58	44.53	12
Hercon 87	10	AZ	93.08	62.24	12
Hercon 87	12	AZ	85.33	56.22	12
Hercon 87	2	WA	149.64	67.80	11
Hercon 87	4	WA	90.58	43.33	12
Hercon 87	6	WA	95.50	59.63	12
Hercon 87	8	WA	99.58	58.90	12
Hercon 87	10	WA	78.00	44.76	12
Hercon 87	12	WA	118.67	68.45	12
Blank			5.67	8.10	24
100ug Standard			69.83	50.77	12

FIGURE 1. EFFECT OF WICK AGE ON GYPSY MOTH MALE CAPTURE WASHINGTON



EFFECT OF WICK AGE ON GYPSY MOTH MALE CAPTURE ARIZONA

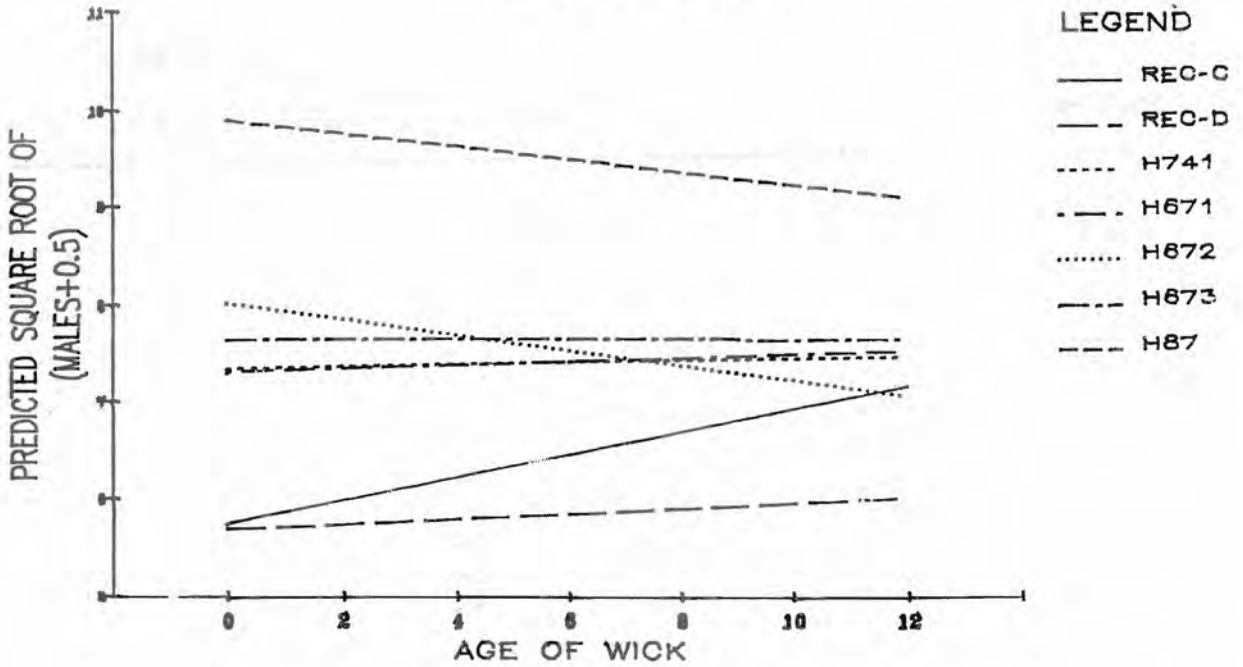
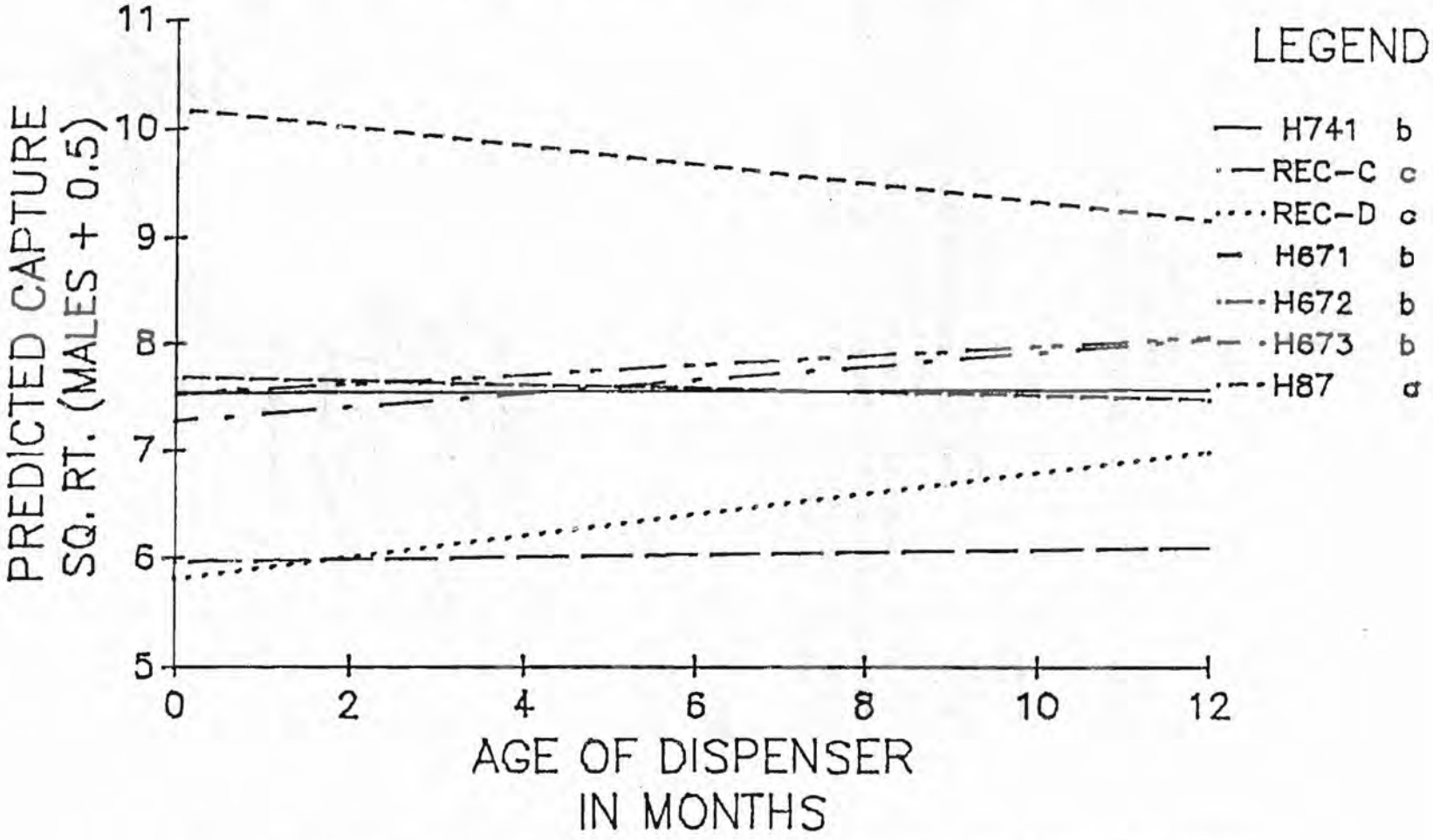


FIGURE 2. EFFECT OF WICK AGE ON GYPSY MOTH MALE CAPTURE



Fitted regression lines for treatments followed by the same lower case letter are not different at 5% level of significance. The best model to describe the data are the males + 0.5 = $B_o + B_1$ (wick age) + B_2 (Date of capture).

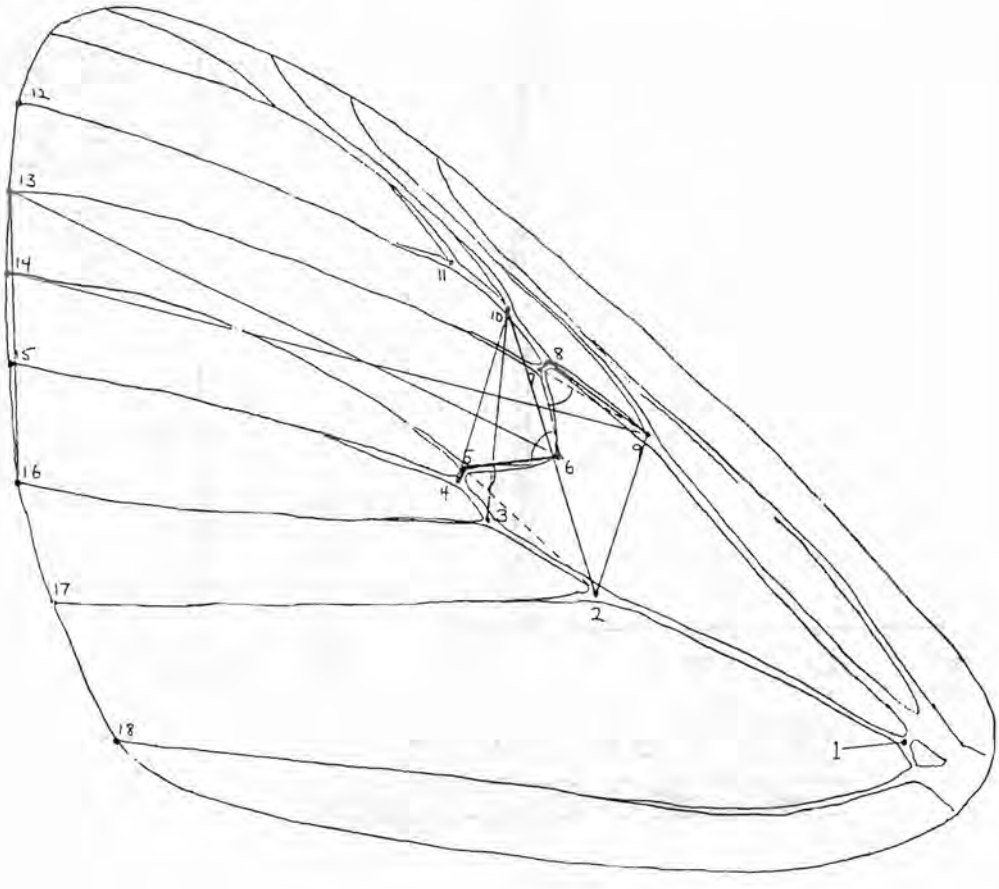
Project Number: GM 88.2.4
Project Title: Morphometric Analysis of Gypsy Moth Populations
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: P. C. Kingsley and G. L. Bernon

OBJECTIVE

Develop a cost-effective technique to document over-flooding ratios in F_1 sterile male release sites by quantifying characteristics of wing venation as an indicator of morphometric differences between populations.

METHODS

Wings from male gypsy moths were mounted in 35mm slide frames, projected, and digitized such that distances between vein intersections could be calculated (see figure below). These values were then transformed to a relative scale, eliminating the influence of wing size. Using discriminant analysis, classification data sets were compiled for pairs of gypsy moth populations using a subset of the total number of distance variables. Unknown wings were then statistically tested for membership into either population.



PRELIMINARY RESULTS

When a classification data set was constructed from laboratory reared (LAB DIET) and field collected (MAINE FOLIAGE) moths, discrimination of test insects from the same populations was excellent (see below). Discrimination was poor however, when populations of foliage fed laboratory moths (LAB FOLIAGE) and diet fed field collected moths (MAINE DIET) were compared, indicating an influence of diet, or rearing conditions, on these morphometric characters. When classification data sets were developed using foliage reared laboratory (LAB FOLIAGE) and wild (MAINE FOLIAGE) populations, discrimination of test moths was good.

	Classified Data Sets		Test Data Sets			
	Lab Diet	Maine Foliage	Lab Diet	Maine Foliage	Lab Foliage	Maine Diet
Number of wings	68	78	17	18	28	38
# Misclassified	0	1	0	1	16	32
% Error	0	1.3	0	5.6	57.1	84.2
Distance	25.84					

	Lab Foliage	Maine Foliage	Maine Foliage	Lab Foliage
Number of wings	23	78	18	5
# Misclassified	0	3	1	0
% Error	0	3.9	5.6	0
Distance	11.98			

Lab Diet - NJSS Generation 32, diet reared
Maine Foliage - Belgrade, Maine, 1987, field trapped
Lab Foliage - NJSS Generation 32, foliage fed
Maine Diet - Belgrade, Maine, 1988, field collected as egg masses, diet reared

DISCUSSION

The effect of diet, or laboratory rearing conditions, on wing morphology was unexpected and has complicated what we had hoped would be a relatively simple procedure. If the type of foliage does not influence these characters, or if the results we see are simply due to rearing conditions (temperature, container, lighting, etc.) then we can still be optimistic about this techniques ultimate utility.

Project Number: GM 88.2.5
Project Title: Sterile Male Demonstration Project, Fort Collins, CO.
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: V. C. Mastro, A. Pellegrini-Toole, C. P. Schwalbe
Cooperators: O. Barham, D. Leatherman

The objective of this project was to evaluate the utility of the F_1 egg mass release technique for treatment of an isolated, sparse population. Gypsy moth was first found in Fort Collins, Colorado in 1985 when four male moths were captured in traps. Delimitation surveys in 1986 and 1987 succeeded in identifying two isolated infestations. One of these isolated infestations (east infestation) was chosen for treatment with a release of F_1 10k egg masses (i.e., progeny of males treated with 10 krads x normal laboratory females). In 1987, 77 males were caught with 2 traps/acre (7.5/ha). At this trapping density, approximately 75% of the male population should have been trapped, indicating a total male population of 102 males. Assuming that the sex ratio was 1:1, that all females mated and oviposited in 1987 and that all egg masses survived the winter, 102 wild egg masses should have been present in the spring of 1988. Note that it is possible that at this trapping density, mating success may have been reduced. However, we conservatively did not attempt to factor this into our release. Six egg masses were collected in the fall of 1987, and they contained a mean of 450 eggs with a mean of 99.5% embryonation. If all eggs overwintered, then an average egg mass would produce 447 first instar larvae. From egg masses held outside in Fort Collins,we found average hatch rates much lower (x = 19.4%) which resulted in an average of only 87 first instar larvae/egg mass. If it is assumed that all wild egg masses produced an average of 87 first instar larvae, then (102 E.M x 87) 8,874 newly hatched would represent the wild spring population of gypsy moth. Between April 12 and 14 we released 44,119 F_1 egg masses. Making the following assumptions, the listed outcomes could be projected.

	850	850	850
Eggs/F_1 egg mass hatch rate	x .40 optimal	x .30	x .20
neonates/egg mass	340	255	170
# egg masses released	x44,119	x 44,119	x 44,119
total no. F_1 neonates	15,000,460	11,250,345	7,500,230
F_1 larval survival differential	x .50	x .50	x .50
	7,500,230	5,625,173	3,750,115
Expected F_1:wild ratio	1232:1	922:1	661:1

Overflooding ratios in the adult stage would be expected to be lower than the projections because of asynchrony of F_1 hatch, placement of some F_1s on non-preferred host and also because a portion of the 44,119 F_1 egg masses were used to treat a buffer around the known infested area. However, it was expected, given all of the variables involved, that the release would result in an overflooding ratio of at least 200:1 that would markedly reduce the

native population.

Table 1. Results of monitoring sterile F_1:wild overflooding ratios in Fort Collins, CO, 1988.

Technique		n	F_1:wild	% fertile
Male chromosome analysis		226	224:2 (112:1)	0.9
Male mating evaluation		923	56.7:1	1.7
F_1 monitor female egg mass evaluation		177	176:1	0.6
Female mating evaluation		NOT COMPLETE		
Post season egg mass evaluation		NOT COMPLETE		
Males trapped	core area	117		
	associated	26		
	Total	143		

RESULTS AND DISCUSSION

Hatch from a single wild egg mass began on May 1 and was completed on May 12. F_1 egg masses began hatching on April 15 and were completed on May 31.

Results available to date are presented in Table 1. Overflooding ratios thus far projected indicate that a high degree of overflooding was achieved. Even at the lowest estimate of overflooding (approximately 56:1) the wild population would be suppressed. Only 143 males were captured in or close to the release area in 1988. At the trap density deployed, nearly 25% of the males should have been captured. If this capture rate is accurate, then less than 600 males were present in 1988. Given the large number of F_1s released, larger captures of males would be expected. Observations in the release area offer at least a partial explanation for the low survival rate. Beginning June 6, numbers of larvae resting under burlap bands began to decrease dramatically. Bands were also noted to have holes in them and there was one observation of starlings pecking on the bands. Within days of the first observation, the decline was so rapid that it was difficult to find any larvae. The impact of this "disappearance" on the control afforded by the F_1 release is unknown. If the "predation" was indiscriminate of larval type (F_1 or wild) the only impact should be a general lowering of the overall population (and not the overflooding ratio). If F_1 or wild insects are predisposed to predation, the overflooding ratio could have been shifted.

