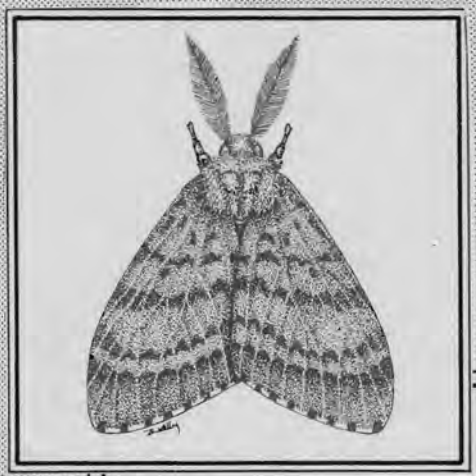


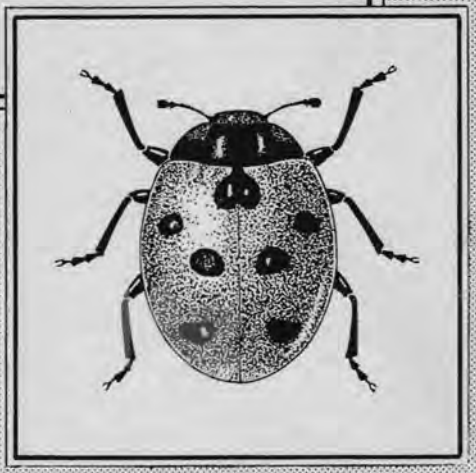
1987

United States Department of Agriculture

Otis Methods Development Center

Progress Report

Animal and Plant Health Inspection Service



October 1, 1986 - September 30, 1987

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

October 1, 1986 - September 30, 1987

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

Jessie J. Baker	Supervisory Biological Technician
Joyce A. Finney	Biological Technician
Othel T. Forrester	Entomologist
Billy G. Fulp	Electrician
Richard W. Hansen	Research Assistant
William C. Kauffman	Research Assistant
Philip C. Kingsley	Entomologist
Victor C. Mastro	Entomologist
Winfred H. McLane	Pesticide and Application Technology Section Leader
Edward C. Paszek	Agriculturalist
Charles P. Schwalbe	Center Director
John A. Tanner	Entomologist
Joseph G. R. Tardif	Equipment Specialist
Alida Pellegrini-Toole	Research Assistant
Rebecca Upton	Secretary
Marlene Vermette	Secretary

Project Number	Project Title	Project Leader	Report Type	Page
ABC 6.1.1	Artificial Diets for Predatory Coccinellids	Forrester	Interim	1
ABC 7.1.1	Laboratory Study of Aphid Suitability for *Coccinella septempunctata* Larvae and Adults	Kauffman	Preliminary	5
ABC 7.1.2	Field Determination of Standard Predation Levels for *Coccinella septempunctata* in Selected Crops; Cooperative State Agreement	Kauffman		12
ABC 7.1.3	Field Suppression of Pea Aphid and Cereal Aphids by the Predator, *Coccinella septempunctata*	Kauffman		20
ABC 7.1.4	Evaluation of *Coccinella septempunctata* Efficacy as an Aphid-Suppressive Agent in Selected Agroecosystems	Kauffman	Preliminary	26
AW 1.1.1	Evaluation of the Alfalfa Weevil Parasite Redistribution Program	Kingsley	Interim	27
BT 7.1.1	Development of Browntail Moth Rearing Techniques (Or How to Learn to Stop Worrying and Love the Browntail)	P.-Toole	Interim	33
CPB 5.1.1	Rearing *Edovum puttleri* an egg parasite of *Leptinotarsa decemlineata*	Kingsley	Final	34
CPB 6.1.1	Development of an Artificial Diet for Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say)	Forrester	Interim	40
GM 5.1.1	1987 Field Trials with Early Dimilin Applications in West Virginia	McLane	Final	43
GM 5.1.2	1987 Field Trials with *Bacillus thuringiensis* BT	McLane	Final	56
GM 8.1.3	Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth	McLane	Interim	61

Project Number	Project Title	Project Leader	Report Type	Page
GM 5.2.1	Comparison of 1987 Disparlure with Various Concentrations of Lure	Paszek	Interim	86
GM 5.2.4	Sterile F_1 Demonstration Project - Darke Co., Ohio	Mastro	Interim	88
GM 5.2.3	Sterile F_1 Demonstration Project - Bellingham, Washington	Mastro	Interim	89
GM 6.2.1	Sterile F_1 Demonstration Project - Southern Shores, Dare Co., North Carolina	Mastro	Interim	91
GM 6.2.2	Sterile Male F_1 Demonstration Project - Allen Co., Ohio	Mastro	Interim	100
GM 6.2.3	Sterile F_1 Demonstration Project, Manor, WA	Mastro	Interim	103
GM 6.2.4	Sterile F_1 Demonstration Project - Portland, Oregon	Mastro	Final	109
GM 7.2.1	Comparative Location Patterns of Late-stadium Gypsy Moths	Hansen	Interim	111
GM 7.2.2	Sterile Male Demonstration Project, Gates Co., North Carolina	Mastro	Interim	118
GM 7.2.3	Competitiveness of Gypsy Moth Adults	Mastro	Interim	126
GM 7.2.4	Disparlure Dispenser Comparison Aging Study - 1987	Paszek	Interim	140
GM 7.2.5	Disparlure Dispenser Comparison - 1987	paszek	Interim	144
GM 7.2.6	Trap Design Comparison	Interim	Interim	145
GM 1.3.1	Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility	Tanner	Interim	146

Project Number	Project Title	Project Leader	Report Type	Page
GM 3.3.1	Development and Evaluation of Improved Rearing Techniques	Tanner	Interim	153
JB 4.1.1	Evaluation of Traps and Other Techniques for Controlling Japanese Beetles in and Around Airports and Nurseries	McLane	Interim	162

Project Number: ABC 6.1.1
Project Title: Artificial Diets for Predatory Coccinellids
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leader: O. T. Forrester

Twenty-three artificial diets were formulated and tested using the exotic Coccinellidae Hippodameia variegata (HV) and Propylea quatuordecempunctata (P14). Three of these diets produced weight gain and oviposition in teneral females (Table 1). In tests with HV, significant differences were obtained with the controls Acyrthosiphum pisum (Pea aphid), Schizaphis gramina (greenbug) and artificial diet type III and I (Table 2). First oviposition occurred in 5 days when fed pea aphids and in 6 days when fed greenbug or diet type I or III.

Table 1. Diets which resulted in weight gain and oviposition when fed to HV and P14.

Ingredient	1	2	3
Homogenized Raw Pork Liver	60gm	60gm	60gm
Sucrose	40gm	24gm	40gm
Wheast	5gm	-	5gm
Pollen substitute	5gm	-	-
Glycerol	5ml	-	5ml
Olive Oil	5ml	-	5ml
Yeast Extract	-	-	5ml
Water	5ml	-	5ml
Vanderzant Vitamin Mix	-	-	.5
Palm Oil		-	-

Table 2. Effect of diet on weight gain and fecundity of teneral female HV.

Diet	n	Mean weight gain (mg)	Mean no. eggs/day (21 days)
Pea aphid	5	5.54 a	36.70 a
Greenbug	4	4.03 b	27.65 b
Type III	5	2.65 c	3.65 c
Type I	4	2.02 c	3.62 c

Means within columns followed by the same letter are not significantly different (P> 0.05 Duncan's Multiple Range Test).

HV were maintained on artificial diets I and III for 29 days. On days 30 and 32 the diets were supplemented with pea aphids. The mean number of eggs per day on type I diet increased from 3.6 to 15.4 eggs and on type III increased from 3.7 to 12.9. These results suggest that the artificial diets are sufficient for post storage feeding of HV and could reduce aphid feeding to 1 or 2 days before release.

In tests with P14, fecundity was significantly higher on the greenbug control than on diet type I or II. There were no significant differences in weight gain (Table 3).

Table 3. Effect of diet on weight gain and fecundity of teneral female P14.

Diet	n	Mean weight gain (mg)	Mean no. eggs/day/female (21 days)
greenbug	5	4.14 a	12.64 a
Type II	5	3.60 a	4.25 b
Type I	4	3.44 a	4.48 b

Means within columns followed by the same letter are not significantly different (P> 0.05 Duncan's Multiple Range Test).

First oviposition occurred after 5 days when fed greenbugs and after 6 days when fed diet type I or II. Coccinella septempunctata (C7) field collected as pupae and eclosed in the laboratory were used to evaluate the effect of diet on weight gain. There was no significant difference in weight gain between greenbug or pea aphid. Weight gain on artificial diet was significantly lower than on aphids (Table 4).

Table 4. Effect of diet on weight gain in C7 females.

Diet	n	Mean weight gain (mg)
Pea aphid	10	27.79 a
Greenbug	10	26.59 a
Type II	9	19.18 b

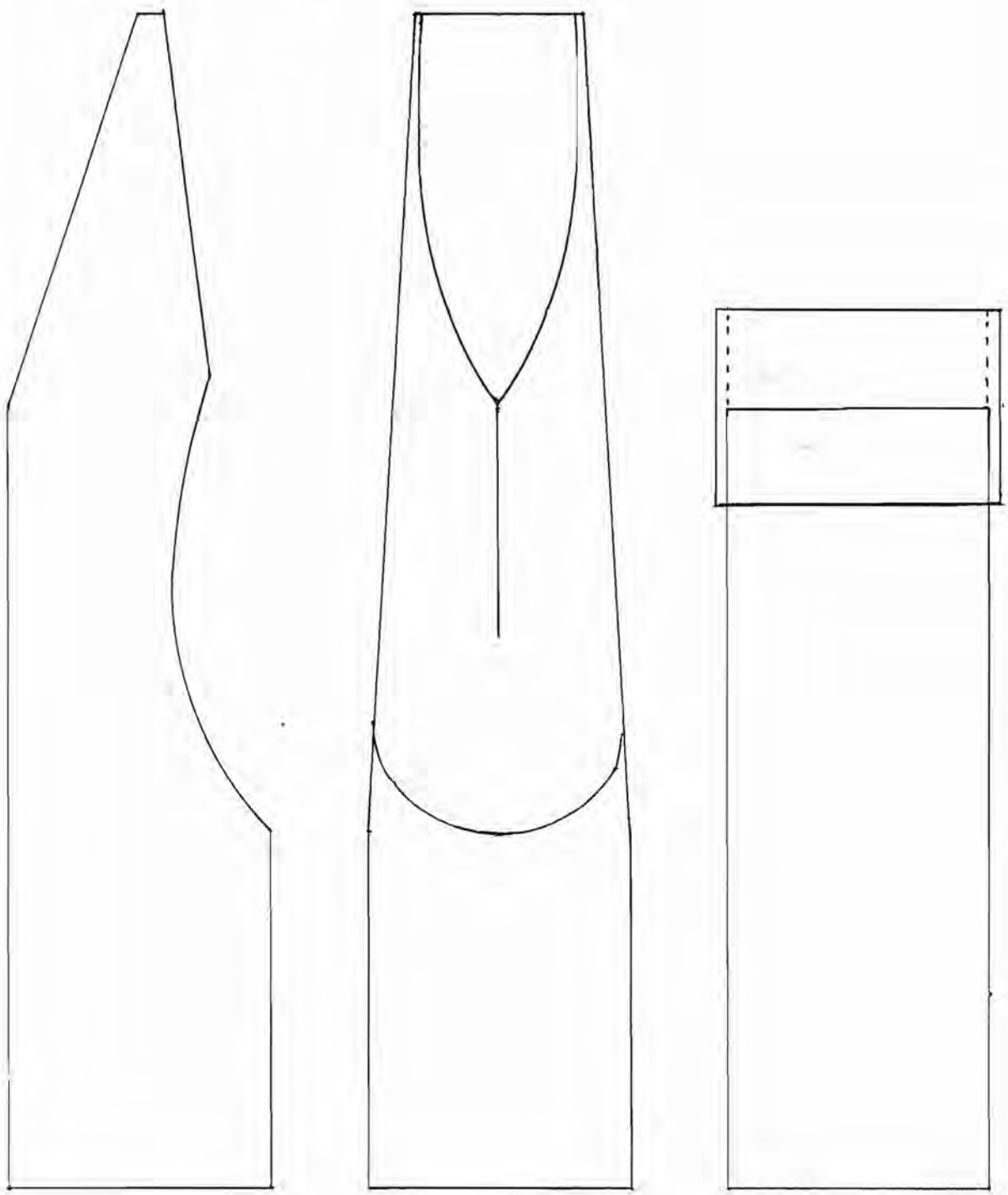
Means within columns followed by the same letter are not significantly different (P> 0.05 Duncan's Multiple Range Test).