Following are listed sites where wild insects were collected.

Male Mating Evaluation

Insect number	Location
657	605 Whedbee, Weeping Willow
809	515 Mulberry, Weeping Willow
1007	521 Smith, Cottonwood
1605	518 Whedbee, Mountain Ash
1655	510 Whedbee, Plum
1670	515 Mulberry, Weeping Willow
1679	521 Smith, Cottonwood
1681	521 Smith, Cottonwood
1689	521 Smith, Cottonwood
1690	521 Smith, Cottonwood
1863	515 Mulberry, Weeping Willow
1939	521 Smith, Cottonwood
1941	521 Smith, Cottonwood
1944	521 Smith, Cottonwood
1947	521 Smith, Cottonwood
1960	518 Whedbee, Mountain Ash

Male Chromosome Evaluation

Insect number	Location
202	515 Mulberry, Weeping Willow
1193	515 Mulberry, Weeping Willow

Project Number: GM 88.2.6
Project Title: Comparative Performance of Young F_1-Sterile Gypsy Moth Larvae on Foliated Willow Cuttings.
Report Period: Jan. 1, 1988 - March 30, 1988
Report Type: Interim
Project Leader: R.W. Hansen

INTRODUCTION

Application of the inherited sterility, or F_1-sterile, technique requires that released F_1-sterile insects be "competitive" with their feral counterparts during egg, larval, pupal, and adult stages. F_1-sterile insects can be considered competitive if their survival rates, development, various behavioral patterns, and other physiological characteristics are similar to those of wild insects.

We have examined the relative growth and development of F_1-sterile gypsy moth larvae on foliage and artificial diets, and larval behavior patterns in field and laboratory situations. The dispersive abilities of F_1-sterile and feral first instars appear similar (Mastro et al. 1984, 1985). However, fewer first-stadium F_1-sterile larvae become established on oak foliage than do nonirradiated lab-strain or feral neonates (Lance et al. 1983, 1984; Montgomery, unpubl. data). In F_1-sterile first instars, this phenomenon may represent a relative inability to locate host leaves, initiate feeding, or a combination of both factors. The following experiments continue surveillance of young F_1-sterile larval competitiveness, examining short-term survival, development, and growth on plant material.

METHODS

Rearing material was prepared by clipping ca. 30-cm branch tips from several dormant weeping willow (Salix babylonica L.) trees (Jan. 1988). Cuttings were disinfected in a bleach solution, and the apical and about half of the lateral buds excised. The basal 10-12 cm of each cutting was immersed in a very dilute solution of liquid fertilizer in distilled water. Cuttings were maintained at about 21°C, 60% RH, with a 16:8 (hrs L:D) photoperiodic cycle. Generally, new leaves and roots began to appear within 14 days. Cuttings were used for gypsy moth rearing when leaves approximated the size of those encountered by first instars under typical field conditions. Cuttings were maintained in water culture throughout each experiment.

Three gypsy moth larval treatments were included: a) F_1-sterile (standard laboratory strain; irradiated male parent); b) standard laboratory strain ("New Jersey" or NJ, G_{32}); and c) wild insects (parental eggs collected in Rhode Island; one generation in laboratory culture). Egg masses were removed from cold storage (5°C) after at least 150 days chill, and held at ca. 22°C. First instars were collected within 48 hrs of hatch. For each treatment, neonates were weighed to the nearest 0.1 mg, and groups of five were placed on foliated willow cuttings enclosed in translucent glass-topped paper tubes (ca. 8.5 cm diameter). Larvae were reared in an environmental chamber at ca. 23°C and 50% RH. Fluorescent lighting provided a 14:10 (L:D) photoperiod.

Three experiments were performed. Experiments 1 and 2 included only F_1-sterile and New Jersey strains; wild insects were added during experiment 3. Larvae were again weighed after five days on foliage, and survival and development were also recorded. During experiments 2 and 3, larvae were returned to the rearing tubes, with fresh willow twigs if needed, and weighed again 10 days after introduction.

Survival was compared between and among treatments using Chi-square contingency table analysis. Larval development (mean stadium) and larval weight were compared between treatments using Student's-t test (experiments 1 and 2), or among treatments using single-factor ANOVA (experiment 3). Following ANOVA, the Student-Newman-Keul (SNK) procedure was used to identify treatment differences. Significance was assessed at a=0.05.

RESULTS

During experiment 1, survival after five days was similar for F_1-sterile and NJ larvae (Table 1). However, F_1 survival was significantly reduced after five days in experiment 2 (X^2=5.16, P<0.03), and after five and 10 days in experiment 3 (X^2=23.73, P<0.001 and X^2=31.70, P<0.001, respectively). Ten-day survival data shows that most mortality occurred during the first five days, regardless of treatment.

All larvae remained in the first stadium after five days, for all three experiments. During experiment 2, F_1 and NJ mean stadia were similar (1.4 and 1.5, respectively) after 10 days. After 10 days of experiment 3, mean stadia differed among treatments (F=6.97, P<0.01). F_1-sterile mean stadium (1.19) was significantly less than NJ (1.43) or wild (1.67) mean stadia.

Table 2 summarizes larval weight data. At the start of each experiment, F_1-sterile larvae weighed significantly less than NJ or wild first instars (t=2.24, P<0.03; t=2.67, P<0.01; and F=3.50, P<0.05, respectively). Additionally, F_1 larvae weighed less than NJ or feral larvae after five days of experiment 3 (F=5.75, P<0.01). Median weights are also included in Table 2, and may be of particular interest with second instars (day 10, experiments 2 and 3). Second-instar weights were often skewed, with data "clumped" at the lower and upper ends of the weight ranges.

DISCUSSION

F_1-sterile first instars generally exhibited lower survival than nonirradiated lab-strain or feral insects. Lance et al. (1983) also documented reduced first-instar survival among F1 larvae reared on black oak foliage. Most first-instar mortality also occurred within the first five days of the experiments.

Reduced F_1-sterile neonate survival could be a consequence of reduced larval weight; F_1 first instars weighed, on average, about 13% less than their NJ and wild counterparts. Smaller first instars may possess smaller food reserves, perhaps reducing their ability to climb and locate foliage shortly after hatching. Capinera and Barbosa (1976) report that larger first instars have greater dispersive capabilities, due, in part, to increased crawling speed and

silk production (ballooning). Field tests of F_1-sterile neonate dispersal were inconclusive (Mastro et al. 1985), but F_1 first instars appeared less likely to disperse to targets at 3- and 6-m distances. However, reduced dispersive abilities were unlikely to affect larval survival in the small test arenas employed in these experiments.

Alternatively, F_1-sterile neonates may have been less able to consume young willow foliage, perhaps due to smaller size (smaller mandibles, reduced musculature). Other physiological factors may impair utilization of stored food reserves by F_1 neonates, predisposing them to starvation shortly after hatch.

In any event, reduced first-instar survival appears a characteristic of F_1-sterile gypsy moths. Somewhat slower development (mean stadium) and reduced growth (mean larval weight) may characterize young F_1 larvae that do survive.

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Table 1. Survival of gypsy moth larvae on willow twigs at 5 or 10 days after introduction.

Exp.	Treatment	N (start)	Survival(%)		
			Day 5	Day 10:	
				from start	from day 5
1	F_1	48	89.6	--	--
	NJ	34	85.3	--	--
2	F_1	60	63.3*	60.0	94.7
	NJ	60	83.3	75.0	90.0
3	F_1	48	50.0*	35.4*	70.8*
	NJ	50	74.0	70.0	94.6
	Wild	60	91.7	86.7	94.5

* Significant reduction in larval survival for indicated treatment (Chi-square contingency table analysis; a=0.05)

Table 2. Gypsy moth larval weights at start of experiments, and 5 or 10 days after intruduction on willow twigs.

		Weight (mg) at start				Weight (mg) at day 5				Weight (mg) at day 10			
Exp.	Treatment	N	Mean[a]	Median	Range	N	Mean	Median	Range	N	Mean	Median	Range
1	F_1	48	0.82 (0.07)*	0.8	0.5-1.5	43	3.19 (0.41)	3.3	0.7-5.7		----		
	NJ	34	0.96 (0.11)	1.0	0.5-1.9	29	3.77 (0.57)	4.1	1.2-6.7		----		
											First instars		
2	F_1	60	0.84 (0.05)*	0.8	0.5-1.4	38	2.04 (0.34)	1.75	0.7-5.3	20	3.34 (0.47)	3.75	1.3-4.9
	NJ	60	0.95 (0.06)	0.9	0.6-1.6	48	1.78 (0.17)	1.7	0.7-3.2	22	3.68 (0.52)	3.9	1.1-5.6
											Second instars		
										16	8.66 (2.07)	7.3	4.8-17.9
										23	7.39 (0.98)	6.6	4.2-15.1
3	F_1	48	0.84 (0.05)*	0.8	0.6-1.2	23	1.57 (0.20)*	1.5	0.9-2.6	13	2.73 (0.48)	2.6	1.6-4.3
	NJ	50	0.93 (0.06)	0.9	0.6-1.7	37	2.29 (0.43)	1.8	0.9-6.8	20	3.35 (0.65)	3.8	1.2-5.4
	Wild	60	0.94 (0.06)	0.9	0.6-1.6	54	2.59 (0.36)	2.05	1.0-5.6	17	3.76 (0.81)	4.4	1.4-6.2
											Second instars		
										3	4.83 (1.86)	5.5	3.0-6.0
										15	9.89 (3.06)	7.45	3.2-20.2
										34	7.99 (1.14)	7.6	4.1-15.5

[a] Numbers in parentheses equal twice the standard error.

* Marked mean weights are significantly lower (a=0.05) than weights for other treatment(s), based on Student's-t test (exp. 1 and 2) or single-factor ANOVA (exp. 3).

Project Number: GM 88.2.7
Project Title: Comparative Performance of F_1-Sterile Gypsy Moths Under Different Rearing Conditions
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leader: R.W. Hansen, with assistance from J. Cummings and S. Dunn

INTRODUCTION. Larval development (i.e. time spent in the larval stage) is an important factor controlling the relative numbers and synchronous activity of F_1-sterile gypsy moth adults and their feral counterparts. In Lepidoptera, F_1-sterile larvae, the progeny of irradiated fathers, generally develop more slowly than untreated larvae; the magnitude of this growth reduction appears dose-dependent. F_1-sterile gypsy moth larvae have been shown to develop somewhat more slowly than untreated lab-strain larvae on artificial diet or oak foliage. However, these comparisons were based on a single set of rearing conditions. We do not know if the relative relationships between F_1 and untreated larval, or pupal, growth parameters change with different rearing conditions.

The following experiments monitor and compare several larval and pupal growth parameters for F_1-sterile, untreated lab-strain, and feral gypsy moths reared under different temperature and photoperiodic regimes.

METHODS. Three experiments were conducted in 1987-1988. Each involved F_1-sterile (father treated with 10 krad), untreated lab-strain ("New Jersey", or NJ), and feral (sources differed among experiments) larvae. In all experiments, first instars were collected within 48 hr of hatch and individually reared on standard artificial diet.

For experiment 1, F_1, NJ, and wild (egg masses collected in South Carolina) first instars were reared in 40-ml glass vials, each containing a cylindrical plug of diet and sealed with a rubber foam stopper. Twenty larvae of each treatment were reared in environmental chambers at constant 7°, 13°, 20°, 25°, and 30°C (n=15 for wild larvae at 13° and 25°). A 16:8 hr (light:dark) photoperiod and relative humidities of 40-60% were maintained in each chamber. Larvae were reared until pupation and their developmental status monitored daily; fresh diet was supplied as necessary. Parameters recorded were duration of first stadium and length of larval stage (introduction to pupation).

In experiment 2, F_1, NJ, and wild (egg masses collected in West Virginia) were reared in 45-ml clear plastic cups with 15-20 ml of poured diet. Thirty larvae of each treatment were reared in chambers maintained at 15°, 23°, and 30°C (16:8 L:D and >50% RH) and in an outdoor insectary under ambient temperature, humidity, and light regimes (May to July). Insectary temperature and humidity were recorded on a hygrothermograph. Larvae were monitored daily until adult eclosion. Parameters compared were first stadium, larval stage, and pupal stage duration, and pupal weight.

For experiment 3, F_1, NJ, and wild (F_2 egg masses; parents originally collected in Rhode Island) were reared in 45-ml cups. Twenty-five of each treatment were reared in two chambers at 25°C; one chamber had a 16:8 hr

photoperiod and the other a 12:12 hr photoperiod (the 12:12 hr chamber had only 7 F_1 larvae at the start). A third group of larvae was reared in a heated greenhouse under ambient light (Oct. to Dec.), but these insects were eventually discarded due to excessive condensation and mold growth. Duration of larval and pupal stages and pupal weight were compared.

For all experiments, various treatment effects were compared using 2- or 3-factor analysis of variance procedures (fixed effects model). Rearing temperature or photoperiod, larval treatment, and sex were the factors of interest. The Student-Newman-Keuls (SNK) test was used to detect significant differences between group means.

RESULTS

A. Length of first stadium. Larvae reared at 7°C (exp. 1) did not survive the first stadium and were not included in the analysis. During experiments 1 and 2, first instar duration (Fig. 1) varied significantly among rearing temperatures ($F=675.9$, $P<0.001$ and $F=1205.0$, $P<0.001$, respectively) and larval treatments ($F=30.9$, $P<0.001$ and $F=26.5$, $P<0.001$, respectively). Not surprisingly, first stadium duration usually showed an inverse relationship with rearing temperatures. Generally, wild first instars developed most slowly and NJ first instars most rapidly, with F_1 larvae intermediate. However, temperature/treatment interaction terms are significant ($F=22.8$, $P<0.001$ and $F=6.7$, $P<0.01$, respectively), obscuring these relationships.

B. Length of larval stage. Larvae reared at 13°C (exp. 1) and at 15°C (exp. 2) did not pupate and thus were not included in this analysis. Rearing temperature had a significant effect on larval duration during experiments 1 and 2 ($F=604.3$, $P<0.001$ and $F=156.6$, $P<0.001$, respectively; see Fig. 2a,b). Generally, larval duration was also inversely related to temperature; larvae reared outdoors exhibited longer development than those reared at 23°C (exp. 2). Photoperiod (exp. 3) had no significant effect on length of the larval stage ($F=3.5$, $P>0.05$; see Fig. 2c).

Larval duration varied significantly among larval treatments for all three experiments ($F=75.9$, $P<0.001$, $F=109.4$, $P<0.001$, and $F=26.0$, $P<0.001$, respectively). Feral larvae took the longest to develop; F_1 and NJ larvae were not significantly different during experiments 1 and 2, but NJ larvae develop significantly faster during experiment 3. Finally, males exhibited significantly shorter larval stages than did females in all experiments.

However, interaction terms (temperature/treatment, treatment/sex) are significant in experiments 1 and 2. Thus, the general relationships discussed above may not hold true in all temperature and larval treatment combinations.

C. Pupal weight. Pupal weight (Fig. 3) varied significantly among rearing temperatures (exp. 2; $F=37.2$, $P<0.001$); pupae reared at 30°C were heaviest and those at 23°C were lightest, while those reared outdoors were intermediate. Weight also varied among larval treatments, as NJ pupae were heavier than F_1 pupae, which in turn were heavier than wild pupae. Interaction terms were also significant. Photoperiod (exp. 3) had no significant effect on pupal weight ($F=2.7$, $P>0.10$). As expected, female pupae were significantly larger than male pupae in both experiments ($F=745.8$, $P<0.001$, and $F=101.3$, $P<0.001$).

D. Length of pupal stage. In experiment 2, pupal duration (pupal molt to adult eclosion) differed significantly among rearing temperatures ($F=221.7$, $P<0.001$) and was inversely temperature-related (Fig. 4a); pupae reared outdoors were again intermediate. Treatment effects were also significant ($F=4.0$, $P<0.05$) exhibiting the following general relationship: F_1>NJ>WV (wild). However, various interaction terms were also significant, obscuring these general relationships. In experiment 3, photoperiod (Fig. 4b) had no effect on length of the pupal stage. Male pupae took longer to develop than female pupae in both experiments ($F=96.6$, $P<0.001$ and $F=34.0$, $P<0.001$, respectively).

E. Survival. The numbers of insects surviving the first stadium, and producing pupae and adults are summarized in Table 1. Similar numbers of F_1, NJ, and WV completed the first stadium for a given rearing temperature (exps. 1 and 2). Generally, wild larvae produced the fewest pupae or adults at a given temperature or photoperiod.