A method of collecting greenbugs from flats of sorghum or barley using a vacuum has been developed. The device is made of 2-inch OD PCV central vacuum pipe and a 12-inch square of nylon organdy fashioned into an aphid collection bag (Figure 1). This is an improvement over the previous method where the plants were cut at the soil surface and the aphids were dislodged by shaking and brushing through soil screens or tumbling through a motorized screen drum. The vacuum collection does not damage the plant so aphids can be harvested two or more times. This collection method could reduce the cost of producing aphids by at least 50%.

Progress was also made in coccinellid egg harvest. The strips of black plastic trash bags used last year have been replaced by surveyor flagging ribbon. The embossed ribbon provides a better surface for egg attachment.

Figure 1.

SCALE: 1 square = 1/4 inch



Project Number: ABC 7.1.1
Project Subtitle: Laboratory Study of Aphid Suitability for Coccinella septempunctata Larvae and Adults
Report Period: October 1, 1986 - September 30, 1987
Report Type: Preliminary
Project Leader: William C. Kauffman, assisted by Cathy Paris and Paul Iorio

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

Rationale: 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" 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 ensure that C7 individuals will not feed exclusively on one aphid species. Consequently, the past history and 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 aphids is warranted. Such laboratory life table data, if available, would enable one to predict 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. Such knowledge would also greatly improve our present mass-rearing potentials for C7.

Materials and Methods: 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 (i.e., species of aphids) were repeated over time and were conducted under constant laboratory conditions of 26°C (± 1) 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 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 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 one larva 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 (black plastic, 6 cm high x 3-4.5 cm dia) 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 with eggs were held to determine egg viability and time to hatch.

Results: When presented with four types of aphids for predation, C7 fed greenbug belonged to the group with the slowest development of eggs and larvae, whereas these C7 differences among the remaining aphid prey were not significant (Fig. 1). It should be noted that length of egg stage was recorded from the adults resulting from, rather than preceding, larvae reared on that particular aphid. Time from egg hatch to adult eclosion was longer for greenbug-reared larvae than for larvae fed any of the remaining aphids (Table 1). Pupal weight, which is a good indicator of nutritional status of larvae, was highest with pea aphids and lowest with greenbug. Greatest survivorship of C7 immatures through the pupal stage was evident when consuming pea aphids.

In the adult generation, the highest percentage of fecund females occurred when larvae and adults consumed pea aphids (100%), whereas only four of nine females associated with greenbug were fecund (Table 2). Fecundity as measured by total oviposition over 25 days and by daily egg production was highest with pea aphid, intermediate with oat bird cherry and corn leaf aphids, and lowest with greenbug. Plots of daily oviposition schedules of C7 indicated the highest, sustained oviposition by those adults fed pea aphid and a prolonged pre-oviposition interval for greenbug-fed females (Fig.2). Egg hatch with pea aphid and corn leaf aphid prey was higher than with greenbug and oat bird cherry aphid (Table 2). This suggests that the national level derived from predation of various species of aphids results in differential

egg quality in addition to altered oviposition levels. Egg hatch is presumably affected by the volume and quantity of yolk available to the developing embryo.

These laboratory life table studies of C7 larvae and adults, although preliminary, clearly demonstrate that pea aphid and greenbug were, respectively, the most and least nutritionally suitable prey. These results support the hypothesis that past history and current status of aphid prey affect fitness of C7 populations and, therefore, should be considered when utilizing field populations and laboratory colonies for research purposes. Based on these biological data, pea aphid is the preferred aphid for mass-rearing C7.

These experiments will be further replicated and then analyzed by life table parameters including intrinsic rate of increase (r_m), net reproductive rate (R_o), and generation time (t). Such calculations will permit improved quantitative comparisons of aphid species as food resources for C7. In addition, a future experiment will examine the effect of switching the most and least suitable prey species (pea aphid and greenbug, respectively) between the larval and adult generations. The anticipated results will clarify the contributions of both larval and adult nutrition to the ultimate fitness of C7.

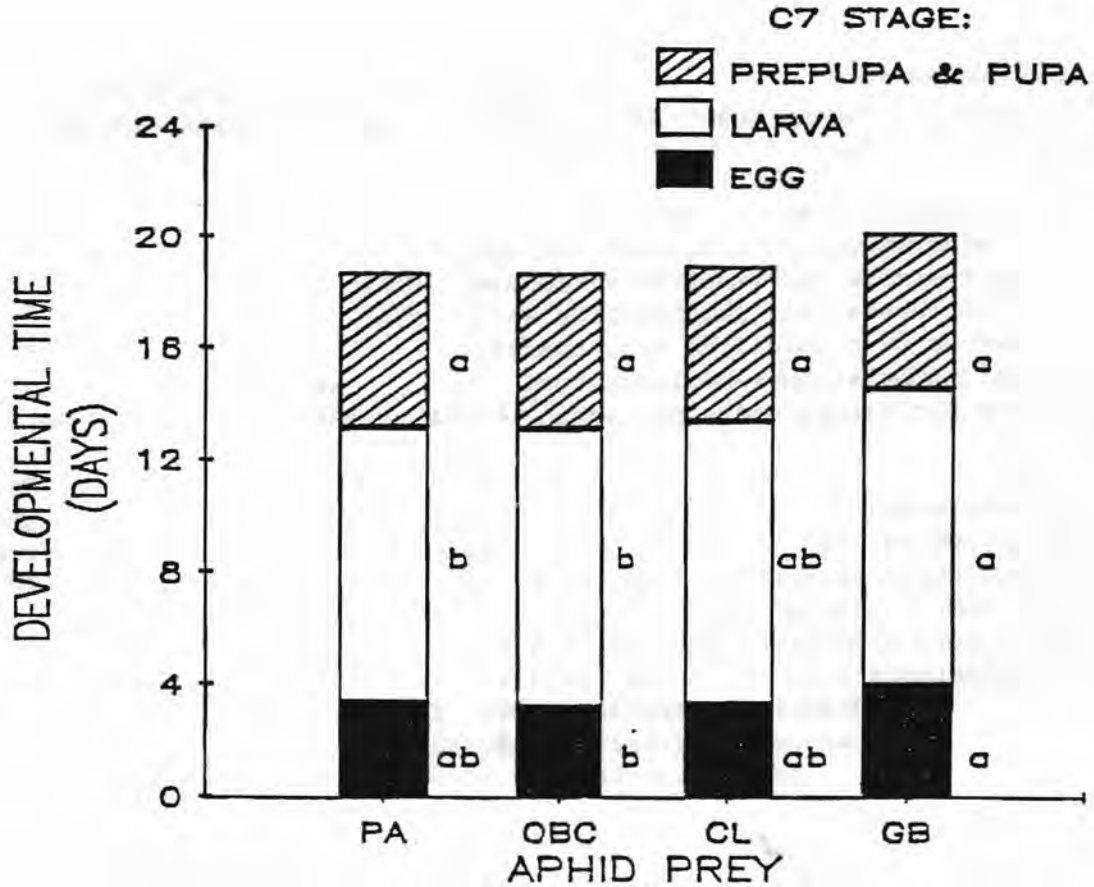


Fig. 1. Length of egg, larval, and prepupal/pupal stages of C7 predators as mediated by aphid prey; horizontal separation of means within each life stage of C7 (ANOVA, $P \leq 0.05$, Duncan's multiple range test).

Table 1. Developmental parameters of C7 larvae associated with four aphid species as prey.[a]

Aphid Prey	No. of larvae (n)	Time (days) from neonate to adult	Pupal weight (mg)	Mortality
Pea Aphid	42	15.4 b	42.8a	9.5
Oat Bird Cherry Aphid	42	15.5 b	36.1 bc	24.3
Corn Leaf Aphid	37	15.7 b	36.7 b	21.4
Greenbug	42	16.2a	33.7 c	14.3

[a]ANOVA (SAS GLM) and vertical separation of means ($P \leq 0.05$, Duncan's); mortality is expressed as a percentage of the total rather than an average.

Table 2. Reproductive parameters of C7 adults fed four aphid species.

Aphid Prey	No. of females (n)	% fecund	Mean[1] Total eggs	Eggs/ day	% egg hatch
Pea Aphid	9	100	669.0a	25.2a	58.9a
Oat Bird Cherry Aphid	10	60	428.8 b	12.4 b	34.0 b
Corn Leaf Aphid	10	60	423.0 b	11.3 b	47.1a
Greenbug	9	44	197.3 c	3.2 c	30.9 b

[1]ANOVA (SAS GLM) and vertical separation of means ($P \leq 0.05$, Duncan's); percentage data transformed by arcsine proportion prior to analysis.

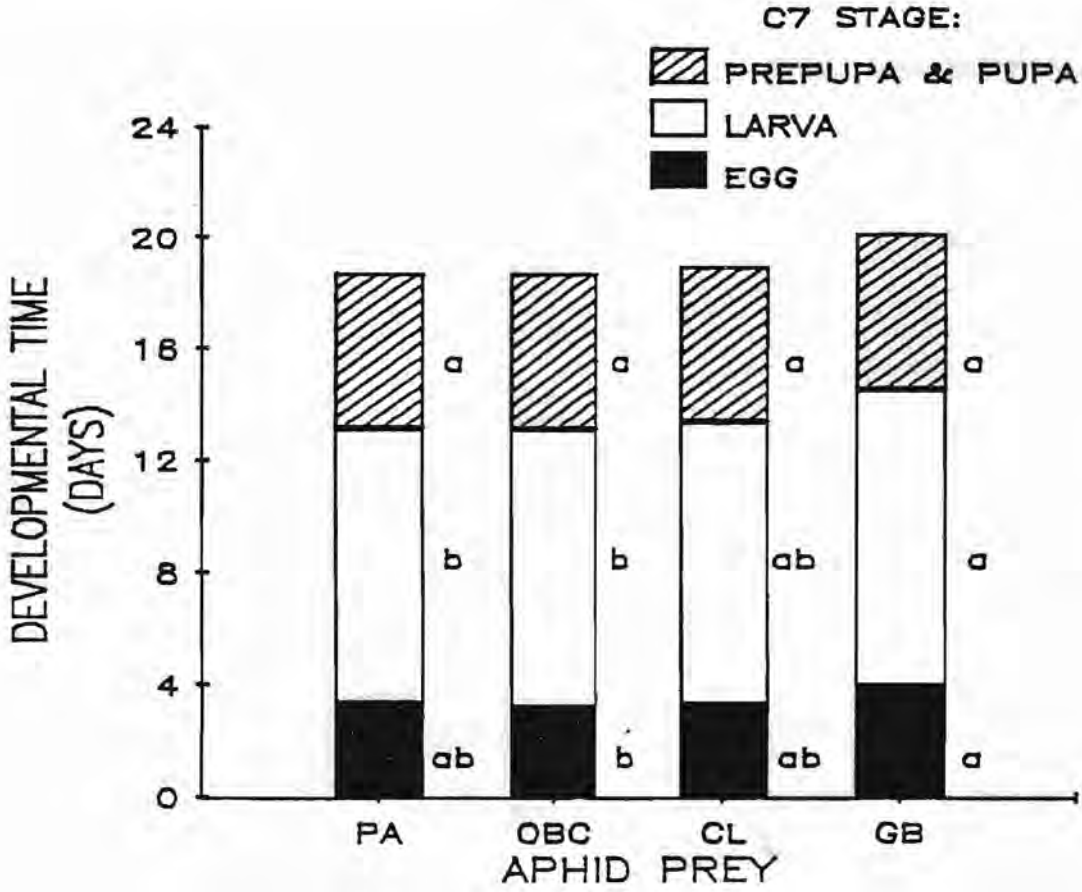


Fig. 1. Length of egg, larval, and prepupal/pupal stages of C7 predators as mediated by aphid prey; horizontal separation of means within each life stage of C7 (ANOVA, $P \leq 0.05$, Duncan's multiple range test).

Table 1. Developmental parameters of C7 larvae associated with four aphid species as prey.[a]

Aphid Prey	No. of larvae (n)	Time (days) from neonate to adult	Pupal weight (mg)	Mortality
Pea Aphid	42	15.4 b	42.8a	9.5
Oat Bird Cherry Aphid	42	15.5 b	36.1 bc	24.3
Corn Leaf Aphid	37	15.7 b	36.7 b	21.4
Greenbug	42	16.2a	33.7 c	14.3

[a]ANOVA (SAS GLM) and vertical separation of means ($P \leq 0.05$, Duncan's); mortality is expressed as a percentage of the total rather than an average.