DISCUSSION and CONCLUSIONS. In general, these experiments support the "general hypothesis" than parental irradiation induces relatively longer larval development in F_1-sterile progeny. NJ gypsy moth larvae had shorter first stadia and overall larval and pupal development, and produced larger pupae, than F_1 larvae. Feral larvae generally exhibited the longest larval development and smallest pupae; this is not surprising, given an initial exposure to artificial diet and rearing conditions. Lab-strain insects (NJ and F_1) represented generations 32 or 33 in an "artificial" mass-rearing program.

Performance parameters exhibited positive (pupal weight) or negative (first instar, larval stage, and pupal stage duration) relationships with rearing temperature. Performance under ambient environmental conditions (exp. 2) was somewhat less that at a constant temperature of 23°C; not surprisingly, mean insectary temperatures during the period were ca 20°C. Altering a 24-hr photoperiod by four hours had no impact on gypsy moth performance.

The general relationships in larval and pupal performance exhibited by F_1-sterile, NJ, and feral gypsy moths are clouded by significant interaction terms. The presence of significant interactions was probably exacerbated by small sample sizes. However, they indicate that the relationship between irradiated and untreated larvae and pupae (e.g. F_1 vs NJ) may not remain constant with differing rearing conditions (in this case, temperature). Future experiments will likely address this uncertainty, as well as the effects of natural (foliage) and artificial diets.

Relatively fewer wild larvae successfully developed into pupae and adults at a given rearing temperature or photoperiod. Some of this "mortality" was due to viral (NPV) infection; feral egg masses were not disinfected prior to use. However, only 5-10% of wild larval mortality appeared virus-induced. Diet deterioration may also have played a role, since wild insects generally required longer developmental periods.

Finally, experiments 1 and 2 suggest a lower threshold for first instar development somewhere between 7° and 13°C, and a lower threshold for "complete" larval development somewhat above 15°C. An upper developmental threshold, if it exists, is undoubtedly greater than 30°C.

Table 1. Survival of experimental gypsy moth larvae.

Exp.	Treatment	Rearing temp. (°C)[b]	Number of insects[a]: Start (L1)	Completed 1st stadium	Pupae	Adults
1	F_1	7	20	0	--	
		13	20	12 (60)	0	
		20	20	16 (80)	16 (80)	
		25	20	20 (100)	19 (95)	
		30	20	12 (60)	7 (35)	
	NJ	7	20	0	--	
		13	20	17 (85)	0	
		20	20	20 (100)	19 (95)	
		25	20	20 (100)	18 (90)	
		30	20	15 (75)	9 (45)	
	Wild	7	20	0	--	
		13	15	10 (67)	0	
		20	20	19 (95)	18 (90)	
		25	15	14 (93)	10 (67)	
		30	20	20 (100)	13 (65)	
2	F_1	15	30	24 (60)	0	--
		23	30	28 (93)	27 (90)	27 (90)
		30	30	30 (100)	23 (77)	21 (70)
		Out	30	27 (90)	16 (53)	11 (37)
	NJ	15	30	27 (90)	0	--
		23	30	30 (100)	25 (83)	25 (83)
		30	30	29 (97)	29 (97)	27 (90)
		Out	30	29 (97)	22 (73)	20 (67)
	Wild	15	30	28 (93)	0	--
		23	30	30 (100)	8 (27)	3 (10)
		30	30	30 (100)	20 (67)	20 (67)
		Out	30	30 (100)	11 (37)	5 (17)
3	F_1	16:8	25		18 (72)	15 (60)
		12:12	7		6 (86)	3 (43)
	NJ	16:8	25		16 (64)	12 (48)
		12:12	25		23 (92)	19 (76)
	Wild	16:8	25		10 (40)	7 (28)
		12:12	25		16 (64)	15 (60)

[a] Numbers in parentheses are percentages of initial N
[b] Insects in exp. 3 reared at 16:8 or 12:12 (hrs L:D) photoperiods; both at 25°C

FIGURE 1. Mean length of the first stadium. Vertical bars indicate ± 1SD.

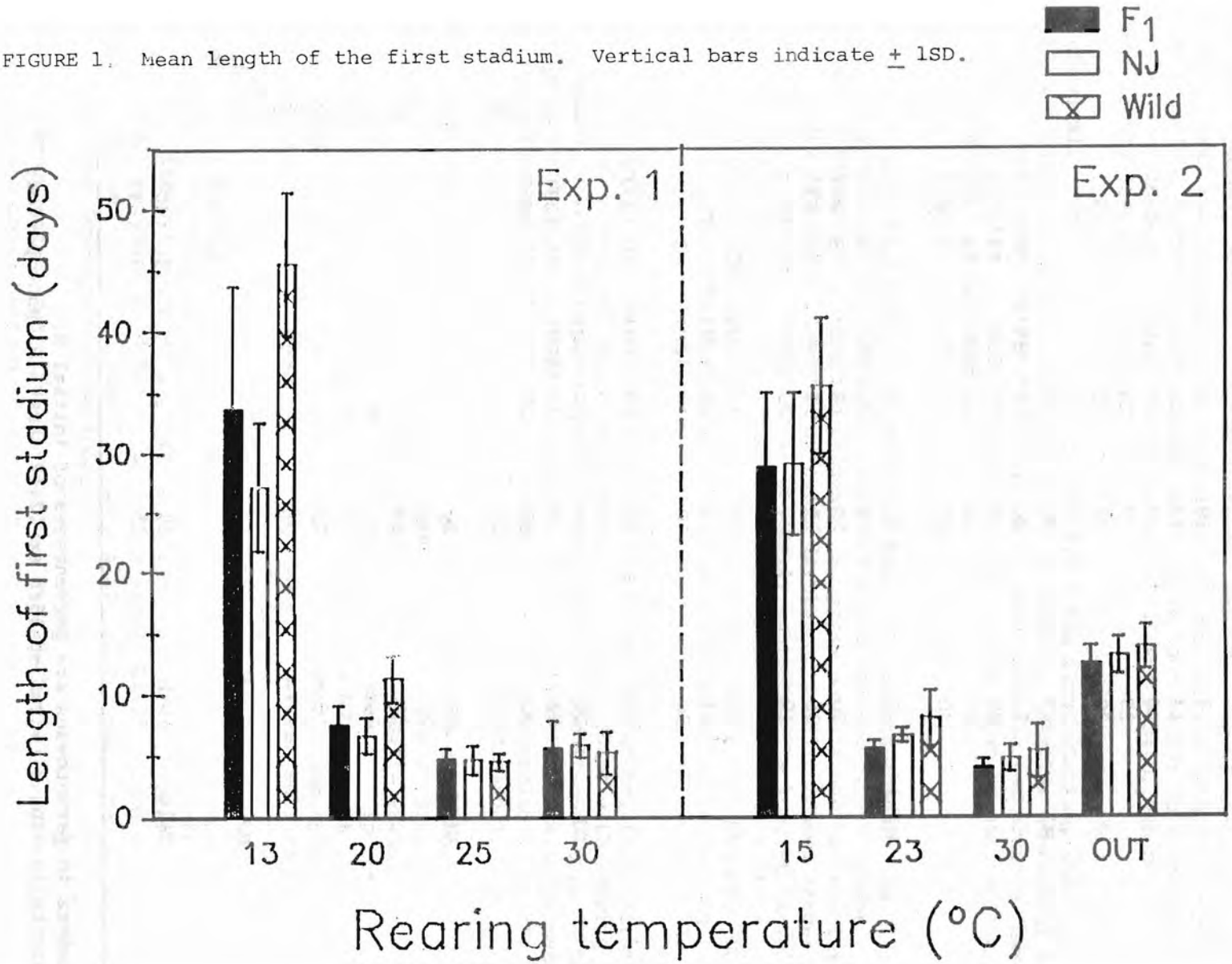


FIGURE 2. Mean length of larval stage for larvae from exp. 1 (A), exp. 2 (B), and exp. 3 (C). Vertical bars indicate ± 1SD.

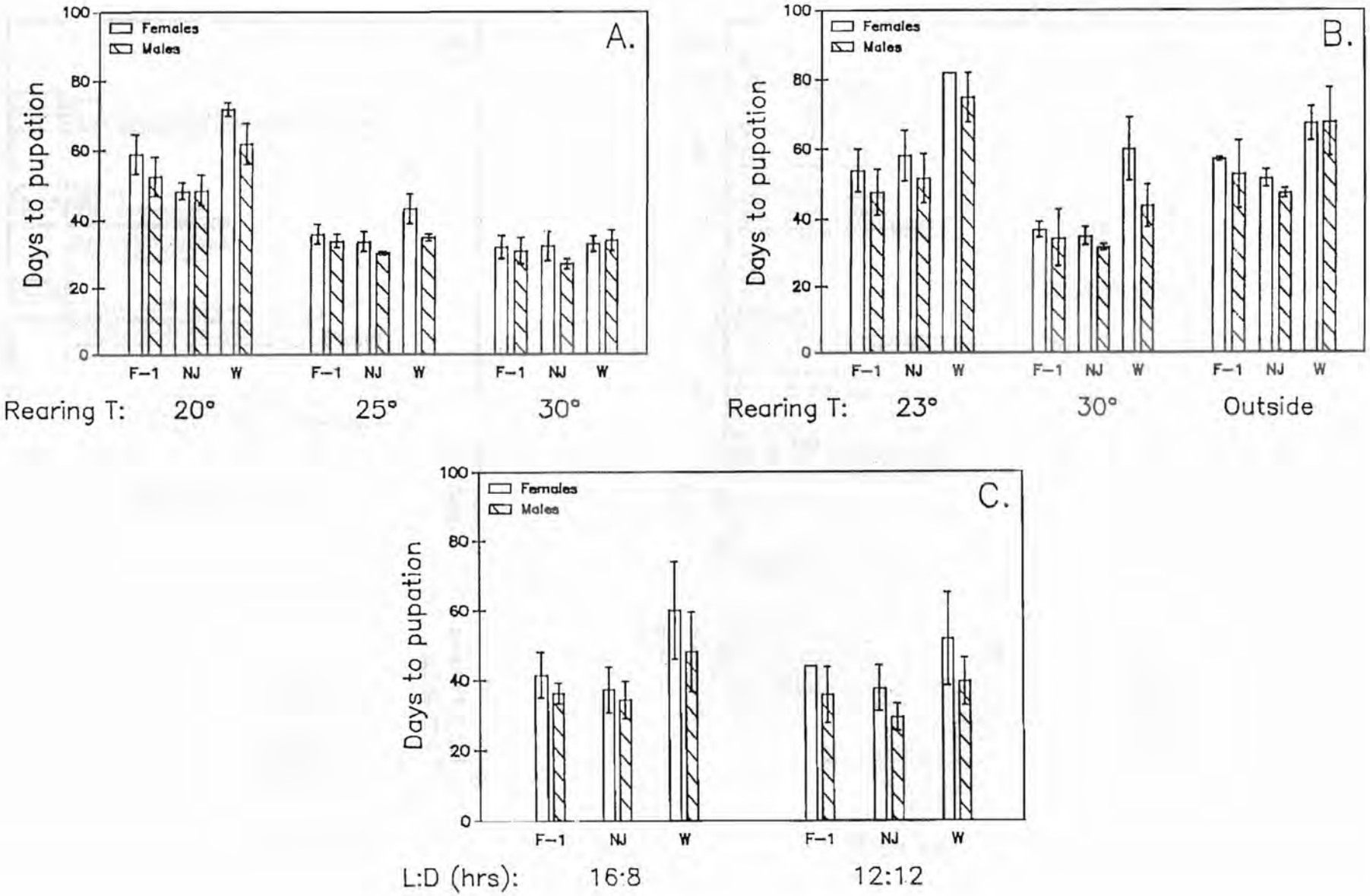
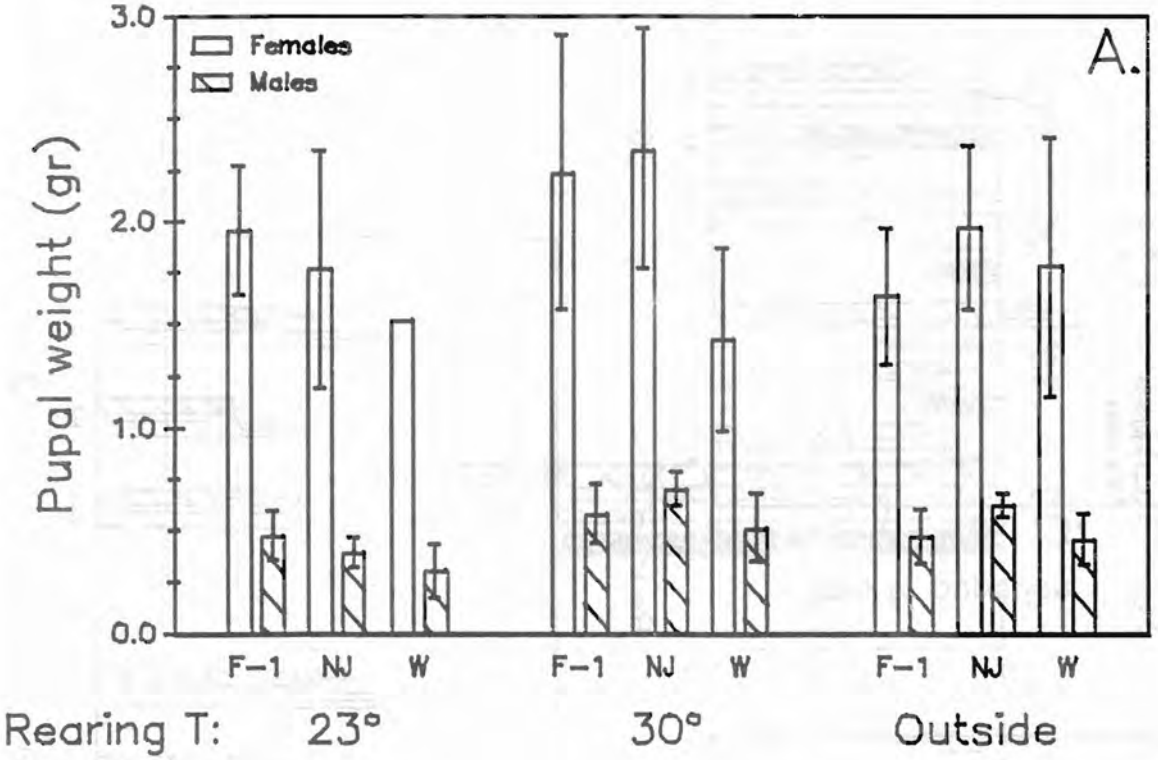


FIGURE 3. Mean pupal weights from exp. 2 (A) and exp. 3 (B). Vertical bars indicate ± 1 SD.



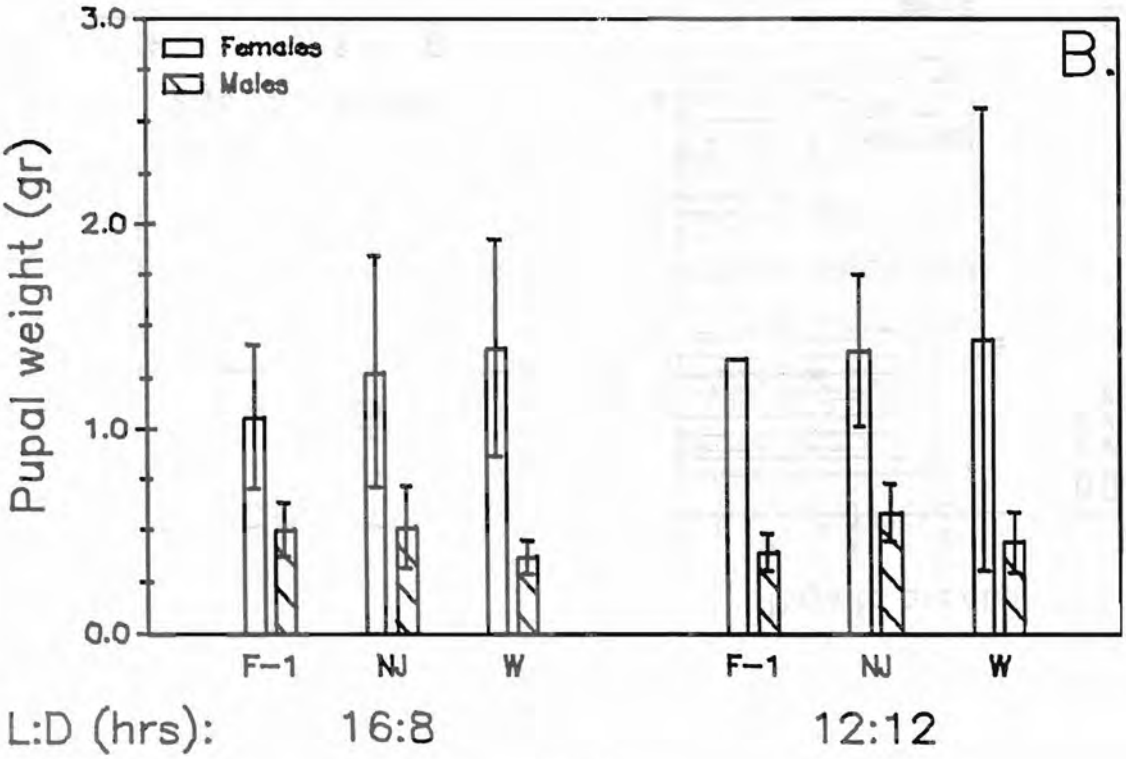
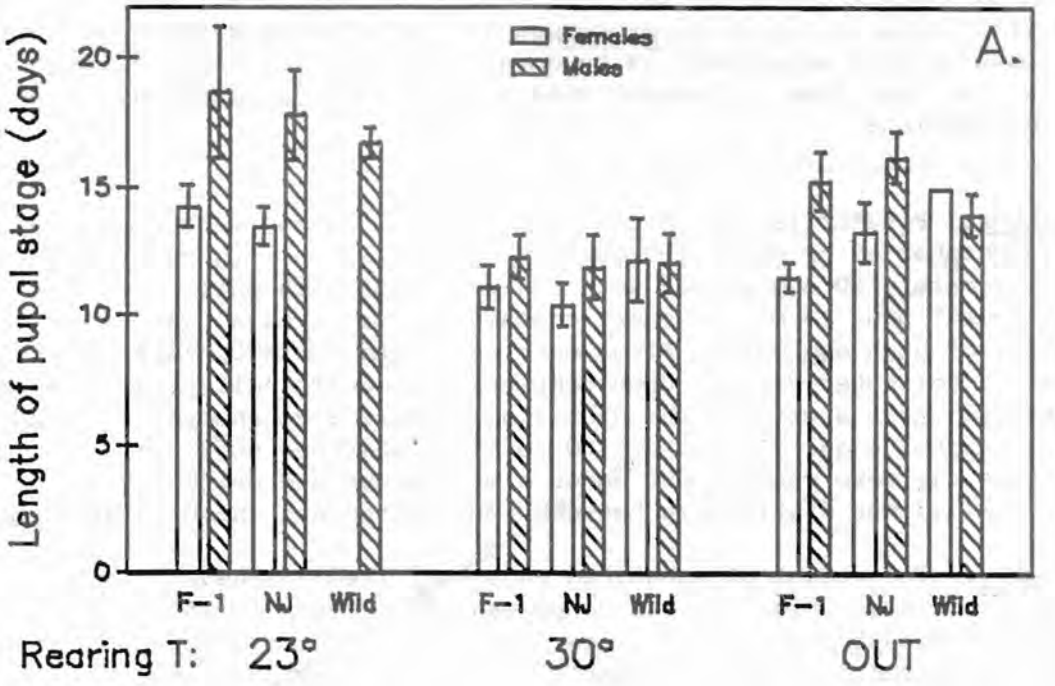
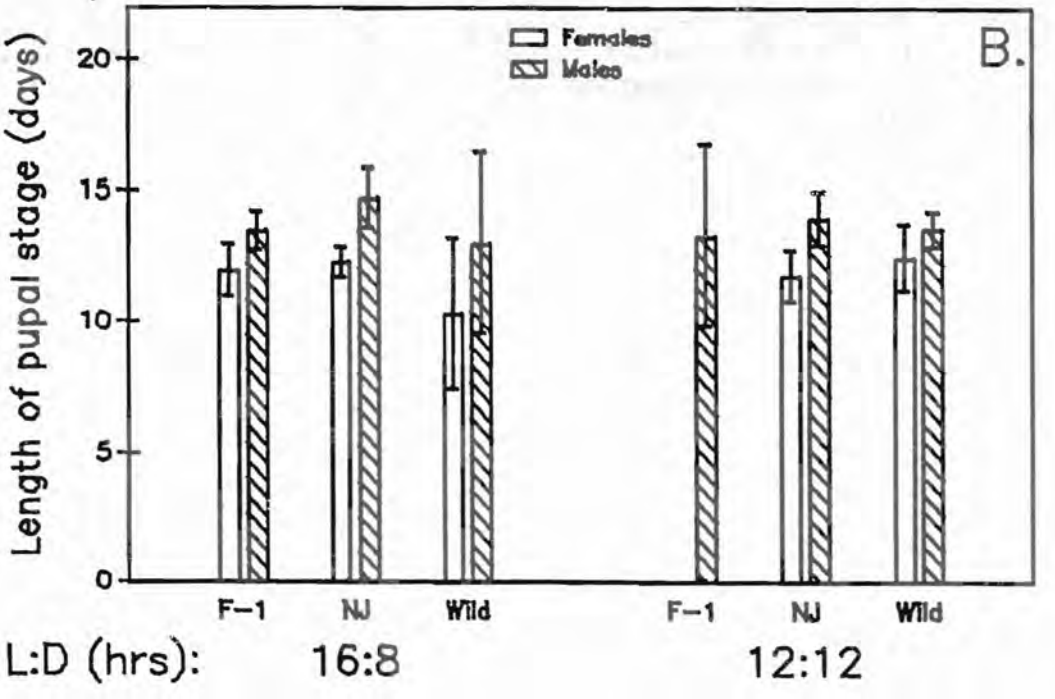


FIGURE 4. Mean length of pupal stage from exp. 2 (A) and exp. 3 (B). Vertical bars indicate ± 1 SD





Project Number: GM 88.2.8
Project Title: Estimating Numbers of Unhatched Eggs From Egg Weights
Report Period: June 1, 1988 - Sept. 1, 1988
Report Type: Preliminary
Project Leaders: R. Hansen, J. Cummings, S Dunn

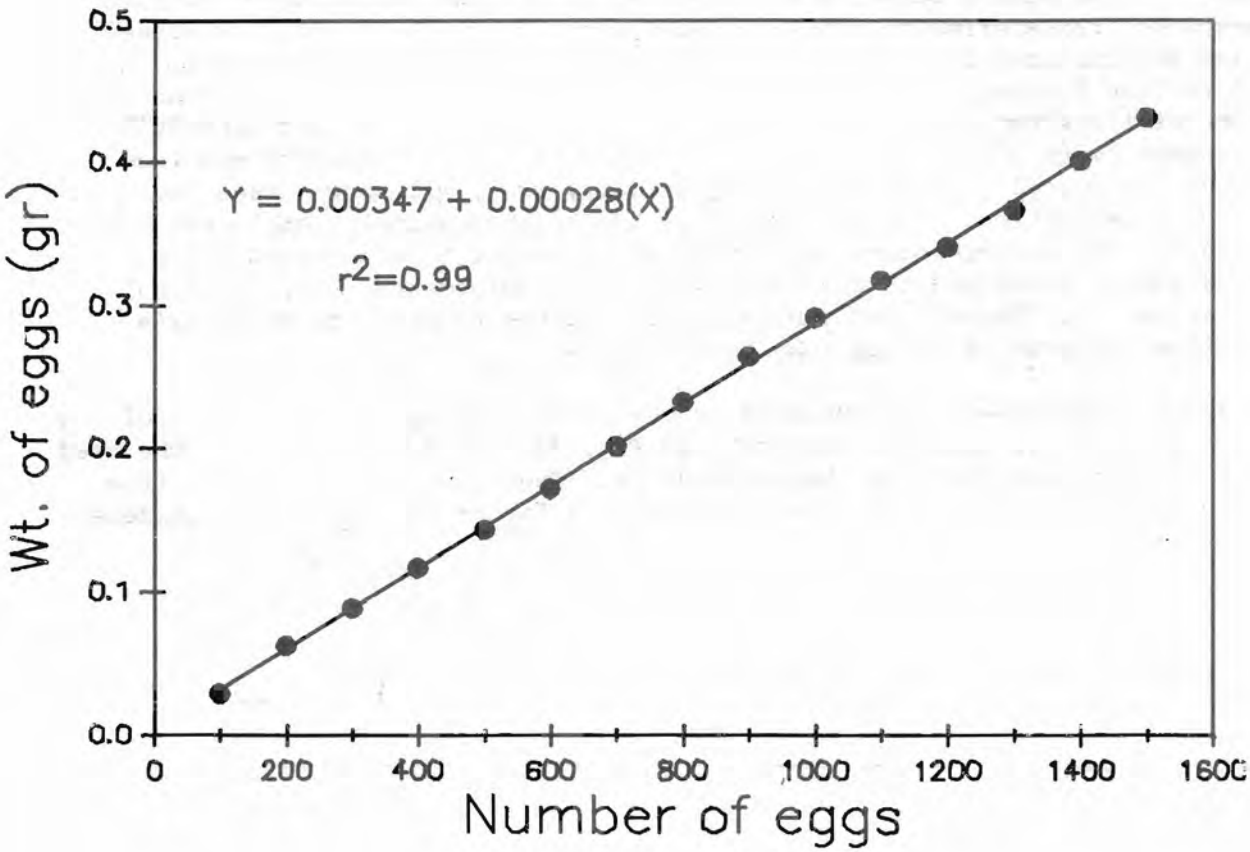
Objective: To determine percent egg hatch, the numbers of unhatched gypsy moth eggs remaining after completion of first instar eclosion must be counted. Counting eggs is a tedious procedure and potentially subject to errors. The purpose of this experiment is to develop an equation that estimates numbers of unhatched eggs from egg weight measurements, thus simplifying hatch rate determinations.

Methods: F_1-sterile (10 krad) gypsy moth egg masses were removed from a 5°C cold chamber after 80 to 180 days exposure (exposure in ca. 7-day intervals). Approximately 20 egg masses were available for each chill duration. A single "standard" plug (4 mm diameter, containing ca. 50 eggs) was removed from the center of each egg mass. Plugs were incubated at 25°C, >60% RH and first instars collected daily. Upon completion of hatch, all plugs were pooled and dehaired under suction. The dehairing process also removed residual fragments from hatched eggs. Groups of 100 to 1500 unhatched eggs, in 100 egg increments, were counted out under a microscope and weighed to the nearest 0.1 mg. We did not distinguish between embryonated and unembryonated eggs.

Results: The weight of unhatched eggs exhibited a linear relationship with numbers of eggs (Fig. 1); the slope was significant ($t=155.6$, $P<<0.001$) but the Y-intercept was not significantly different from zero ($t=2.08$, $P>0.05$). The regression accounts for nearly 100% of the total variation in egg weight.

Predicted numbers of unhatched eggs can be determined from weights by inverting the linear relationship ("inverse prediction"). The relationship thus obtained is: **EGG NO = 3512.6(EGG WT) - 11.8.** The r^2 value for this equation also exceeds 0.99. A BASIC program that calculates predicted egg numbers and their confidence limits from measured egg weights has been prepared. This predictive relationship will be valid for F_1-sterile (10K) eggs in a ca. 0.0-0.45 g weight range. Future work will extend the weight range for which this relationship is valid (i.e. entire egg masses) and compare number/ weight relationships among other gypsy moth "strains" (nonirradiated lab strain, feral insects).

FIGURE 1. Linear relationship between dehaired egg weight (Y) and number (X).

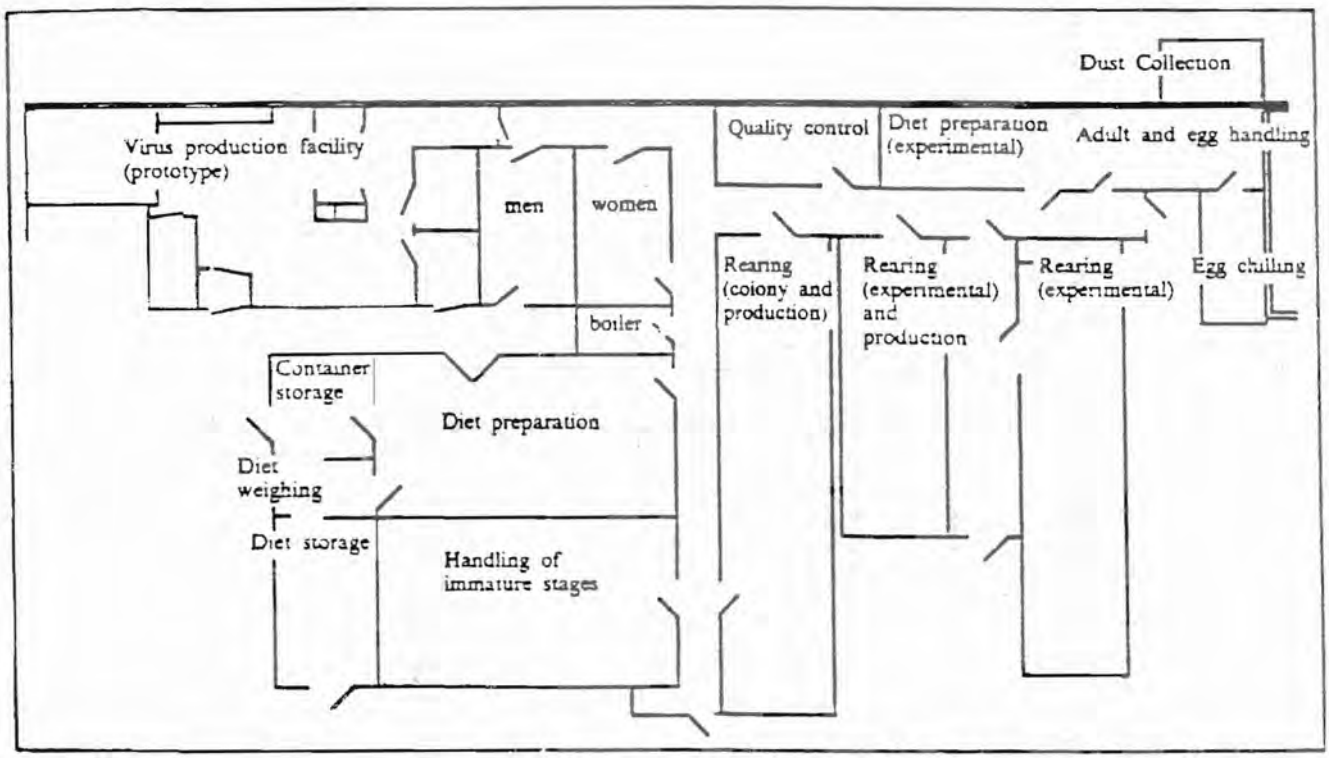


Project Number: GM 88.3.1
Project Title: Rearing Facility: Management and Production
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: G. Bernon and J. A. Tanner

During the last half of FY 1988, plans were initiated to renovate the facility and restructure the staffing and management to accommodate the expanded operation. The new construction will be completed by March, 1989. The rearing operation will more than double in size from the former layout of approximately 4,500 square feet to over 10,000 square feet (Figures 1 and 2).

In July, 1988, Jessie Baker, the biological technician who managed day-to-day operations, transferred to the witchweed program, and the position remained vacant and unfunded for all of FY 1989. Plans for the expanded program in FY 1990 include funding a replacement for this position. In addition, John Tanner will now be primarily responsible for quality control and methods development within the rearing program. Gary Bernon, who transferred from the Mission Biological Control Laboratory in July, 1987, will now manage and supervise production. The employment status of the temporary employees was upgraded. The six biological aids that are critical to a successful program have all been converted to one year renewable appointments. This continuity will reflect our recognition and commitment to the rearing operation as a year-round program requiring long term planning.

Cooperator involvement has expanded to the point that approximately 25% of egg masses produced are sent to cooperators (Fig. 3). We have recognized the need for long term planning from the cooperators. The eight month time lag from egg to usable egg has now been recognized as a factor that must be considered in all future planning.