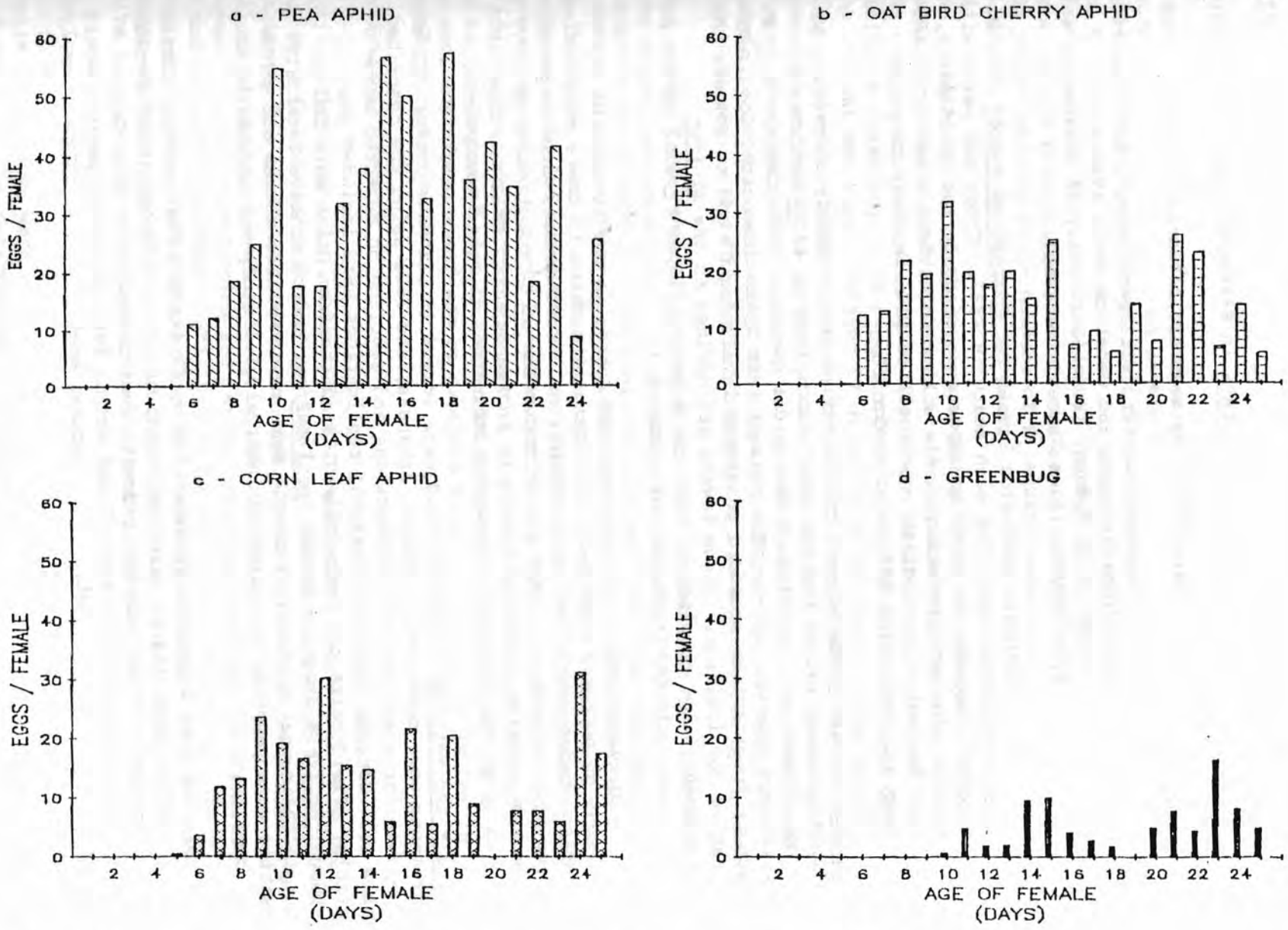


Figure 2. Oviposition schedule of C. septempunctata on several aphid prey.

Project Number: ABC 7.1.2
Project Subtitle: Field Determination of Standard Predation Levels for Coccinella septempunctata in Selected Crops; Cooperative State Agreement.
Report Period: October 1, 1986 - September 30, 1987
Report Type: Preliminary
Project Leaders: William C. Kauffman (Project Coordinator), in conjunction with Frank Gilstrap and Gerald Michels (Texas A&M University), Timothy Kring (University of Arkansas), and John Obrycki (Iowa State University).

Objective: To set standard predation levels for C. septumpunctata (C7) in field cages in selected crops for each state in FY87. Crops and pests will be chosen by state cooperators based on importance of crops and aphid pests. When possible, standard predation levels will also be determined (FY87 and thereafter) for native predominant coccinellids in each state to allow comparisons with predation efficiencies for C7.

Rationale: Stable predator:prey relationships are generally characterized by oscillating equilibria of the two populations (see p. 43 In Huffaker & Messenger, Theory and Practice of Biological Control, 1976; Chapter 5 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 native coccinellids. 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 will determine whether the introduction of C7 complements or antagonizes existing predation levels by indigenous coccinellids, thus providing positive, neutral or negative influences in aphid management.

Materials and Methods: Similar field cage (6x6x6 ft., lumite screen; 10/site) experiments were conducted in Texas, Arkansas, and Iowa using the described techniques for aphid and C7 introductions and sampling on selected crops (Table 1). Control cages and seasonal replications were included when possible. Field collected overwintered C7 adults from Niles were fed on greenbug for 12-14 days (to remove Perilitus sp. and to achieve fecundity) prior to their use in the experiment. Numbers of C7 life stages and aphid densities were recorded at indicated intervals. A study was terminated in a given cage when:

1) aphid densities increased uncontrolled by C7 (termed "escape equilibrium" by Price, 1984) resulting in plant collapse, or 2) aphid densities decreased below detectable levels, presumably due to over-predation by C7. Cages exhibiting cyclic oscillations of C7 and aphids for prolonged periods would best typify successful regulation by a natural enemy.

Results and Discussion:

From Texas (Gilstrap, Michels):

Two cages (1 and 5) exhibited the desired GB/C7 stable equilibrium as predator:prey oscillations which continued for ca. 11 weeks (Figures 1 and 2). The primary reason for this equilibrium was an initial, high oviposition rate during the first week which produced large numbers of larvae within 3-7 days of C7 introduction (Figures 1b and 2b). Predation by larvae and adults appears to have been responsible for the rapid decline of GB in the two early-season peaks, whereas large numbers of F_1 adults were associated with the total demise of the late-season GB peak.

On the contrary, eight of the ten cages exemplified an "escape equilibrium" where GB increased rapidly, followed by C7 increase, then an abrupt crash of aphids and no oscillations (Figure 3). Although C7 slightly reduced GB densities in these cages, GB reproduction was significantly reduced because of a lack of host material. Delayed C7 oviposition and larval development enabled rapid early-season increase of GB. This aphid buildup unrestrained by predation exceeded the sorghum plants' thresholds for herbivory and they succumbed in approximately four weeks. Feeding by C7 larvae was sufficient for their successful development to pupae and adults, however emergence of F_1 adults occurred too late to protect these plants from excessive aphid damage.

In all cages C7 was observed congregating, or "basking", in the upper corners of the cages, particularly in the evenings and at night. Adults usually returned to the plants by 10:00 a.m. It is significant that a higher percentage of eggs and larvae were recorded on plants, rather than on the cage, in cages 1 and 5 than in the eight cages where predator:prey oscillations did not occur (Table 2). Egg placement on cage screening may have contributed to egg and larval mortality via cannibalism by sibling larvae and adults, thus reducing the potential for C7 populations to regulate aphids. This higher degree of cannibalism would appear to be an artifact of the field cage and, therefore, would not be expected to be a significant factor in natural populations.

From Arkansas (Kring):

Sampling efficiency (i.e., recovery) of C7 adults was calculated to be ca. 60%. Very few of the recovered beetles (ca. 10%) were observed actually foraging on the plants, whereas more than 50% were "basking" on the walls or ceilings of the cage, particularly in the cage folds and corners. Consequently, C7 reproduction and larval densities on plants were extremely low and did not contribute significantly to regulation of aphids. The slight reductions in prey rates of increase by C7 were insufficient in all cages to prevent excessive aphid damage culminating in plant death. Results with native coccinellids were similar to those obtained for C7.

From Iowa (Obrycki):

Experiments in Iowa were also unable to achieve the desired predator:prey ratio necessary for determining standard predation levels. This failure was attributed to: 1) high frequency of time spent on the cage by C7 adults, 2) low fecundity of beetles as indicated by a short oviposition period (x of 3-7

days) and low egg production (x of 26-50 eggs/day of oviposition), and 3) high variability in aphid densities resulting from similar infestation levels.

In conclusion, predation by adult C7 in these 1987 field studies was far more variable than anticipated based on similar studies using Hippodamia convergins (HC) by Kring in 1982-83. Thus, the proposed 1988 studies by state cooperators have been modified to release eggs of C7, as well as HC, in order to minimize variability. The objective for 1988 is to estimate the propensity of C7 and HC to reduce aphid populations in cages in the selected crops for each state.

Table 1. Aphid and C7 introduction levels and sampling schemes in selected crops in three states.

		Aphids				C7	
State	Crop	Species[a]	Infestation level (No./plant)[b]	Sampling method	Sampling frequency	Introduction level (No./cage)	Frequency of visual sampling
IA	Alfalfa	PA	400/cage	D-vac	semi-weekly	20	semi-weekly
	Corn	CLA	15	Visual counts; destructive samples	semi-weekly	20	semi-weekly
AR	Sorghum	GB	5	Visual census, 10 plants/cage	alternate days	50	alternate days
		CLA	15	Class values within whorls	alternate days	50	alternate days
TX	Sorghum	GB	5-20	Visual census, 10 plants/cage	semi-weekly	20	semi-weekly

[a] GB = greenbug, Schizaphis graminum; CLA = corn leaf aphid, Rhopalosiphum maidis; PA = pea aphid, Acyrthosiphum pisum.

[b] 30 sorghum or corn plants/cage; normal stand of alfalfa.

Table 2. Percentage of C7 eggs and larvae recorded on sorghum plants in Texas.

Cage	GB/C7 oscillations	$\bar{X}$% on plants	
		eggs	larvae
1 and 5	yes	44	48
8 others	no	28	36