Dust Collection
Virus production facility (prototype)
men
women
Quality control
Diet preparation (experimental)
Adult and egg handling
Rearing (colony and production)
Rearing (experimental) and production
Rearing (experimental)
Egg chilling
boiler
Container storage
Diet preparation
Diet weighing
Diet storage
Handling of immature stages

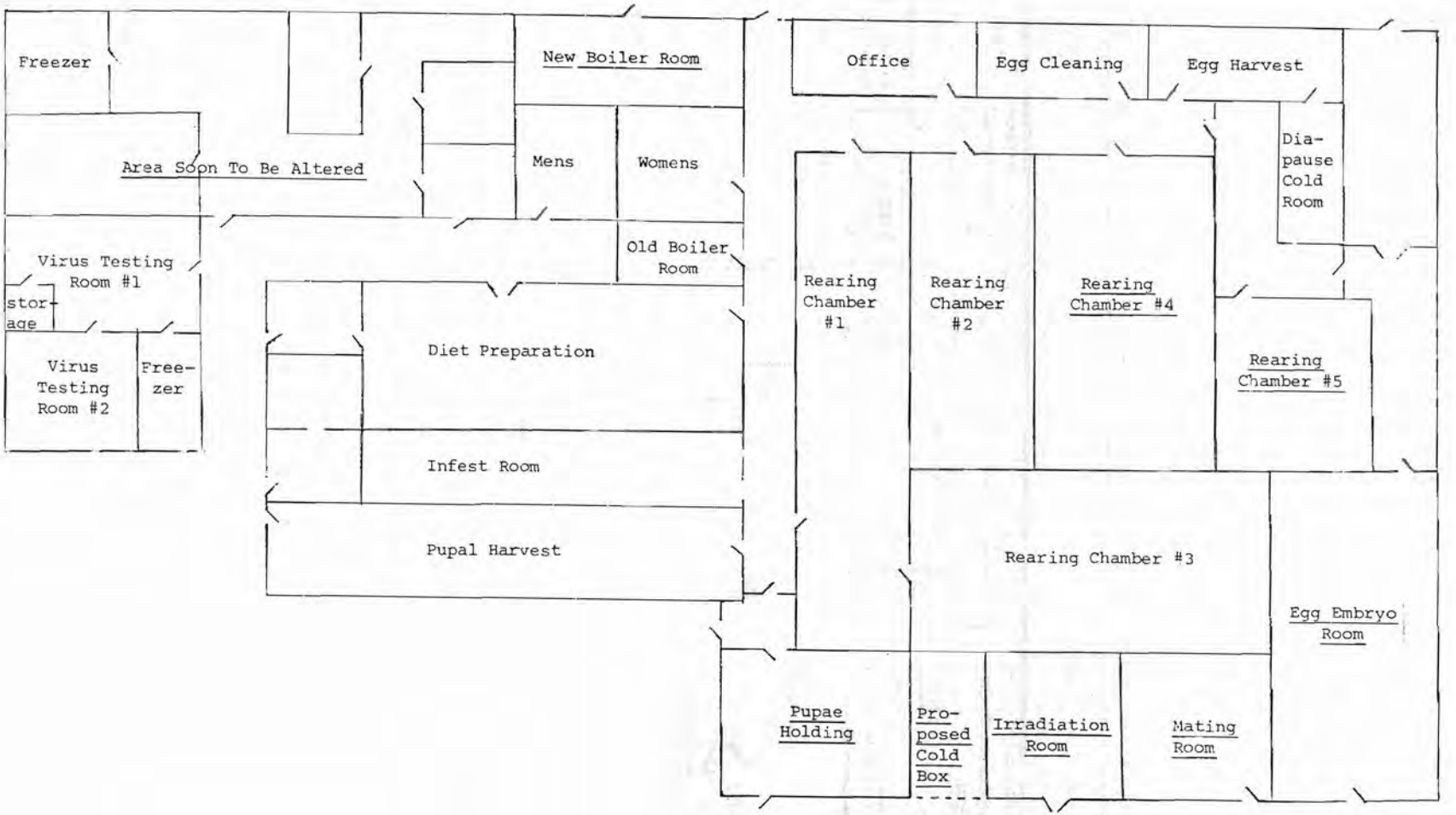

Freezer
New Boiler Room
Office
Egg Cleaning
Egg Harvest
Area Soon To Be Altered
Mens
Womens
Dia-
pause
Cold
Room
Virus Testing
Room #1
stor
age
Old Boiler
Room
Rearing
Chamber
#1
Rearing
Chamber
#2
Rearing
Chamber #4
Rearing
Chamber #5
Virus
Testing
Room #2
Free-
zer
Diet Preparation
Infest Room
Pupal Harvest
Rearing Chamber #3
Egg Embryo
Room
Pupae
Holding
Pro-
posed
Cold
Box
Irradiation
Room
Mating
Room

Figure 3. Cooperators

Cooperator	Agency	Stages shipped	Shipment rate
A. Adamson	Private Company (ESPRO)	eggs	100/week
P. Barbosa	Univ. Maryland	eggs	25/month
J. Brezner	SUNY	eggs	50/month
R. Carde	U. Mass	eggs, larvae	10/week
R. Charlton	Cornell Univesity	eggs, larvae	40/month
J. Elkinton	U. Mass	eggs	variable
A. Hajek	Boyce Thomson Inst.	eggs	20/month
R. Lindroth	U. Wisconsin	eggs	irregular
K. O'Hayer	State of Illinois	eggs	10em/month
T. Pannabecker	Cornell University	larvae	50-100/week
G. Prestwich	State Univ. of NY	larvae	1 tray-2/week
M. Raupp	U. of Maryland	eggs	6em/month
J. Schultz	Penn. State Univ.	eggs	25em/month
S. Shives	State of Virginia	eggs, larvae	32-120/month
D. Smitley	Mich. State Univ.	eggs	10em/month
R. Taylor	Ohio State Univ.	eggs	8em/month
M. Ticehurst	Private Company (NGMMG)	eggs, larvae	variable/month
R. Vogt	Yale University	larvae	1 tray/week
W. Yendol	Penn State Univ.	eggs	120em/month

Project Number: GM 83.3.1
Project Title: Development and Evaluation of Improved Rearing Techniques
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: John Allen Tanner, J.J. Baker, Marc Matthews Jr., Nancy A. Garvey, Susan E. Lane and Kimberly Baker.

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

In 1987-88 the major problem in the rearing program was "straggling" (Tanner, et. al., 1988). The degree of "straggling" now encountered in the rearing program has forced us to severely reduce the size of several rearing programs planned for 1989 rearing year. We are now attempting to identify the cause(s) for "straggling". We are also trying to develop methods that will enable us to "live" with the problem until it can be corrected.

LIVING WITH STRAGGLING

In trying to live with the "straggling" problem, we are now sampling all egg masses 21 days before they are needed and then using only those masses which give rise to normally developing larvae. The sampling method used is similar to the method described by Tanner and Baker (1985). A 1 oz. clear plastic container with a white plastic lid (Comet Products Inc., Chelmsford, Mass.) has been substituted for the 6oz. rearing container. Approximately 11 ml. of diet are placed into each cup. Four holes are poked into each lid to prevent moisture build-up. Samples of each egg mass are reared in these containers and classified as to producing growing or nongrowing larvae.

The use of the 1 oz. cup and plastic lid is expensive and requires a considerable amount of labor to cap and place holes in each lid. We are now testing a 50-cell tray molded from translucent plastic (Bio-Serv Inc., Frenchtown, N.J.) and sealed with a translucent polyester film lidding material coated with a nontoxic hot melt sealable adhesive (Oliver Products Company, Grand Rapids, Mi.). The lidding material is needle-punched to allow gas exchange and prevent moisture buildup (25 holes/in^2).

The size of an individual cell (40x30x15 mm) will provide enough space for food and area for growth to allow all the neonates emerging from a core sample to develop to the 3rd instar. This is adequate to determine if an egg mass is producing developing or nondeveloping larvae.

DETERMINING THE CAUSE(S) FOR STRAGGLING

Laboratory strains are mated in a rearing room maintained at 25$\pm$1°C and 50-55% RH. They are mated by placing 25 pairs of pupae into a gallon glassine lined ice cream container. The adults begin to emerge shortly thereafter and is emergence completed 7 to 12 days later. New Jersey strain females normally emerge synchronously with the males. They will mate and lay an egg mass within 24 hours of emerging. The masses are harvested and placed into refrigeration 42 days after the pupae were placed into the gallon container. The egg masses are 27 to 41 days old when placed into refrigeration. Tanner, et.al., (1985) found that egg masses embryonated 56 days at 50-55% humidity

and chilled 180 days at 7-10°C had a sharp decrease in the percent hatch compared to egg masses embryonated only 28 days. However, the effects of an extended embryonation period on the expression of "straggling" in egg masses was not evaluated. This was attempted in the following experiment.

Methods:

Laboratory egg masses (300/replication) were removed from the standard rearing room (25±1°C, 50-55% R.H.) and placed into chilling (7-10°C) 21, 28, 35, 42, or 49 days after the pupae were placed into the gallon mating container. A total of 50 masses were used for each treatment/replication. Three replications were conducted using three different families of egg masses. Two core samples were taken out of each egg mass after 180 days of chilling. One core sample was placed into a rearing cup (6 oz. fluted) containing 55 ml diet. The rearing cups were held in the standard rearing room. Each cup was opened and the number of larvae in each instar were ascertained 14 days after the start of incubation. Those masses with a mean larval stage of 1.25 or lower were classified as "straggling" egg masses.

The second core sample taken from each mass was placed into individual small plastic dishes. The dishes were held in a high humidity chamber (25°C, 80+% R.H.) until hatch was completed. Then the percent hatch based on the total number of embryonated eggs was determined.

Results:

Figures 1 and 2 respectively show the number of masses that were classified as "straggling" egg masses and the number of masses which produced no hatch. Figure 3 shows the percent hatch. Egg masses selected from the parent infest dates of Nov. 19 and Dec. 3, produced a high number of "straggling" egg masses and masses which did not hatch compared with egg masses from the parent infest date of Nov. 26, which produced no "straggling" egg masses and very few masses which did not hatch. The percent hatch for egg masses from Nov. 19 and Dec. 3 were also lower than the hatch for masses from Nov. 26. The length of the embryonation period did not have any consistent effect on incidence of "straggling". Therefore, whether a mass produces "straggling" or "nonstraggling" larvae seems to be determined either early in the embryonation period or during the rearing of the parents. The data do show that the incidence of "straggling" can vary considerably from week to week. This agrees with the results obtained from the colony core samples. Egg masses from any week of production have been genetically isolated for several generations from the adjacent weeks of production. The parents came from egg masses that were classified as "nonstraggling" and, of course, the parents were "nonstraggling" as larvae. The above data seems to indicate that we should be concentrating our efforts to identify the cause(s) for "straggling" on the methods and conditions used to rear the parents and/or the methods used for selecting and mating the parents.

Figure 1. The number of "straggling" egg masses as affected by the length of the embryonation period.

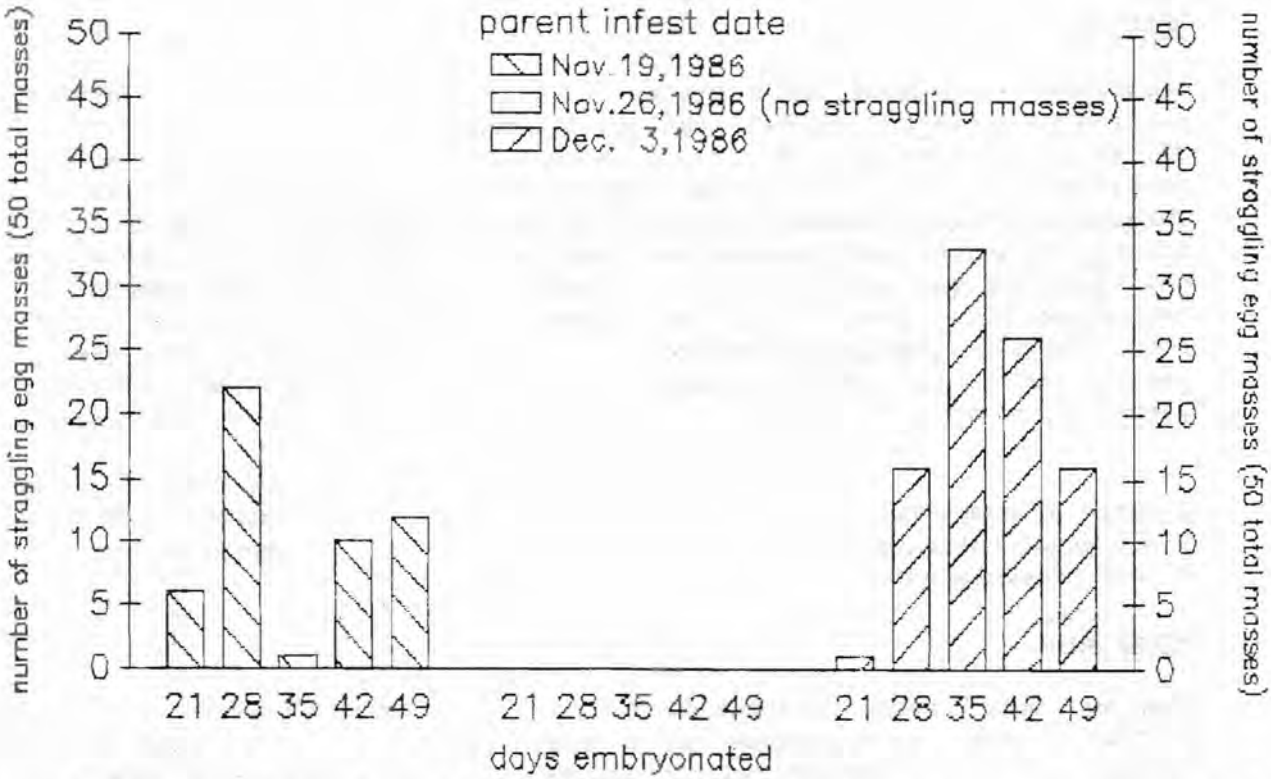


Figure 2. The number of nonhatch egg masses as affected by the length of the embryonation period.