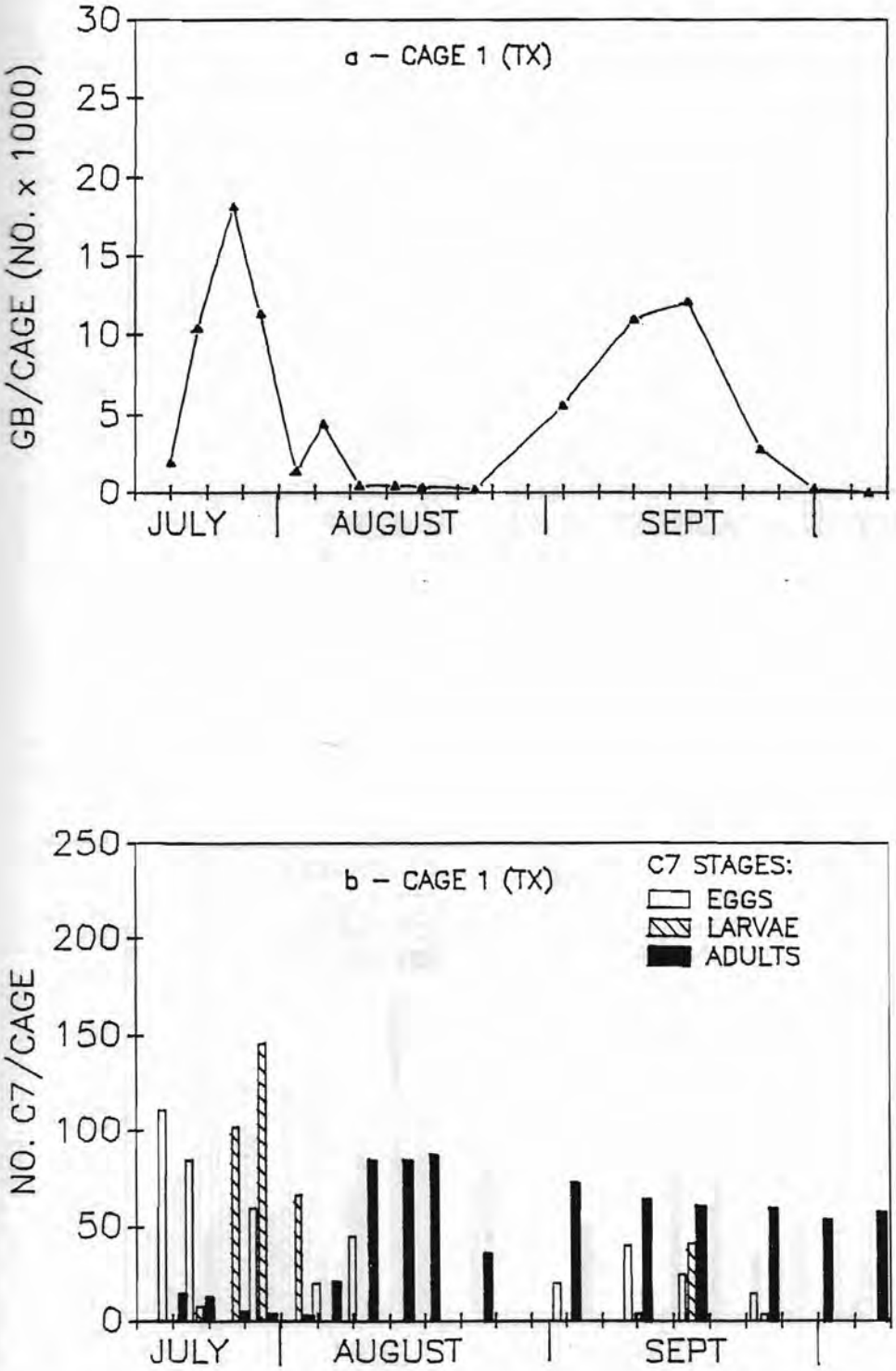
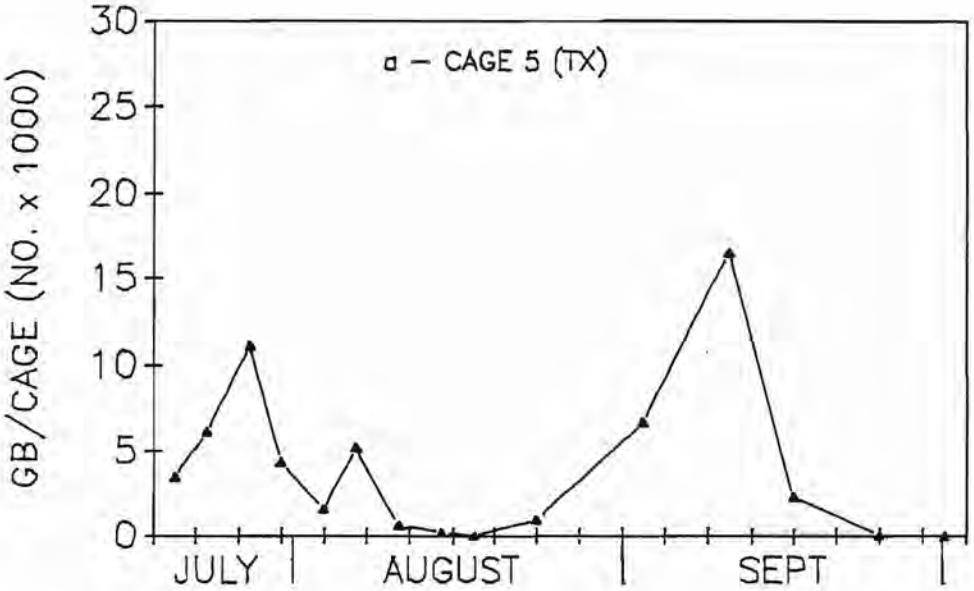


Fig. 1. Greenbug and C7 densities in an equilibrium situation; Bushland, TX.



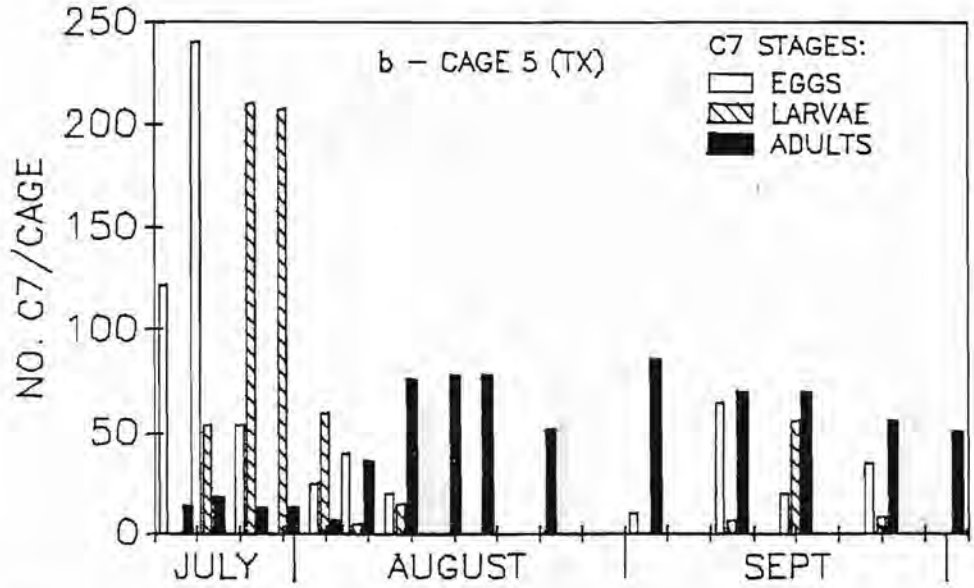


Fig. 2. Greenbug and C7 densities in an equibrium situation; Bushland, TX.

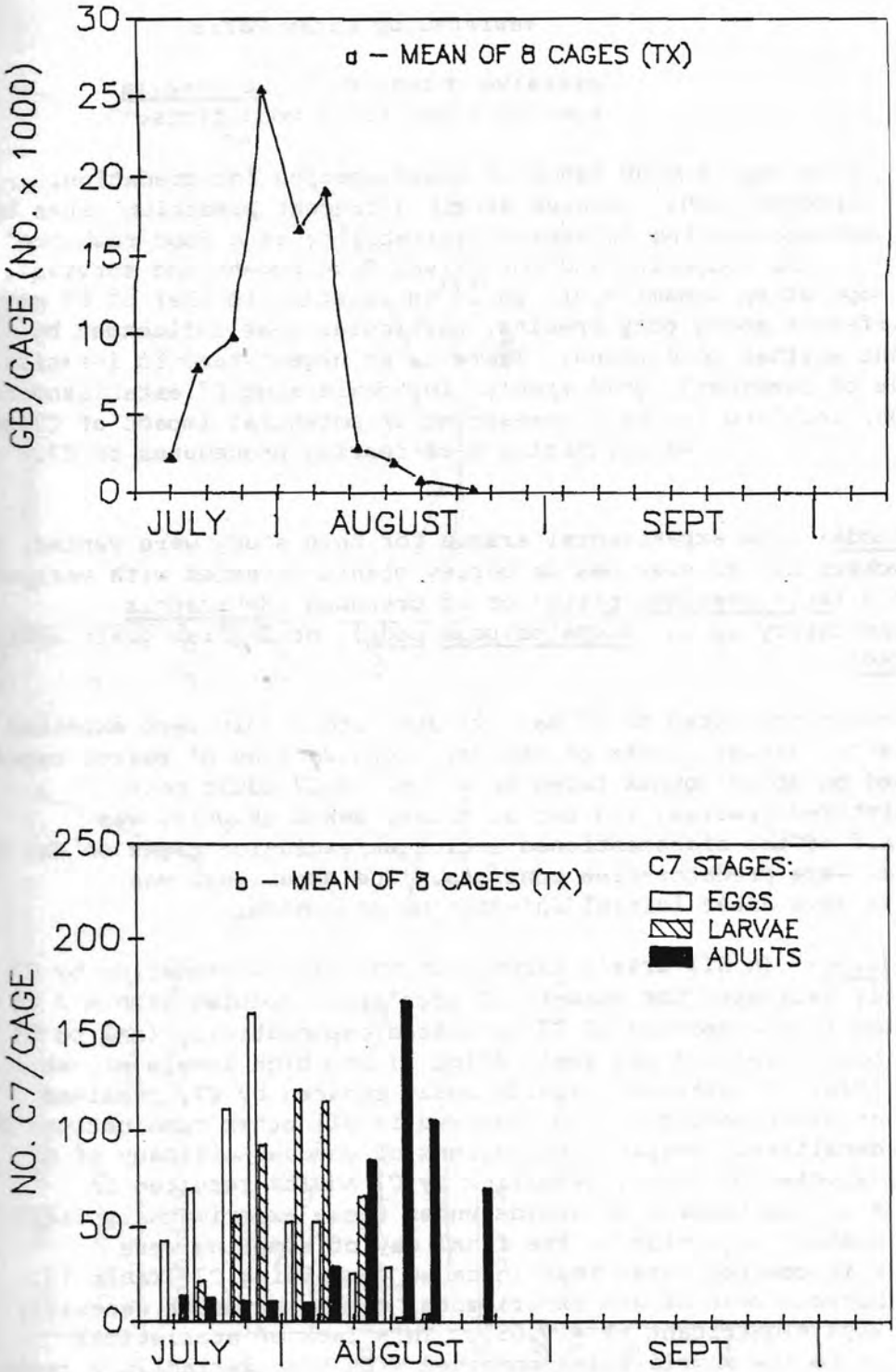


Fig. 3. Greenbug and C7 densities in eight non-equilibrium situations; Bushland, TX.

Project Number: ABC 7.1.3
Project Subtitle: Field Suppression of Pea Aphid and Cereal Aphids by the Predator, Coccinella septempunctata.
Report Period: October 1, 1986 - September 30, 1987
Report Type: Preliminary
Project Leader: William C. Kauffman, assisted by Cathy Paris

Objective: To measure the aphid-suppressive potential of Coccinella septempunctata (C7) on several prey species under field conditions.

Rationale: C7 adults accept a wide range of aphid species for predation. Presumably, these different aphid species elicit different predation rates by C7 as well as demonstrate varying degrees of suitability as a food resource for adult oviposition and longevity and for larval development and survival. In addition, the population dynamics of aphids in relation to that of C7 may be drastically different among prey species, particularly as influenced by fluctuating ambient weather conditions. There is an urgent need to identify the relative value of candidate aphid species for maximizing C7 establishment and redistribution, enabling accurate assessment of potential impact of C7 on field populations of aphids, and optimizing mass-rearing procedures of C7.

Materials and Methods: The experimental arenas for this study were vented, 5-gallon plastic buckets placed over pea or barley plants infested with various levels of pea aphid (Acyrthosiphum pisum) or of greenbug (Schizaphis graminum), oat bird cherry aphid (Rhopalosiphum padi), or English grain aphid, (Macrosiphum avenae).

Replicated experiments initiated on 27 May, 22 June and 9 July were examined on 2-day intervals for visual counts of aphids. Replications of paired cages were obtained based on aphid counts taken on day 6. A C7 adult pair (laboratory overwintered beetles, fed out at Niles, sexed at Otis) was introduced into half of the aforementioned inclusion/exclusion cages on day 6; the remaining cages were predator-free controls. The experiment was terminated 12 or 14 days after initial infestation of aphids.

Results and Discussion: In all trials throughout the study, predation by C7 adults significantly decreased the numbers of each aphid species within 2 to 6 days, whereas aphids in the absence of C7 increased exponentially (n=2 to 6) (Fig. 1-3). Very high levels of pea aphid (Fig. 1) and high levels of oat bird cherry aphid (Fig. 3), although significantly reduced by C7, remained relatively higher at experiment end than observed in all other combinations of aphid species and densities. Despite indications of unequal efficacy of C7 against these four species of aphid, predation by C7 adults resulted in dramatic reductions in the numbers of aphids under these experimental, field conditions. Mean numbers of aphids on the final day of sampling were consistently higher in control cages than in cages containing C7 (Table 1). These differences between control and experimental cages, although generally large, were not always significant ($P \leq 0.05$). This lack of statistical significance was due to low sample sizes combined with high variability among control cages presumably due to widely variable levels of aphid reproduction. Conversely, variability was relatively low among experimental cages due to near total elimination of aphid prey by C7 in nearly all cages.