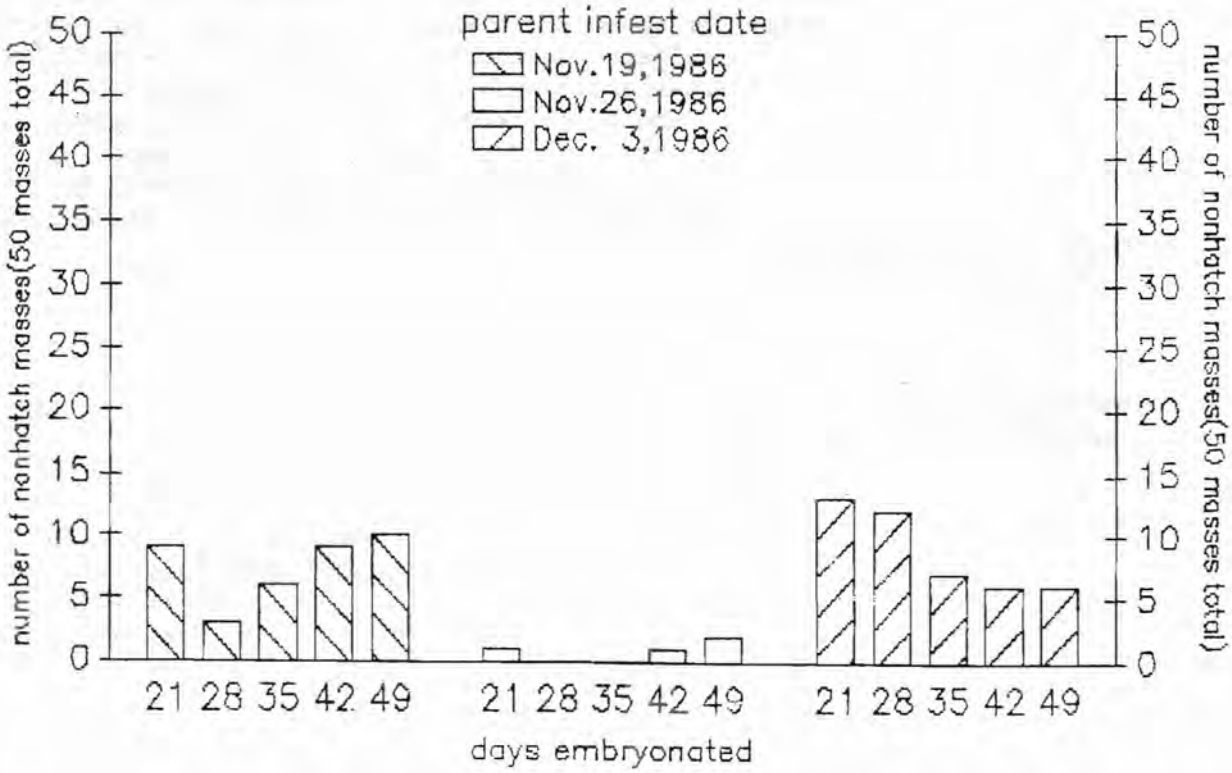
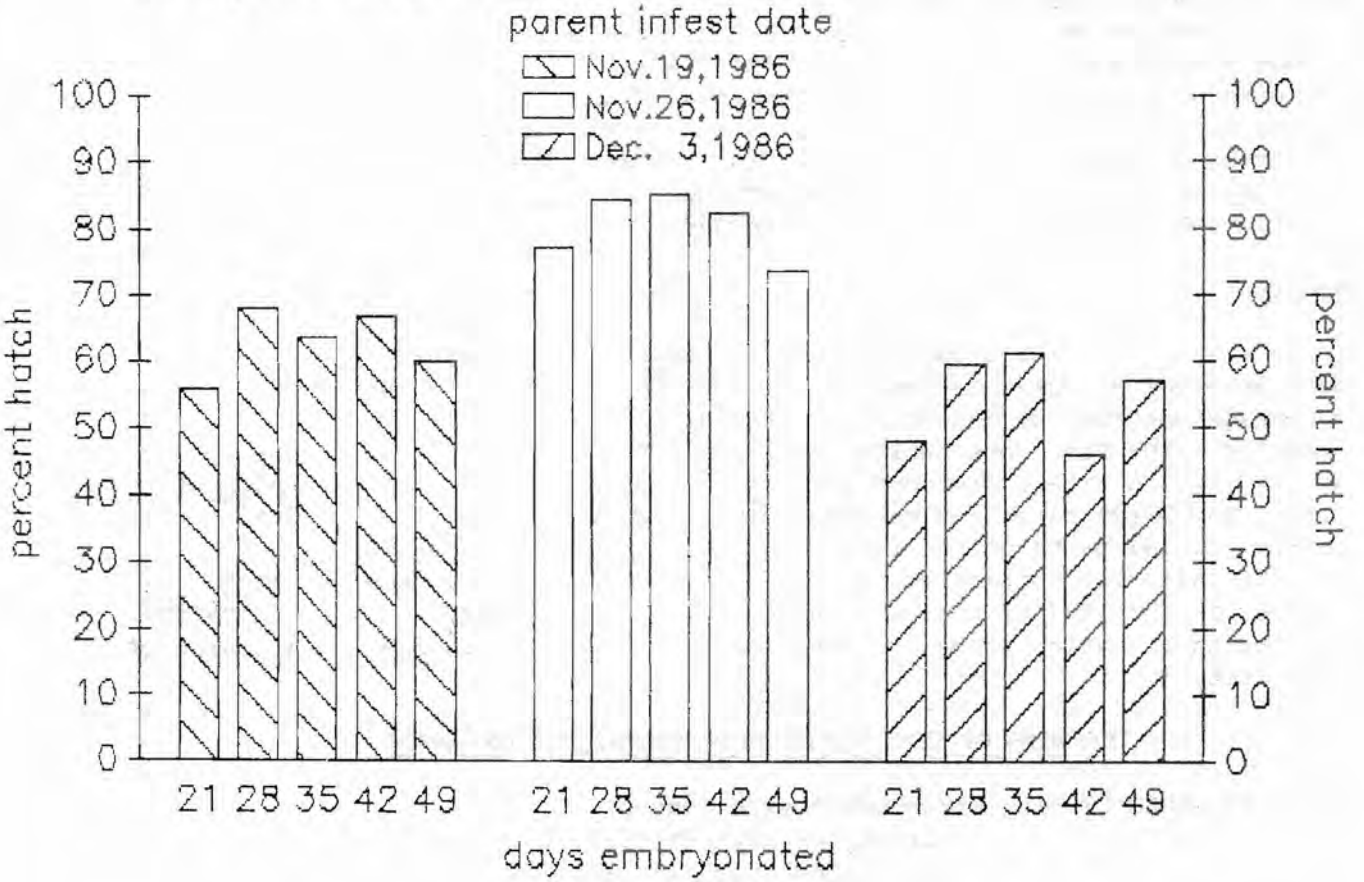


Figure 3. The percent hatch of egg masses as affected by the length of the embryonation period.



EFFECTS OF DIET CONTACT ON HATCH

During the 1987 F_1 rearing program, the hatch of New Jersey egg masses was at times uncharacteristically low (less than 30%). Often larvae which did hatch died shortly thereafter. In a previous test (Tanner and Weeks, 1981) it was reported that egg-diet contact reduced the hatch approximately 6%. These tests were done with the F_{21} generation. The present generation is the F_{32} and it is possible that the eggs have become less tolerant to diet contact or that the diet conditions have changed.

Methods:

Diet cups (20 cups) were selected from a standard production run just prior to the time the eggs were to be dispensed into the cups. The cups were divided into two groups. One group had 20 eggs hand dropped onto the surface of the diet (control). The second group had 20 eggs placed into the cups without any direct contact with the diet. All the cups were capped and those cups with no egg-diet contact were inverted. The cups were held in a standard rearing room (25-26°C, 60-65% R.H.). The cups were opened after 7 days of incubation and counts were made as to the number of hatched larvae, unhatched but embryonated eggs, and unembryonated eggs. The number of cups with fallen diet (inverted cups only) was also recorded.

Results:

Figure 4 shows that diet contact reduced the hatch of the New Jersey eggs. The reduction averaged slightly more than 13%. This reduction was twice the percentage observed by Tanner and Weeks in the F_{21} generation. This test was conducted using five consecutive families of eggs and the mean percent hatch for the eggs in direct diet contact ranged from a low of 59.2% to a high of 77.5%. The 59.2% hatch was considerably higher than the less than 30% observed during parts of the 1987 F_1 program. The above data seems to indicate that diet contact was not the major cause for the reduced hatch observed in the 1987 F_1 program. However, the consistent reduction in hatch dictates that rearing procedures should be adjusted to minimize this source of variability.

EFFECTS OF EGG PREPARATION TECHNIQUES ON HATCH

During the 1988 F_1 program we again experienced periods of low hatch. A test was conducted to determine if any of methods used to prepare the eggs for dispensing into the 6oz. diet filled cups were affecting the hatch.

Methods:

Egg masses destined for use in the production programs were sampled before and after formalin treatment. The masses were sampled a third time immediately after they were removed from the refrigerator (overnight). Samples of eggs were also taken immediately after the egg masses were dehaired and after the eggs went through the dispensing apparatus. Diet rearing cups containing dispensed eggs were selected as a control. Samples taken before dispensing were held in a high humidity hatch chamber. Samples taken after dispensing were divided into two groups. One group was held in a high humidity hatch chamber and the other group was held in the rearing room. The control cups

were held in the rearing room.

Results:

Table 1 shows that dehairing egg masses and placing dehaired eggs directly on the diet are the two major causes for reduced hatch. However, in this test as well as the above test, the percent hatch was never as low as the 20-30% that occurred in the F_1 program.

VARIATIONS IN THE PERCENT HATCH OF EGG MASSES AMONG DIFFERENT FAMILIES OF NEW JERSEY STRAIN GYPSY MOTH

Hatch data taken from the 1988 F_1 program indicates that in many cases when there was an appreciable rise or fall in the percent hatch it occurs when we switch to a new family of eggs. Eggs used in any one week of production or for colony maintenance all come from one family. There are 36 families in any one generation and all the families have been isolated for several generations. The variations in the percent hatch between different families are not known. The low hatch encountered at times may indicate that certain families are less "fit" to survive in the rearing program and should be discarded. We are now randomly sampling each family after 120, 150, and 170 days of chilling to get base-line data.

Methods:

Twenty egg masses were randomly taken from each family after 120, 150 and 170 days of chilling (60 masses total). Three core samples were taken out of each mass and placed into a small petri dish with a tight lid. The dishes were held in a high humidity chamber (25-26°C, 80-85% R.H.) and hatch taken off each workday. The percent hatch was determined based on the number of embryonated eggs.

Results:

We are now collecting the data. Figure 5 shows the results we have thus far. The data indicate that percent hatch can vary considerably among families, however, the data will need to be collected for more than one generation to determine if the fluctuation is due to differences in the genetic "fitness" between the families or due to differences in the rearing conditions the families may have been exposed to.

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Tanner,J.A., and J.J.Baker. 1985. Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1,1984 - September 30,1985:117-121.

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Tanner, J.A., J.G.R.Tardif, J.J. Baker, and M.C. Flynn. 1985. Development and Evaluation of Improved Rearing Techniques. APHIS Laboratory Report, October 1, 1984 -September 30, 1985:122-135.

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Figure 4. The percent hatch of New Jersey eggs as affected by contact with the high wheatgerm diet.

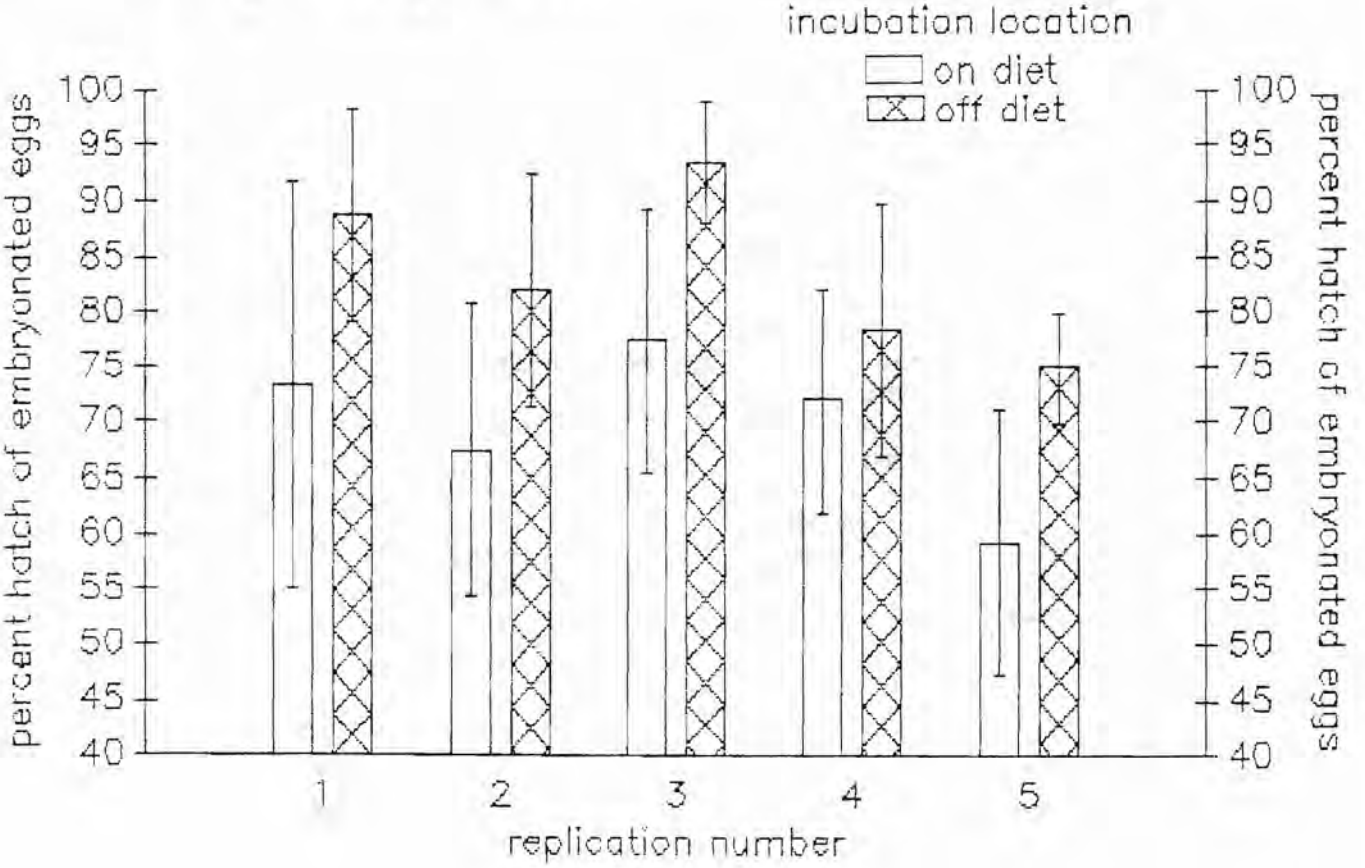


Table 1. The percent hatch of New Jersey eggs as affected by the methods used to surface disinfect, dehair and dispense the eggs.

	Replication number (percent hatch) 1/					
	1		2		3	
	mean	s.e.	mean	s.e.	mean	s.e.
Pre-formalin treatment	73.6ab	15.9	70.9abc	17.7	80.2a	8.5
Post-formalin treatment	80.6a	9.0	79.9a	11.5	82.6a	12.9
Post-refrigeration 2/	81.2a	11.1	76.2ab	21.3	81.7a	6.2
Post-dehairing 3/	69.4b	4.3	68.4bc	4.3	70.5b	5.2
Post-dispensed eggs held in:						
High humidity chamber 4/	67.2b	6.2	65.3c	6.8	66.1c	6.2
Rearing room 5/	67.4b	7.1	65.7c	6.4	69.2bc	6.8
Rearing room on diet (control) 6/	57.9c	8.3	58.1d	11.1	55.8d	7.2

1/ Means within a column not followed by the same letter were significantly different at the .05 level (unparired t-test).

2/ Formalin treated egg masses are held overnight in a refrigerator (7-10°C) and dehaired the following day.

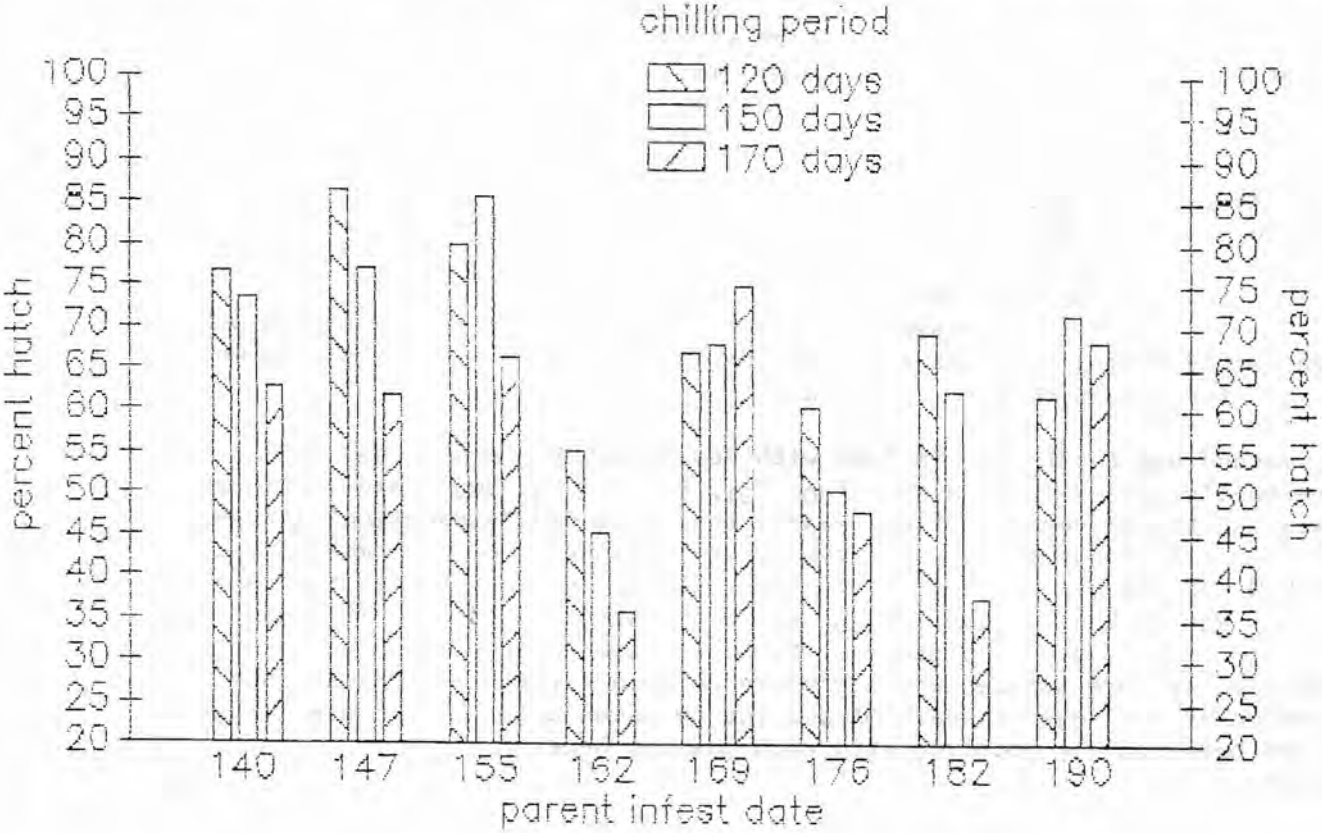
3/ The seta covering the eggs are removed by rubbing them over a screen set over the opening of a vacuum cleaner hose.