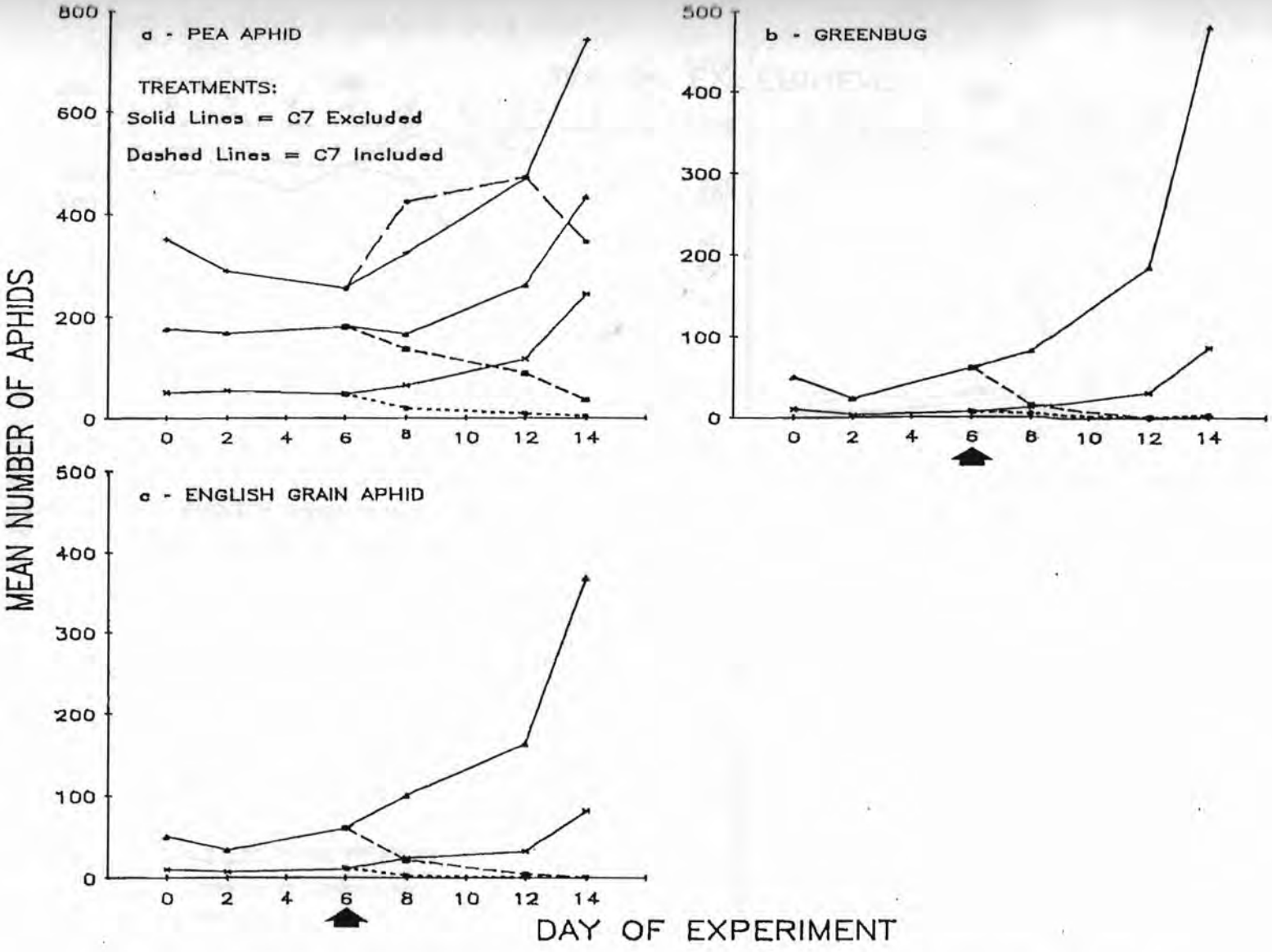


Fig. 1. Trial 1, 27 May to 10 June. Time of C7 introduction designated by ▲.

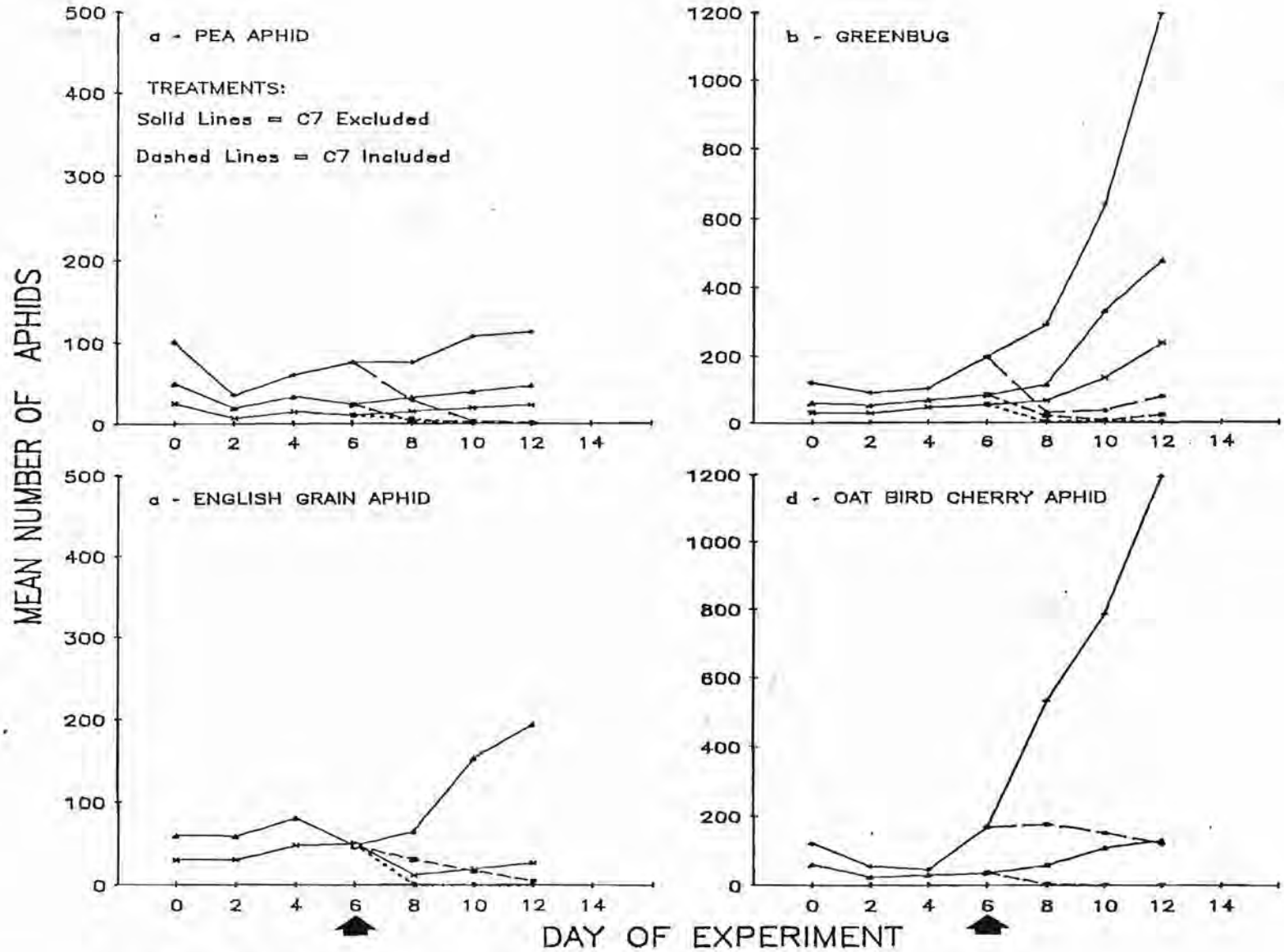


Fig. 2. Trial 2, 22 June to 4 July.

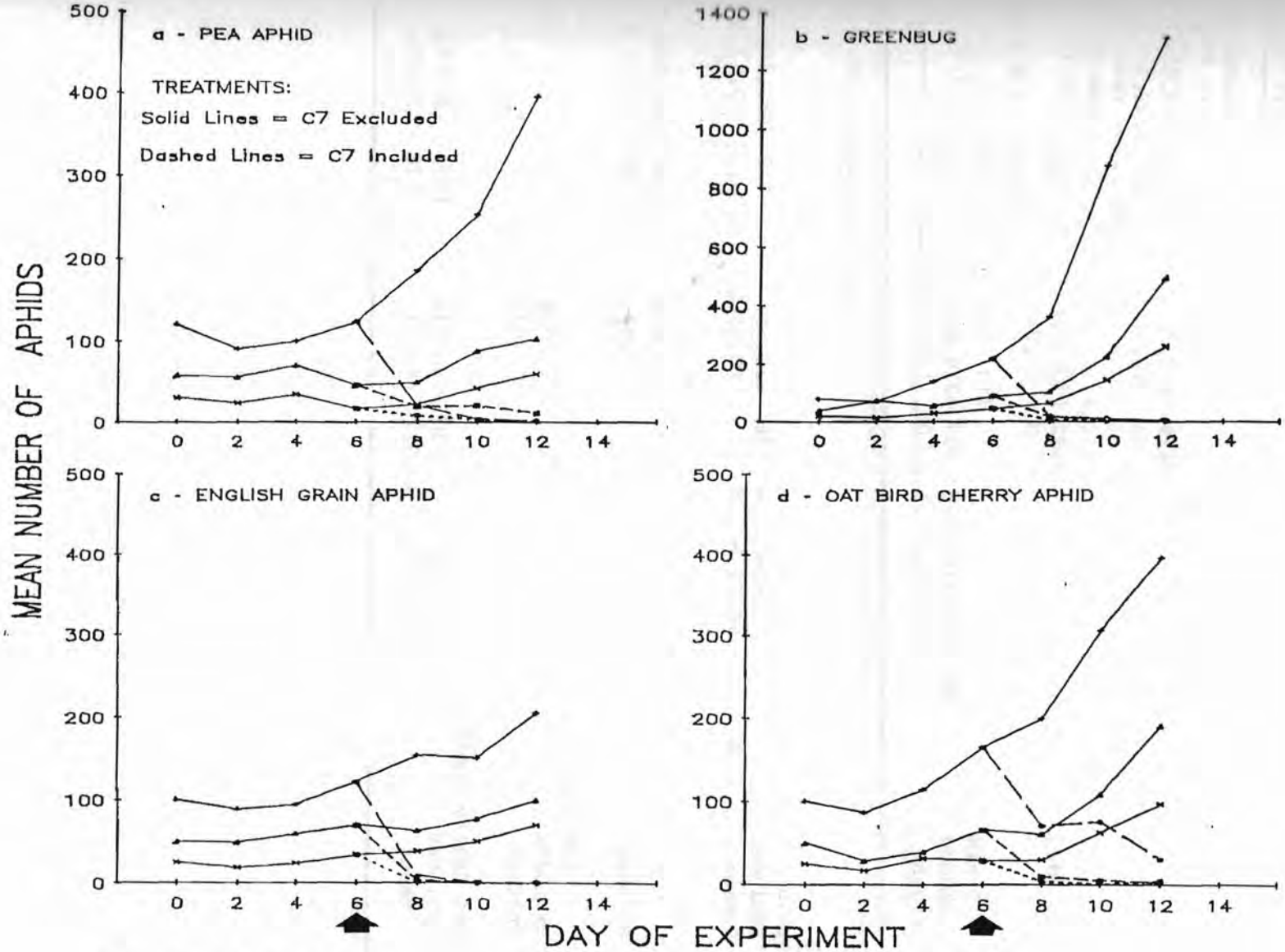


Fig. 3. 9 to 23 July.

Table 1. Contrasts in mean number of aphids at experiment end in cages which contained C7 adults and those which excluded the predators.

Aphid prey	No. of Reps	Relative aphid levels on day 6 (No. replicates, x ± S. E.)[a] Low			Moderate			High	
Trial 1									
Pea Aphid									
-C7 Excluded	3	244	(67.2)a	6	435	(54.5)a	3	743	(316.8)a
-C7 Present	6	3	(1.6) b	4	36	(9.2) b	3	347	(148.2)a
Greenbug									
-C7 Excluded	7	87	(37.1)a	2	482	(309.5)a		N/A	
-C7 Present	6	4	(1.7)a	3	0	(0.0)a			
English Grain Aphid									
-C7 Excluded	4	82	(56.4)a	3	367	(218.8)a		N/A	
-C7 Present	5	0	(0.0)a	4	0	(0.0)a			
Trial 2									
Pea Aphid									
-C7 Excluded	4	24	(5.4)a	4	48	(12.5)a	4	113	(31.5)a
-C7 Present	4	1	(0.7) b	4	0	(0.0) b	4	0.5	(0.3) b
Greenbug									
-C7 Excluded	4	238	(152.2)a	4	480	(51.4)a	4	1199	(287.7)a
-C7 Present	3	0	(0.0)a	5	23	(21.8) b	4	80	(75.9) b
English Grain Aphid									
-C7 Excluded	2	27	(11.0)a	2	194	(47.5)a		N/A	
-C7 Present	2	0	(0.0)a	2	5	(5.0)a			
Oat Bird Cherry Aphid									
-C7 Excluded	6	22	(8.0)a	3	130	(46.0)a	3	1191	(440.6)a
-C7 Present	6	0	(0.0) b	3	0.7	(0.7) b	3	120	(74.6)a

Table 1. Continued

Trial 3									
Pea Aphid									
-C7 Excluded	4	60	(24.6)a	5	103	(19.0)a	3	397	(78.9)a
-C7 Present	4	1	(0.8)a	4	11	(6.3) b	4	0.5	(0.5) b
Greenbug									
-C7 Excluded	4	262	(38.2)a	4	496	(95.9)a	4	1316	(385.0)a
-C7 Present	4	1	(0.4) b	4	4	(3.5) b	4	0	(0.0) b
English Grain Aphid									
-C7 Excluded	4	70	(4.5)a	4	100	(23.4)a	4	205	(26.7)a
-C7 Present	4	0	(0.0) b	4	0	(0.0) b	4	0	(0.0) b
Oat Bird Cherry Aphid									
-C7 Excluded	5	96	(32.2)a	5	191	(30.6)a	3	396	(67.7)a
-C7 Present	3	5	(2.9)a	3	2	(2.0) b	3	31	(26.7) b

[a] ANOVA (SAS GLM) and vertical separation of means within species of aphid ($P \leq 0.05$, pairwise t test).

Project Number: ABC 7.1.4
Project Title: Evaluation of Coccinella septempunctata Efficacy as an Aphid-Suppressive Agent in Selected Agroecosystems
Report Period: October 1, 1986 - September 30, 1987
Report Type: Preliminary
Project Leaders: William C. Kauffman

In 1986, USDA, APHIS initiated the Aphid Biological Control (ABC) Project which targeted a variety of aphid species damaging to agriculture, particularly in cereal grains, alfalfa, corn and vegetable crops. This biocontrol project involves three exotic species of aphidophagous coccinellids - Coccinella septempunctata (C7), Hippodamia variegata (Hv) and Propylea quatuordecimpunctata (P14) - which were introduced from Eurasia and subsequently established in the U. S. by USDA, ARS (Newark, DE). Since the initial introductions, C7 establishments have been confirmed in several eastern and midwestern locales, whereas Hv and P14 have been reported in sites in Montreal, Canada and the New England border.

Therefore, the objectives of this ABC Project are to: 1) distribute C7 into areas where not presently established, 2) colonize Hv and P14 in cereal grains where a new pest, the Russian Wheat Aphid (RWA), presently infests small grains or is a potential pest therein, and 3) evaluate the ability of these coccinellid predators to suppress aphid populations in selected agroecosystems.

The following evaluation program goals will be accomplished by employing diversified approaches possible through cooperative research with university and USDA-ARS scientists: 1) further understand the predator:prey relationship and its complex components so to optimize agricultural benefits of predators, 2) assess the ecological impact of C7 on aphid populations and, ultimately, the economic benefits to growers, and 3) perfect the evaluation criteria and procedures for C7 as a model for appraising other beneficial insects of aphids (i.e., Hv, P14, indigenous coccinellids and parasitoids).

Project Number: AW 1.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasite Redistribution Program
Report Period: October 1, 1986 to September 30, 1987
Report Type: Interim
Project Leader: Philip C. Kingsley

After seven years of sampling up to 150 alfalfa fields for alfalfa weevils and their parasites, we are in the final stretch of the AW Evaluation Project.

Last year we discontinued sampling in the eastern most area (Pennsylvania, New Jersey) but set up a new area in the Western Region. Thirty fields were placed in southern Oregon and northern California to study the effect of the program on western strain weevils. Analysis of adult weevils from the study sites sent to the Mission Biological Control Laboratory this spring, indicate that these sites are to a large extent western strain alfalfa weevil. Our plans for 1988 are to survey the California/Oregon area along with thirty fields in Nebraska and Ohio. Weevils from evaluation sites in Nebraska have also been analyzed for strain and demonstrate an east-west transition between the two strains in this area.

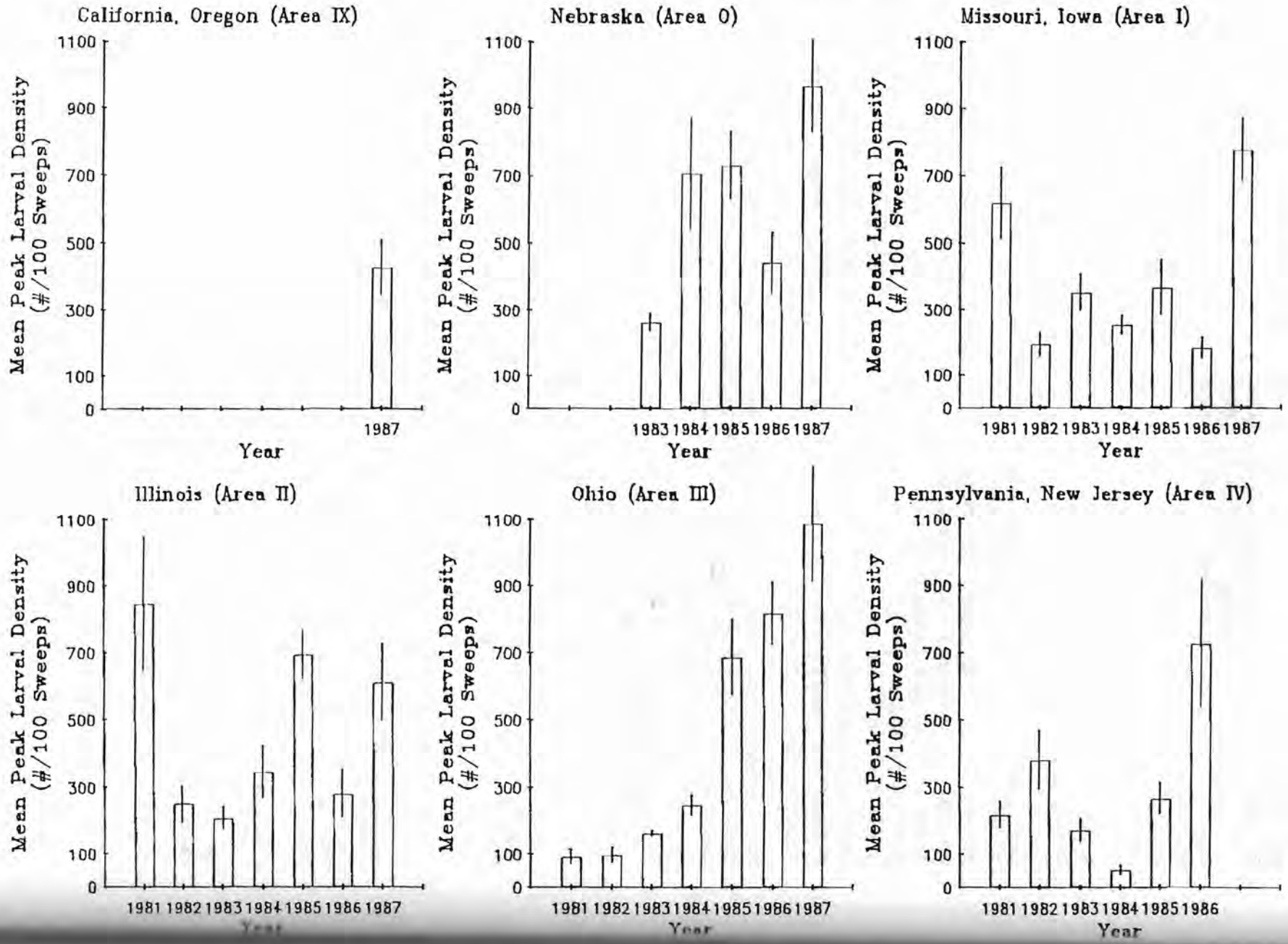
Final analysis of data from the seven survey years is presently underway. Some early trends are encouraging, especially in parasite establishment. For example, the two centrally located areas, Missouri Iowa (Area I) and Illinois (Area II) have both shown a doubling in the average number of parasite species during the 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 56.5% in 1986 (Figure 3).

Spraying for AW control has also shown an encouraging downward trend in these two Midwestern areas. 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 consistently declined in these areas (Figure 1). Assigning a cause and effect relationship between parasites and spraying is therefore difficult at this point in the data analysis.

We have enlisted the help of Dr. Joseph Moffet, an economist from the University of Massachusetts, to work with us and the Economic Research Service in determining the long term economic effects of this national biological program.

We hope to conclude the analysis of data collected so far and produce a report/publication this year. A second publication summarizing the economic aspects of the program should follow, as will a paper on the surveying of areas with western strain weevils.

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



Average number of AW parasite species by area and year.