4/ 80-85% R.H., 25-26°C, eggs held in small petri dish.

5/ 55-60% R.H., 25-26°C, eggs held in small petri dish.

6/ 55-60% R.H., 25-26°C, eggs placed on diet in 6 oz. rearing cup.

Figure 5. The percent hatch observed in different families of New Jersey strain gypsy moth after 120,150,and 170 days of chilling (7–10C.).



Project Number: GM 81.3.1
Project Title: Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: John Allen Tanner, J. J. Baker, Marc Matthews, Jr., Nancy Garvey and Susan E. Lane

The purpose of this project is to monitor the development of the laboratory strains and to determine if it falls within acceptable ranges. Data are collected for each strain/generation and are used to detect changes from normal development so that corrective action can be taken.

Budgetary restraints have forced us to limit collection of developmental data to those insects used to maintain each strain (colony). Data are collected only on larval development, female pupal weights, sex ratio, pupal yields, adult female emergence and deformity and female fecundity in the New Jersey strain.

Larval "straggling" was a major problem during the 1985 rearing year (Tanner and Baker, 1985). To reduce, or possibly eliminate the "straggling" problem, egg masses used for colony maintenance were selected based on the mean larval stage (MLS) of 11 day-old larvae hatch from egg samples taken 21 days prior to the scheduled use of the masses (Tanner, et. al., 1986). Core samples were taken weekly from 100 masses and based on the highest MLS's, 18 masses were selected for use in colony maintenance.

This method was first used in 1985 with New Jersey F_{28} egg masses and has subsequently been used with F_{29}, F_{30}, F_{31}, F_{32} and F_{33} egg masses. Figure 1 shows that the percentage of egg masses that produced "straggling" larvae (MLS 1.00 - 1.25) dropped during the first two generation this method was employed. However, in the F_{31} generation the percentage rose to the same level it was in the F_{28} generation and is now averaging around 18% thus far in F_{33} generation. Another disturbing trend is shown in figure 2. The percentage of core samples which produce no mean larval stage either due to non-hatch of the eggs or death of all the neonates before they were 11 days-old has been roughly doubling with each passing generation since the F_{29} generation.

Tables 1 and 2 also show some disturbing trends. The percentage of larvae surviving to the pupal stages has been slowly dropping since the F_{28} generation, however, in the F_{32} generation the percentage tumbled almost 8% from the F_{31} generation level. The number of male pupae per female pupa also increased in the F_{32} generation and may indicate that the decrease in survival was due to an increase mortality in the female larvae. The considerable decrease in the weights of the female pupae in the F_{32} generation further indicates that the females were not as healthy as in previous generations. Adult female emergence in the F_{32} generation was at its lowest level since the F_{28} generation and adult female deformity was at its highest level. Lastly, in the F_{32} generation the number of eggs deposited per female and the number of eggs per colony mating were at their lowest level since we began to use the colony core method.

The above data indicate that the New Jersey strain has deteriorated considerably in the F_{32} generation. It is not known what is causing this deterioration, however, the use of only 18 egg masses to maintain each week of colony production and the isolation of each production week leads one to suspect that inbreeding may be a prime factor.

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Tanner, J. A., and J. J. Baker. 1985. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1984 - September 30, 1985:117-121.

Tanner, J. A., J. J. Baker, H. A. Hamilton and B. A. Fontes. 1986. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1985 - September 30, 1986:122-125.

Table 1. Performance of New Jersey Colony insects.

Parameter	Generation 1									
	28		29		30		31		32	
	mean	SEn=36)	mean	SE(n=37)	mean	SE(n=34)	mean	SE(n=35)	mean	SE(n=35)
Larvae per cup	9.5	0.08	8.5	0.17	7.7	0.08	7.8	0.04	7.9	0.05
Mean larval stage 2	2.93	0.03	2.79	0.04	2.88	0.03	2.76	0.04	2.87	0.04
Female pupal weights (gms)	2.21	0.03	2.21	0.04	2.38	0.04	2.32	0.03	2.01	0.04
% survival neonate to pupae	91.9	0.87	91.0	1.15	90.5	0.98	90.2	1.32	82.5	1.22
Developmental time to 50% pupation (DT50)										
Female	29.0	0.11	28.6	0.10	28.0	0.13	27.6	0.17	27.4	0.21
Male	27.0	0.12	26.6	0.11	25.6	0.12	25.2	0.16	25.2	0.15
Sex ratio M:F	1.0:1	0.02	1.0:1	0.02	1.0:1	0.02	1.0:1	0.02	1.1:1	0.03
% adult female										
emergence	88.6	2,35	95.6	0.80	93.7	1.14	93.2	1.29	89.7	2.93
deformity	4.3	0.75	6.6	0.83	4.2	0.59	2.6	0.65	8.9	1.53

1 Generation 28 - started April 1, 1985 through November 20, 1985.
Generation 29 - started November 27, 1985 through August 13, 1986.
Generation 30 - started August 18, 1986 through April 14, 1987.
Generation 31 - started April 21, 1987 through December 31, 1987.
Generation 32 - started January 7, 1988 through September 9, 1988.

2 9th Post-neonate infest date.

Table 2. Reproductive data of New Jersey colony insects.

Parameter	Generation 1									
	28		29		30		31		32	
	mean	SEn=36)	mean	SE(n=37)	mean	SE(n=34)	mean	SE(n=35)	mean	SE(n=35)
% of females depositing an egg mass	80.9	3.03	84.6	2.71	74.8	3.69	84.8	2.19	81.6	2.63
# eggs deposited per female	958.6	22.38	995.7	19.13	1021.4	27.61	1001.5	23.94	834.5	36.38
# colony eggs per colony mating	761.5	24.23	873.2	23.28	833.1	23.40	794.9	22.39	634.7	29.36

1 Generation 28 - started April 1, 1985 through November 20, 1985.
Generation 29 - started November 27, 1985 through August 13, 1986.
Generation 30 - started August 18, 1986 through April 14, 1987.
Generation 31 - started April 21, 1987 through December 31, 1987.
Generation 32 - started January 7, 1988 through September 9, 1988.

Figure 1. The percentage of New Jersey laboratory strain gypsy moth egg masses that produced "straggling" larvae.

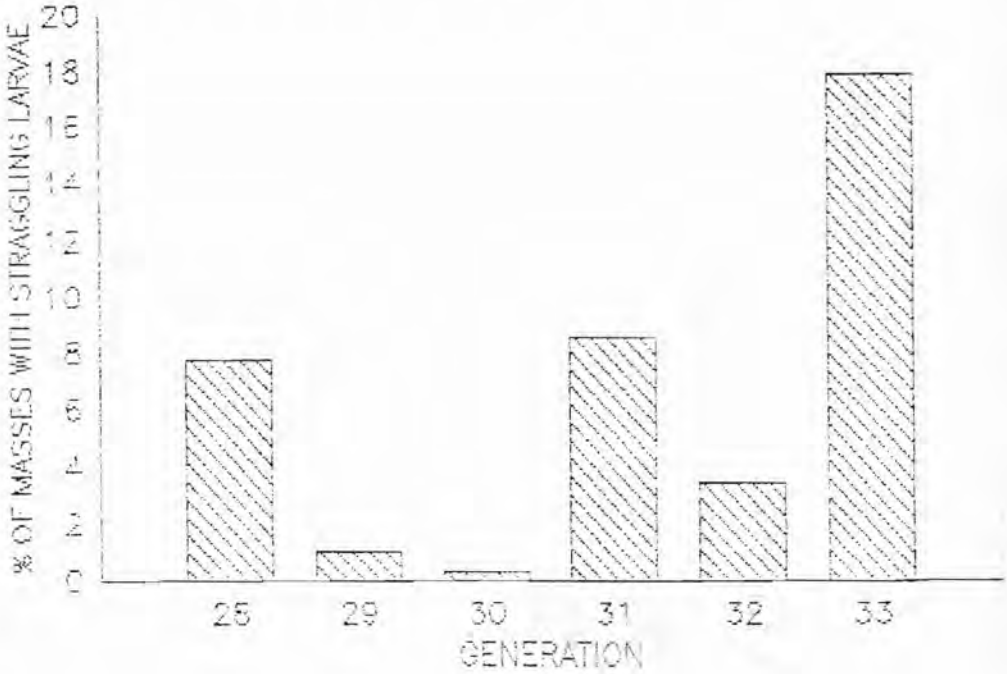
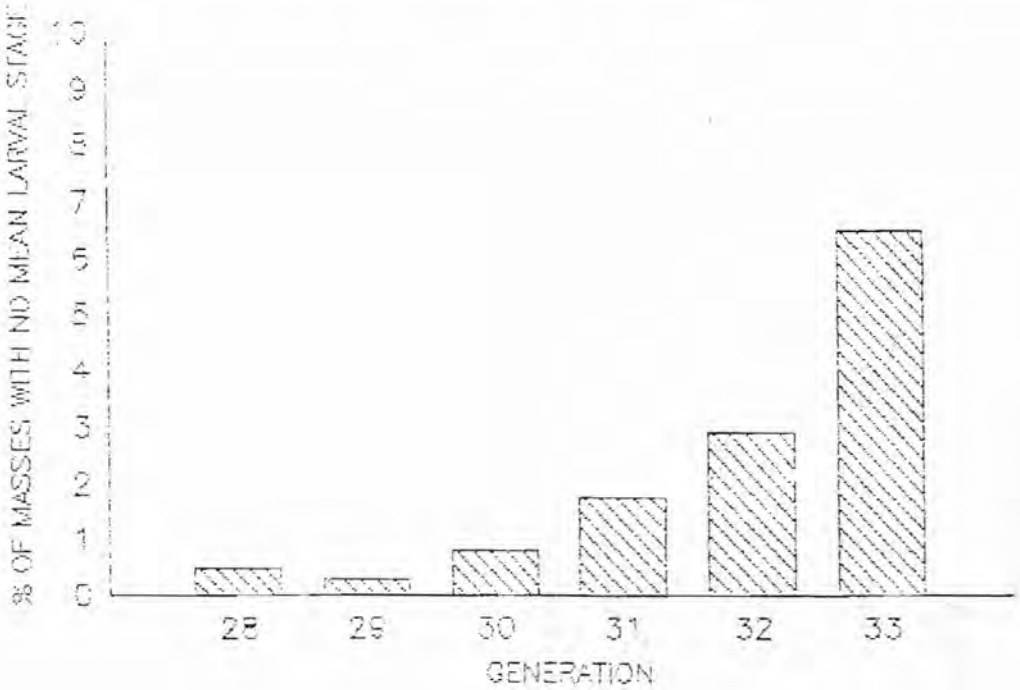


Figure 2. The % of New Jersey colony core samples that produced no mean larval stage due to non hatch of the eggs or death of all the neonates.



Project Number: JB 84.1.1
Project Title: Evaluation of Traps and Other Techniques for Controlling Japanese Beetles in and Around Airports and Nurseries
Report Period: October 1, 1987 - September 30, 1988
Report Type: Interim
Project Leaders: W. H. McLane, J. A. Finney and T. Ladd

Currently, nurserymen in the Northeast are unable to export nursery stock (other than bare root) to Canada because no effective regulatory treatment is available as a basis for certifying it free of Japanese beetle grubs. Nationally, the export of general landscape nursery stock to Canada has decreased from 18.3 million dollars in 1981 to 10.1 million dollars in 1986. A major decline occurred after 1983 when chlordane supplies were depleted. There is a desire on the part of Canadian distributors to buy nursery stock from American producers since many species are not economically grown in Canada due to the short growing season. Therefore, developing certification methods would be well received by Canadian and U. S. nurserymen.

A meeting was held on March 12, 1987 in Albany, New York, for the purpose of outlining a protocol for demonstrating that nurseries can be certified Japanese beetle-free based on management techniques. The crucial aspect of the approach involved maintaining the nursery in a weed-free condition such that acceptable plant hosts for grub development are unavailable. Additionally, incorporation of soil insecticide would be prescribed in the summer. It was the opinion of the experts discussing this approach that such a management practice would completely eliminate Japanese beetle grubs from the soil in the nursery, even when populations of Japanese beetles occur in the environs of the nursery. A work plan was drafted and presented to the Canadians for comment. A positive response was received and field demonstrations started in New York, New Jersey and Ohio with the objective of demonstrating by fall, 1987, that these techniques can eliminate the problem. If workable, this technique could be adapted to nursery operations in the Northeast and would be useful for growing balled and burlapped nursery stock for export to Canada.

During 1987, the technique was tested at 3 major nurseries in the Northeast. Diazinon and Isofenphos were the insecticides tested in combination with clean cultivation. Three dosages of each material were tested, each being applied at only one time interval during August. Each treatment was replicated four times with each replication representing 3 small (6x8 ft.) trees. During mid-September, all trees were removed and soil around each checked for Japanese beetle grubs.

Live grubs were found in each treatment series indicating that the treatments were less than 100 percent effective (see Annual Report, October 1, 1986 - September 30, 1987).

A good number of grubs were found deeper in the soil than anticipated (6-9 inches). Soil analysis indicated that nearly all residue was in the top 0-3 inches of soil. Grubs may have been well below the upper soil surface at time of treatment resulting in no contact being made.

The results of last year's cooperative efforts to eliminate Japanese beetle larvae from field-grown nursery stock indicated that considerable research yet

needs to be done before treatment protocols, based on research, can be recommended to the nursery industry. Therefore, a series of tests were recommended and conducted during 1988.

1. Sub-soil injection of insecticides: A sub-soil injector equipped with a digital-flowmeter was used to place measured amounts of different insecticidal solutions at depths up to 12 inches below the soil surface. The injector, connected to a 100 gallon sprayer (boom removed), required a tractor with a standard three-point hitch and power take-off. For preliminary tests, the injector was used to inject the soil surrounding infested trees originally used as buffers during 1987 tests. Insecticides were injected on April 5 and trees were examined for the presence of larvae during late May to early June.

2. Sub-soil injection of nematodes: Strains of soil-dwelling nematodes that have been shown to be highly pathogenic to Japanese beetles in turf were tested against larvae using the trees and procedures mentioned above.

3. Evaluation of registered insecticides: Four registered insecticides were evaluated against larvae during June-July. One to three applications were made during the season and the results of the treatment procedures determined during September-October.

4. Evaluation of slow-release chlorpyrifos: A formulation of chlorpyrifos (Dursban) that releases its toxicant over a period of several months was tested.

This work was conducted at Princeton Nurseries at their Allentown, New Jersey, site. This site has a substantial resident population of Japanese beetles. An area of about 1500 sq. ft. was used (3 blocks, 100 x 50 ft). Four hundred small trees (6-8 ft.) were planted in the test site.

Objective: To evaluate selected registered insecticides for their ability to eliminate Japanese beetle from field-grown nursery stock.

Background: Female Japanese beetles deposit their eggs in soil over a period of several months. In the northeastern part of the country, oviposition begins in late June and continues until late August to early September. In areas where nurseries are located, larvae are frequently found in close association with the roots of ornamental plants and trees. These larvae are found at levels considerably deeper than those in turf (50% may be found at 6-inch depths or lower). Hence, rescue or clean-up treatments using insecticides applied in late summer or fall are ineffective owing to the depths at which larvae are found. Amounts of surface-applied materials sufficient to kill third-stage larvae cannot penetrate 6-12 inches into the soil. Consequently, insecticides must be evaluated for their ability to establish barriers to larval survival. That is, they must be maintained in soil in amounts sufficient to kill larvae at time of hatching. Since currently available insecticides lack the residual qualities necessary to maintain an effective barrier for an entire season, sequential applications of insecticides, able to supply overlapping periods of toxicity in the soil during egg laying, should be evaluated.

Procedure: Four insecticides registered for control of Japanese beetle larvae

were evaluated: Chlorpyrifos (Dursban), Isofenphos (Oftanol), Bendiocarb and Trichlorfon (Dylox). All are organophosphate compounds. Trichlorfon is soluble in water; a characteristic which may aid in its effectiveness in nursery situations.

Candidate compounds were applied to the surface of soil surrounding nursery plants, worked-in, and watered-in. Individual plots consisted of two small trees spaced 3-4 feet apart within rows. Insecticides were applied to plots at rates indicated on labels for control of larvae in turf as well as at two and four times these amounts. One series of plots was treated with a single application in late June; a second series was treated in early June and about three weeks thereafter. A third series received the two earlier applications as well as a final application 3-4 weeks after the second.

Effectiveness of insecticide application was determined by digging up treated plants after the end of the adult flight season and examining plant roots for the presence of larvae.

This research was a cooperative effort between APHIS, ARS and Princeton Nurseries. Dr. Ladd of ARS was the lead scientist.