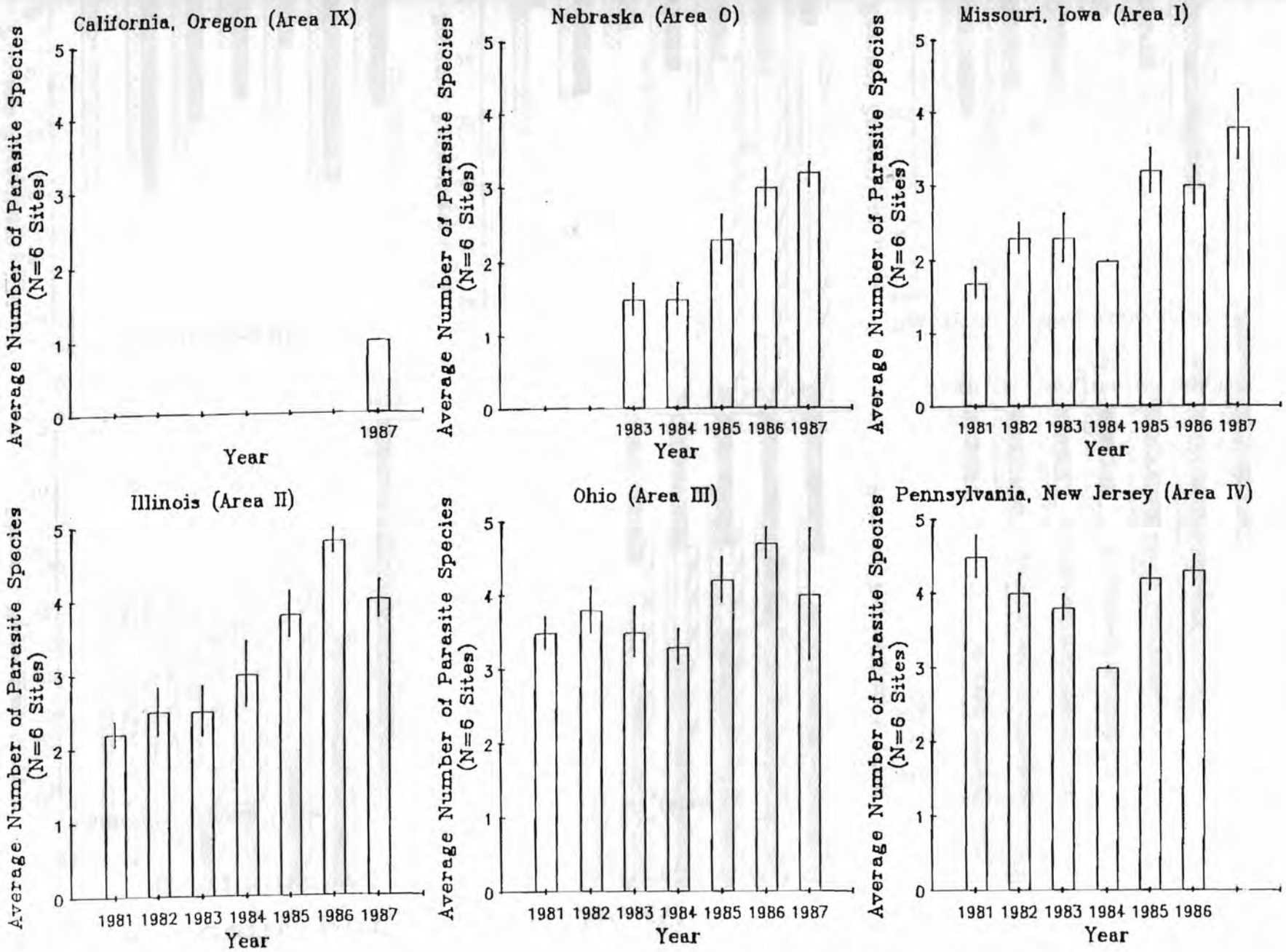
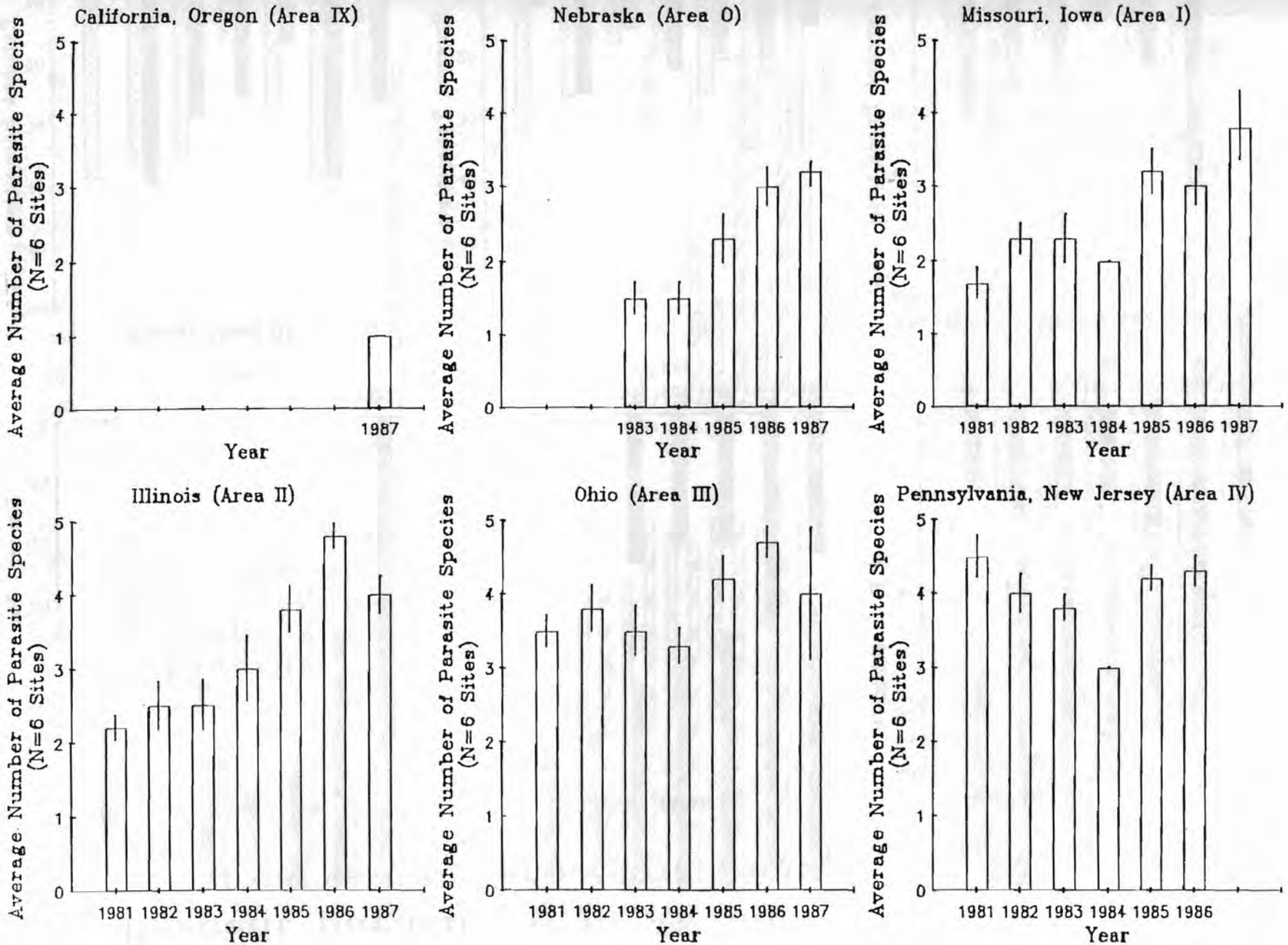
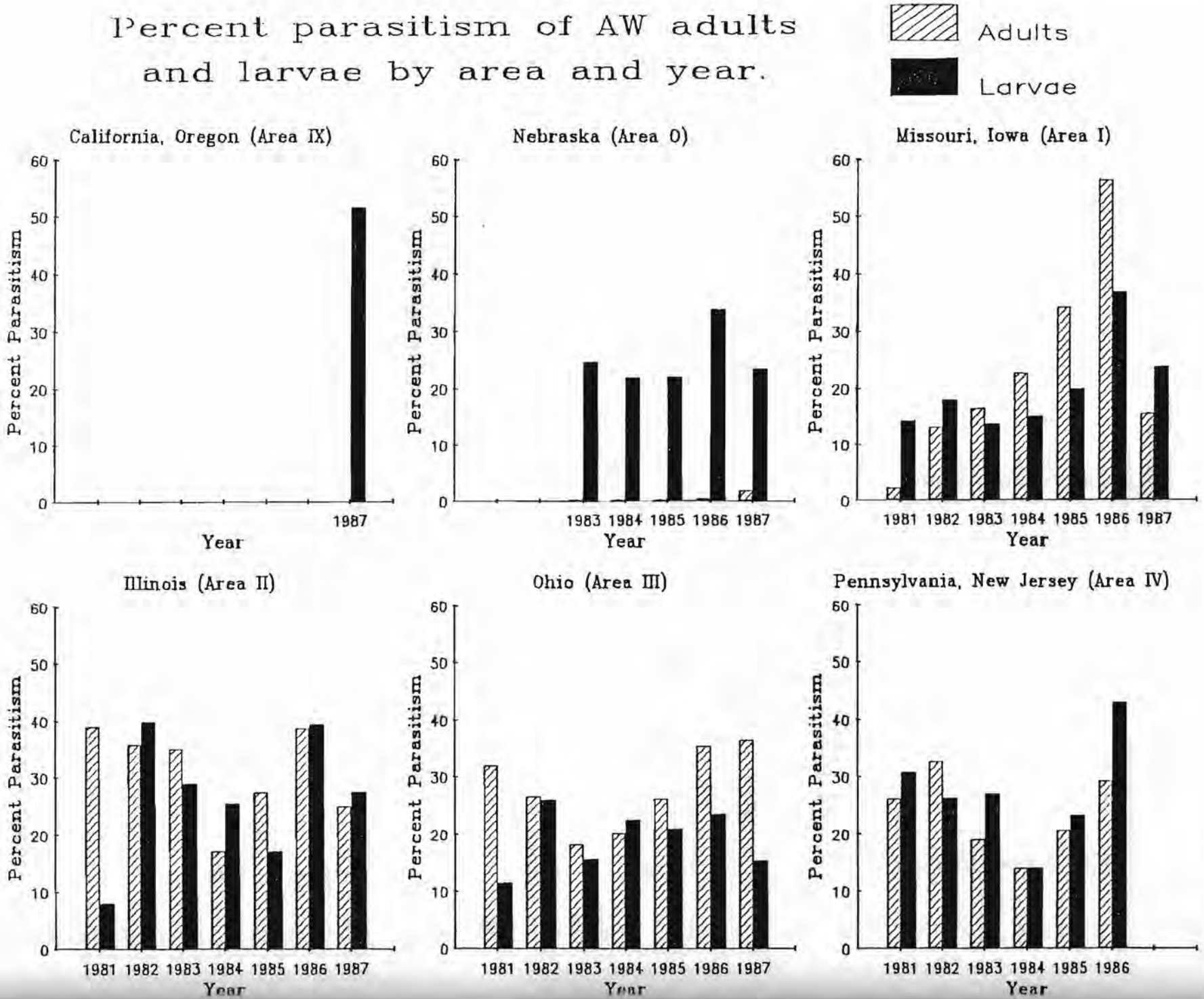


Figure 2

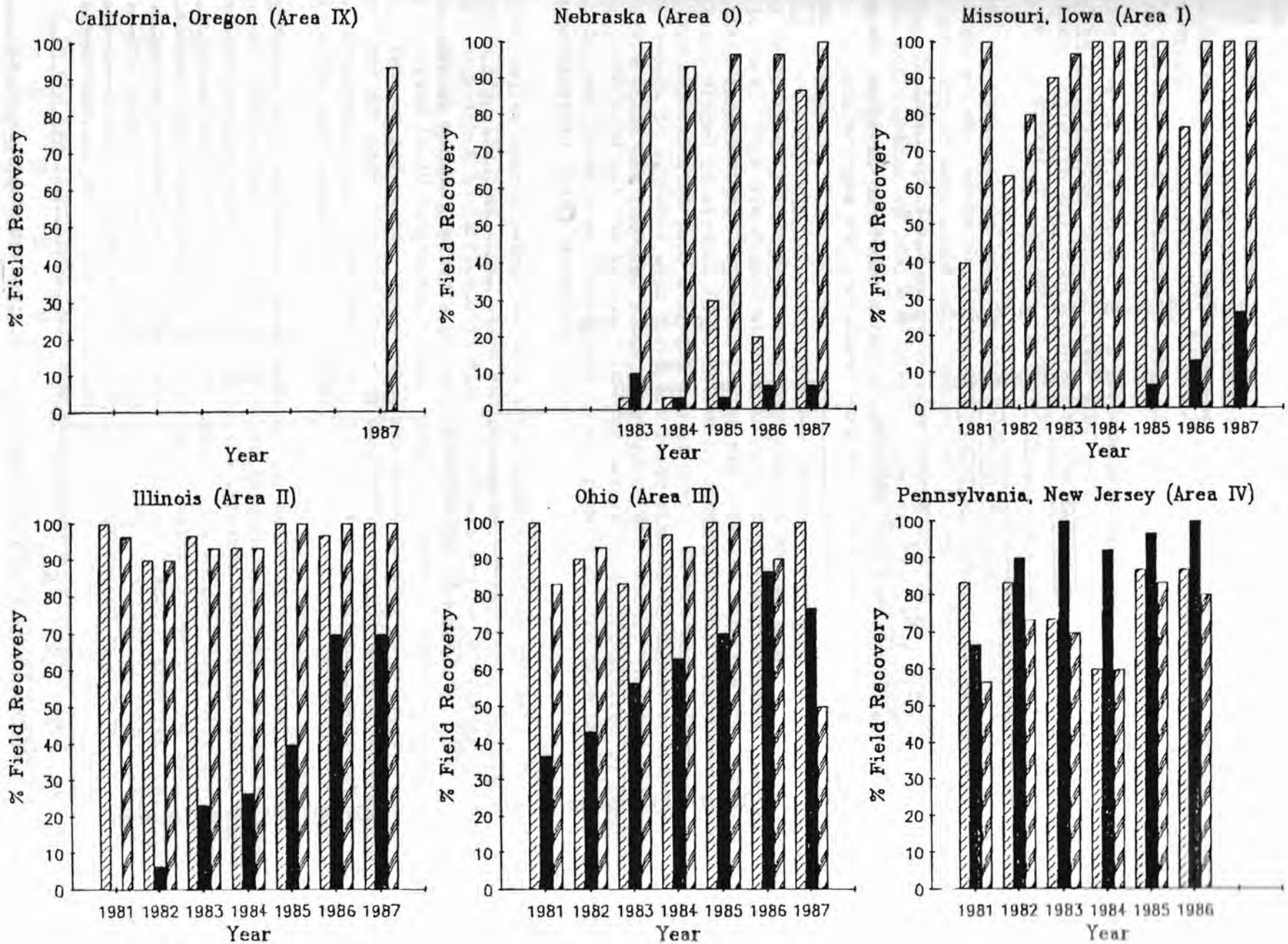
Average number of AW parasite species by area and year.