RESULTS

Subsoil injection of insecticides (Table 1): Three insecticides were injected as indicated into the soil surrounding field-grown nursery stock using an injector, equipped with a digital flowmeter, donated by Lesco, Inc. of Rocky River, Ohio. Regardless of volume injected or pattern of injection, none of the materials tested eliminated all larvae from soil surrounding the roots of the nursery stock. Saturation of soil surrounding the test plants, however, may not always have occurred since injected materials often surged to the surface of the ground instead of remaining below the soil surface.

Evaluation of registered insecticides (Table 2): Of the four insecticides listed in Table 2, only Bendiocarb completely eliminated larvae from soil surrounding the roots of the field-grown stock. The compound was effective following three applications at 4 lbs. AI/acre and after two and three applications of 8 lbs AI/acre. All insecticides in these tests were applied as broadcast sprays to the soil surfaces, lightly raked, and watered-in.

Evaluation of slow-release chlorpyrifos (Table 3): Dursban ME, a slow-release formulation of chlorpyrifos, did not eliminate Japanese beetle larvae from field-grown nursery stock. As with other surface-applied materials, Dursban ME was lightly raked into the soil and watered-in.

Sub-soil injection of nematodes (Table 4): The data in this table indicate that the injection of nematodes into the roots of field-grown nursery stock had no effect upon the survival of larvae there. 1988, however, was a year of severe drought, and a lack of soil moisture may have adversely affected the ability of the nematodes to survive and infect larvae in the plots.

From the results of research described, it seems evident that one insecticide, Bendiocarb, should be investigated more closely to determine if it can continue to eliminate larvae form field plantings. We intend to continue this research in 1989.

Table 1. Mean numbers of Japanese beetle larvae found in soil surrounding the trunks of small trees injected[a/] with aqueous preparations of insecticides[b/].

Insecticide (formulation)	Gallons/tree	Injection radius	Average number of grubs/tree[c/]
Chlorpyrifos (4EC)	2	3-in.	0.5e
Chlorpyrifos (4EC)	2	6-in.	0.5e
Chlorpyrifos (4EC)	3.6	3-in.	1.0c
Chlorpyrifos (4EC)	3.6	6-in.	0.75d
Isofenphos (2F)	2	3-in.	1.25b
Isofenphos (2F)	2	6-in.	0.75d
Isofenphos (2F)	3.6	3-in.	0.5e
Isofenphos (2f)	3.6	6-in.	0.5e
Bendiocarb (76% WP)	2	3-in.	0.75d
Bendiocarb (76% WP)	2	6-in.	1.0c
Bendiocarb (76% WP)	3.6	3-in.	0.5e
Bendiocarb (76% WP)	3.6	6-in.	1.25b
Control, water	2	3-in.	1.5a

a/ Aqueous preparations injected on all 4 sides of trunks at depths of 3, 6, and 9 inches. About 1/12th of indicated volumes (8.3%) injected at each locus.

b/ 1 lb. AI/100 gallons of water.

c/ Means followed by the same letter are not significantly different at the 5% level.

Table 2. Mean numbers of Japanese beetle larvae [a] found in plots (2 trees per plot) at the Princeton Nurseries, Allentown, New Jersey, treated with successive applications of insecticides during the summer of 1988[b].

Insecticide	Lbs AI/acre	No. of applications 1	2	3
Chlorpyrifos 4E	4	3.0b	6.5a	3.5abc
Chlorpyrifos 4E	8	4.3b	4.5ab	5.3abc
Isofenphos 2F	2	13.3a	3.5ab	5.8a
Isofenphos 2F	4	5.3b	4.8ab	5.5ab
Bendiocarb 76% WP	4	3.0b	3.0ab	0.0c
Bendiocarb 76% WP	8	3.3b	0.0b	0.0c
Trichlorfon 80 SP	8	8.0ab	4.8ab	1.8abc
Trichlorfon 80 SP	16	7.0ab	6.3ab	0.3bc
Control		6.0ab	9.0a	5.5ab

a/ Means followed by the same letter are not significantly different at the 5% level.

b/ 1st application, June 28; 2nd application, July 19; 3rd application, August 9. Plots examined September 13-14

Table 3. Mean numbers of Japanese beetle larvae found on September 13, 1988, in plots (2 trees per plot) at the Princeton Nurseries, Allentown, New Jersey, that had been treated on June 28, 1988, with Dursban ME, a slow-release formulation of Chlorpyrifos.

Lbs AI Dursban ME per acre	Mean no. grubs/plot
4	4.8a
8	3.5
(Control) 0	8.5

Table 4. Average number of Japanese beetle larvae per tree, six weeks after application of nematodes to plots on April 5, 1988.

Treatment	No. larvae[a/]
Heterorhabditis HP88-injected	3.75ab
Heterorhabditis HP88-surface	.50 b
Steinernema feltiae - injected	7.25a
Steinernema feltiae - surface	2.50 b
Control	1.50 b

a/ Means followed by the same letter are not significantly different P=.05.

Project Number: RWA 88.1.1
Project Subtitle: Russian Wheat Aphid Biological Control Survey
Report Period: October 1, 1987 - September 30, 1988
Report Type: Preliminary
Project Leader: William C. Kauffman, compiled jointly with K. O. Bell

USDA-APHIS undertook in 1988 a new initiative in classical biocontrol of the Russian wheat aphid (RWA), Diuraphis noxia, through foreign exploration for natural enemies. Several exotic species of parasitoids and predators have already been introduced through quarantine and field released as a result of these cooperative efforts of USDA-APHIS, the CIBC, British Museum, USDA-ARS and university/state scientists. Selected beneficial species which show promise for RWA suppression are now being cultured by two USDA-APHIS Biological Control Laboratories for mass rearing in support of field releases.

It is anticipated that establishment and colonization of these introduced species will augment present levels of parasitism and predation of aphid populations in small grains. This enhancement of existing natural enemies is hoped to lower the relative population densities of RWA throughout its geographic range in the United States. Therefore, a region-wide field survey of RWA densities and natural enemies associated with RWA in small grains is needed in order to measure the agricultural benefits of this investment in biological control. This concept of a systematic survey coordinated by APHIS has been enthusiastically supported by states involved, and the methodology has been refined following preliminary testing in a few selected states in 1988.

The objective of this regional biocontrol survey is:

Provide information on RWA and natural enemies before and after releases of exotic natural enemies and to assess the impact of these releases on RWA and other aphids in small grains. Specifically, the survey will:

a) Identify the species and abundance of natural enemies, both indigenous and exotic, that exist in RWA-infested fields.
b) Estimate relative population densities of RWA and co-habiting aphids in small grain fields infested with RWA.

Aphid and beneficial arthropod data will be gathered by a combination of chemical extraction and visual census of plant tillers and by sweep netting. These varied sampling techniques will minimize time spent in the field and increase accuracy of the survey. Sorting and species identification of collected specimens will be handled by APHIS laboratories. Instructional materials and collecting equipment will be supplied as needed by the APHIS Otis Methods Development Center. The survey will, ideally, involve 4-15 fields in all RWA states (presently 15) through the growing season. These fields will be selected based on proximity to field releases of natural enemies and, when possible, should be in conjunction with fields used in the suction trap network. USDA, APHIS had hoped to initiate this comprehensive survey in 1989. However, the complexity of the activities and the need for significant back-up lab support to process samples and some uncertainty as to parasite taxonomy, prompts us to defer large-scale implementation of this survey until 1990. Thus, any field activities in 1989 will be directed towards perfecting the methods that will be used in the survey. The outlook for adequate funding in 1990 to perform this important survey is very good.

A. FIELD SELECTION

1. The survey coordinator in each state should select wheat fields with moderate to heavy infestations of RWA for this multi-state survey. Within a "generally infested" area, choose 2 to 5 fields within a 10-20 mile radius; this constitutes one geographic location. Fields in a geographic location must be at least 25-50 miles from releases of exotic natural enemies prior to and during the first two years of the survey (1990-1991), thus providing baseline data. Niles Biocontrol Laboratory is the current repository for these release records. Releases of beneficial species (coordinated by APHIS) will be conducted in the vicinity of these survey fields so to create an "after release" situation for comparison.

2. When possible, repeat this field selection in 2 to 3 geographic locations which are at least 50 miles apart and preferably on a north-south gradient throughout the state.

3. Winter wheat fields should be selected whenever possible, however, spring wheat may be substituted if it is the primary wheat crop in the state. The criterion for candidate fields should be an existing population of RWA in that field (fall or early spring determination). Such fields may be accompanied by the typical RWA symptoms (curling, pink/purple leaf blotching or streaking often bordered by white). Select fields of growers who are less likely to apply pesticides for RWA control. Field statistics, pesticide usage and the occurrence of grazing by cattle should be recorded on page one of the survey forms.

4. Selected fields are to be surveyed as follows:

   1) every two weeks throughout the growing season, weather permitting, from tillering through harvest,
   2) between 8:00 AM and 12 noon,
   3) when the temperature is above 45 degrees F (exceptions in winter time),
   4) at least 2 days following a heavy rain, and
   5) after the prescribed waiting period following pesticide application.

B. HOW TO SURVEY WITHIN THE FIELD

1. Select 10 sampling sites arranged diagonally in the field. Walk 10 paces into the field for the first site, 10 paces further for the second site, etc., for a total of 10 sites. The sites selected in the field should differ from week to week.

2. At each site, take the following four distinct types of samples in the sequence indicated for insect and crop data.

a. Tillers for Chemical Extraction

(Aphids, larval and adult predators)

Using a scissors, cut at the base 10 tillers (= stems originating at crown of plant) at random within a 5 foot radius. After cutting the first tiller, immediately transfer it to a 1 gallon plastic ziplock bag in such a way that disturbance and escape of insects is minimized. For example, tillers longer that 12 inches can be inserted into the bag while cutting off the tiller. Follow this procedure until all 10 tillers are collected in the bag, then affix a gummed label (label A 1-10, indicating date, field, field site, etc.). Accumulate these 10 plastic bags (1 per site) in a large paper bag, then place the bag into an ice chest to prevent overheating until processing.

Insects should be extracted as soon as possible at the field edge after all sampling (#2a-d) is completed. If extraction is not possible at the field because of inclement conditions (i.e., windy, precipitation, etc.), this process should be done immediately back at laboratory/station/office. Use the chemical extraction can (specified below) in an open, well-ventilated location protected from the wind or under an exhaust hood. Care should be taken to prevent inhalation of this chemical or contact with one's skin.

Chemical Extraction Can - Apparatus and Methodology:

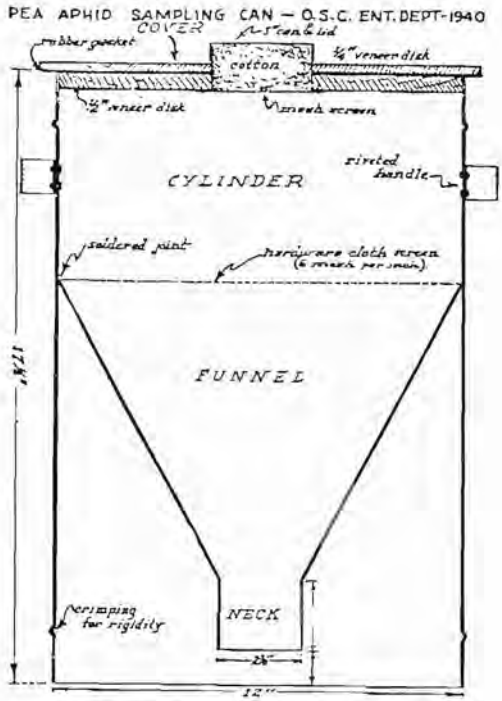


FIG. 2.—Diagram of can showing details of construction

Reference:

Gray, K. W., and J. Schuh 1941. J. Econ. Entomol. 34:411-415

These extraction cans will be constructed as units consisting of 10 extraction cans (i.e., need one 10-can unit to sample a field) and will be supplied by Otis Methods Development upon request from participating states.

Procedure for extracting arthropods:

1) Saturate the cotton wicks located in extraction can lids with 4-Methyl-2-Pentanone ($CH_3COCH_2CH_2$; Methyl isobutyl ketone; a flammable, toxic irritant). Attach glass collection vials containing 70% ethanol (EtOH) to the bottom of the funnel.

2) Dump wheat tillers and their contents from each field site into an extraction can and replace the can lid. Transfer "A" labels from ziplock bags to corresponding vials.

3) After 5 minutes, gently shake each can 10 times so that the most active insects fall into the alcohol vial. Remove the lid, then proceed to examine tillers for mummified and diseased aphids, as directed in part b below.

4) Now replace lids and vigorously shake the cans 50 times to complete the dislodgement of aphids and predators.

5) Species and abundances of RWA and other aphids, small predators and larval stages of predators (especially coccinellids) will be determined at the Niles Biocontrol Laboratory.

b. Tillers for Removal of Mummies and Diseased Aphids

Carefully examine (one by one) each of the 10 tillers used in the chemical extraction process (above) in search of mummified or diseased aphids. These aphids are uncharacteristically brown or black, are immobile and remain attached to the leaves. Visually search the tillers, unrolling any damaged by RWA, directly over the open extraction can so to catch any insects which drop form the tiller. Cut away the leaf from around the mummy or diseased aphid with a small dissecting scissors and place mummies into gelatin capsules (preferably #00, 0 or smaller) with one mummy or diseased aphid per gelatin capsule. Combine all gelatin capsules from a particular site in the field into a labeled container (label B 1-10) held at room temperature. This visual examination may be done at the field or immediately upon returning to the laboratory, so as to preempt emergence of adult parasitic species. From these collections, rates of parasitism and species of parasitoids (i.e., parasitic wasps) as well as insect pathogens will be determined for aphid hosts in the sample (Nile Laboratory).

c. Sweep Net Sample

At each field site in areas not disturbed by tiller sampling,

take one sweep net sample consisting of 5 sweeps (180° sweep arc) with a standard 15-inch muslin sweep net. Empty the sweep net contents into a 1 gallon plastic ziplock bag (label C 1-10). Transport bags from the field in an ice chest. Immediately freeze bags, then transfer the contents of each bag to a glass vial containing 70% ethanol (again label C 1-10). The target insects in this sample are primarily lady beetle adults and larvae, but also nabids, lacewings, nabids, etc.

d. Plant Stage and Tiller Density

At each of the 10 sites in the field, use a throwstick to randomly choose a 1-foot section of row and record the following data:

1) Count the number of tillers in the 1-foot row sample, then use Appendix A to calculate number of tillers on a per sq. ft. basis.

2) Record the overall stage of plant development in the 1-foot row sample using the Zadok's scale (Appendix B).

Combine the following samples from a single field in a sealed shipping container:

- 10 chemical extraction can samples (labels A 1-10)
- 10 sets of mummies and diseased aphids enclosed in gelatin capsules (labels B 1-10)
- 10 sweep net samples (labels C 1-10)
  (Note that A-C represents sample type and 1-10 represents field site)

and send to:

Robert V. Flanders
USDA, APHIS, PPQ
Biological Control Laboratory
2534 South 11th Street
Niles, MI 49120
(616) 683-3563, (FTS) 333-8212

Insect counts and species identification data compiled at the Niles Laboratory will be sent by Niles to the individual state as soon as possible. It is then the responsibility of each state to enter this sampling data into the NAPIS database promptly upon receiving the laboratory diagnostic report from the Niles Laboratory.

The Niles Laboratory will also send a photocopy of this diagnostic report for survey analysis to:

Bill Kauffman
USDA, APHIS, SCI & TECH
Otis Methods Development Center
Building 1398
Otis ANGB, MA 02542
(508) 563-9303 (FTS) 828-9355

Coordination for this RWA Biological Control Survey will be a cooperative effort between Otis Methods Development Center and Niles Biocontrol Laboratory.

APPENDIX A: CALCULATING TILLERS PER SQ. FOOT FROM TILLERS IN A 1 FOOT SECTION OF ROW AND THE ROW WIDTH.

Multiply the appropriate factor (corresponding to row sparing) times the average number of tillers counted at each of 10 sites. Record these tiller densities in the space provided on the forms.

Row spacing, inches	Multiplication factor
2	6.0
4	3.0
6	2.0
8	1.33
10	1.2
12	1.0
14	0.86
16	0.75
18	0.67
20	0.60

APPENDIX B: ZADOK'S ONE DIGIT CODE FOR PRINCIPLE GROWTH STAGES OF CEREALS

Code	Growth Stage Description	
(0	Germination)	Stages prior to data recording.
(1	Seedling growth)	
2	Tillering	
3	Stem elongation, including flag leaf appearance	
4	Booting	
5	Inflorescence emergence	
6	Anthesis (blooming)	
7	Milk development	
8	Dough development	
9	Ripening	

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