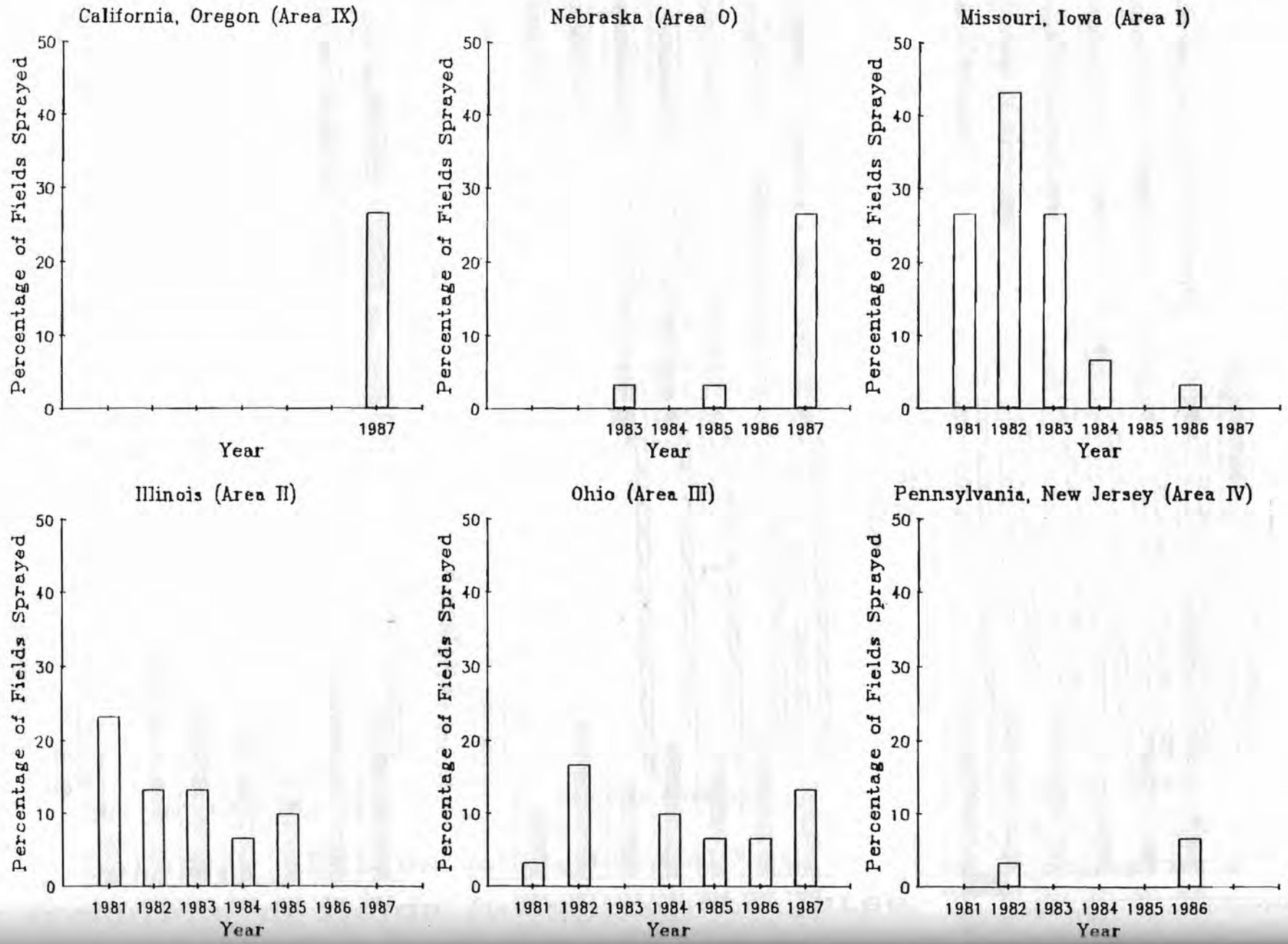
Percent parasitism of AW adults and larvae by area and year.



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



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



Project Number: BT 7.1.1
Project Title: Development of Browntail Moth Rearing Techniques (Or How to Learn to Stop Worrying And Love The Browntail)
Report Period: November 1985 - September 30, 1987
Report Type: Interim
Project Leader: A. Pellegrini-Toole, V. C. Mastro and S. Thomas

The objective of this project is to successfully rear browntail moth, Euproctis chrysorrhoea, on artificial diet in sufficient numbers to facilitate isolation and identification of the female pheromone. This project began in the winter of 1985 and is ongoing.

Initial browntail web collections were made in November of 1985 at High Head, Provincetown, MA. Since the larvae overwinter in communal hibernaculae, a large quantity of webs could be collected and stored at 5°C and used as needed. To interrupt diapause, webs were placed into 16 oz. containers and kept in an incubator at 25°C ± 2° with a light:dark cycle of 14:10 hours. Larvae are infested onto diet after a sufficient number (ca.500) emerged from webs. Several methods and containers were tried before settling on the present method.

Thirty to forty milliliters of gypsy moth diet is poured into 6 oz. fluted plastic cups. Cups are capped with paper lids after the diet has cooled and held until infestation. Browntail larvae are infested onto the diet using a paintbrush dipped in alcohol (90%) to reduce contamination. Approximately 60-80 larvae are infested into a single cup of diet. It has been determined that better growth is achieved when there are large numbers of larvae in a cup. The larvae which are infested range from 3rd to 4th instar. The larvae are held at 25°C ± 2° and 33% RH for 7-10 days before being transferred to fresh diet. As the larvae grow, reductions in cup population are made until there are approximately 30-40 larvae/cup.

Pupation begins approximately 44 days after emergence from the web. Pupae are collected from the diet cups, sexed and placed into 8 oz. cups. Eclosion takes place within 14-21 days. To facilitate female gland extractions during normal working hours, pupae are placed into a second incubator at 25°C ± 2° and a reversed 14:10 light:dark cycle. Cups are checked daily for eclosion, females removed and placed into a new 8 oz. cup with date of eclosion. Gland extractions are made when the female is at least 1 day old and no older than 2 days. Extractions are done with hexane 2, 4 or 6 hours after lights out. Extracts are then stored in the freezer.

To determine if a continuous laboratory colony could be maintained, in the summer of 1987 matings of laboratory reared insects were done. Resulting egg masses were hatched out (21 days) and larvae infested onto diet. It was hoped that diapause could be prevented. The larvae were watched carefully for signs of diapause, e.g. heavy webbing within cup, decrease in feeding and activity. As the larvae reached 3rd and 4th instar, signs of diapause appeared. At this point, the light:dark cycle was changed to 16L:8D (Danilevskii 1965). After 13 days the insects became much more active and proceeded to pupate. Additional matings producing egg masses were done, these larvae are being reared in the new light:dark cycle and show no signs of diapausing.

It is safe to say that the original objective has been met, browntail can be reared in large quantities to the adult stage.

Project Number: CPB 5.1.1
Project Title: Rearing Edovum puttleri an egg parasite of Leptinotarsa decemlineata.
Report Period: October 1, 1986 - September 30, 1987
Report Type: Final
Project Leader: P. C. Kingsley

A significant portion of our work completed this year was presented in a poster display entitled "Tricks for Rearing Edovum puttleri (Hymenoptera: Eulophidae), an egg parasite of the Colorado Potato Beetle". See Appendix 1.

Appendix 1.

Presented as a poster at the Annual Meeting of the Entomological Society of America; Boston, MA December 2, 1987

Tricks for Rearing Edovum puttleri (Hymenoptera: Eulophidae), an Egg Parasite of the Colorado Beetle

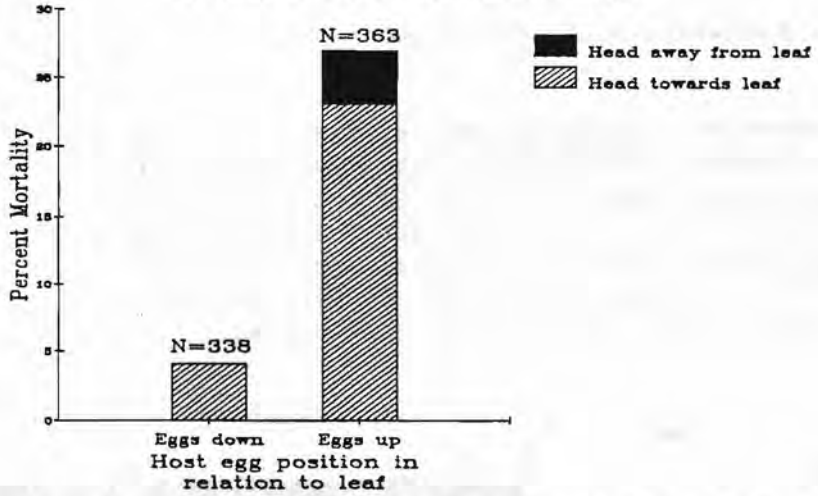
P. C. Kingsley, O. T. Forrester and C. P. Schwalbe

The eulophid egg parasite, Edovum puttleri Grissell is presently being reared in large numbers at several institutions for experimental field releases against the Colorado Potato Beetle (CPB). Over the last three years, the USDA's Animal and Plant Health Inspection Service (APHIS) has reared over a million E. puttleri at the Mission Biological Control Laboratory in Mission, Texas. At APHIS's Otis Methods Development Center, we have been looking at ways to more efficiently rear this insect and its host.

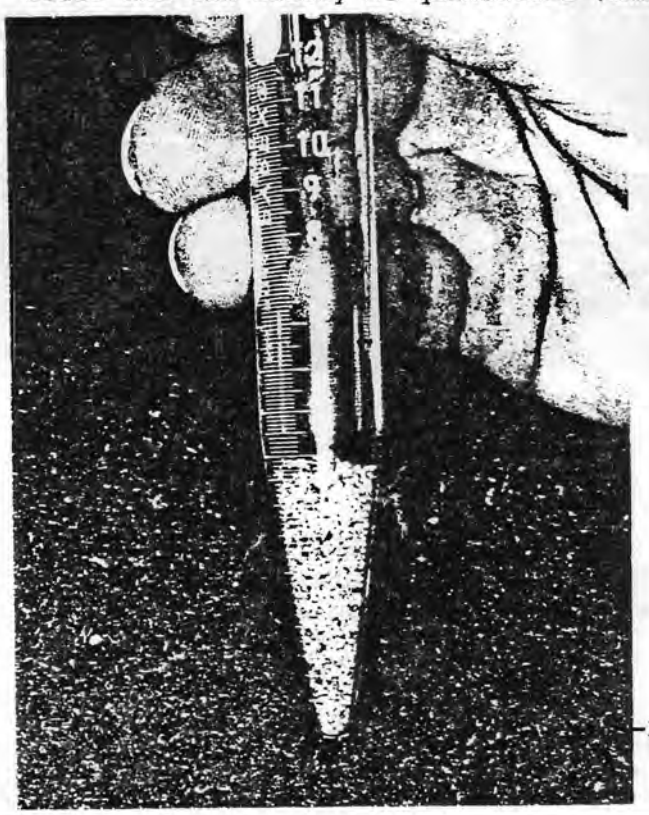
Two factors lead to a significant mortality while rearing this parasite in the laboratory. First, CPB normally lay their eggs vertically in masses on the underside of the leaf.

Secondly, in the late stages of development E. puttleri cannot reverse its orientation in the host egg. This is important because wasps pupating with their heads towards the leaf and not towards the exposed end of the egg will be less likely to emerge successfully. This orientation is dictated by gravity. That is, the large majority of wasps position themselves geotropically with their heads down. If parasitized egg masses are held with the eggs up during rearing, approximately 23% will be unable to emerge due to this phenomenon.

Mortality of Edovum puttleri at the time of emergence due to its orientation in host egg.



The following egg handling procedure was devised in part to avoid this mortality when rearing these wasps in the laboratory. Eggs can be removed from leaves by brief agitation in hexane. After the supernatant with kairomone is decanted, the residual eggs are transferred to a buchner funnel with a small amount of solvent. The supernatant is then evaporated over the eggs returning a portion of the kairomone. Eggs extracted in this manner are loose and can easily be quantified (1ml=approximately 1000 eggs.



A significant saving in handling time can be achieved by devitalizing host eggs with a 20 KRAD dose of gamma radiation before parasitism. This way, removal of unparasitized host larvae becomes unnecessary. Irradiated eggs were not significantly different in terms of parasite acceptance.

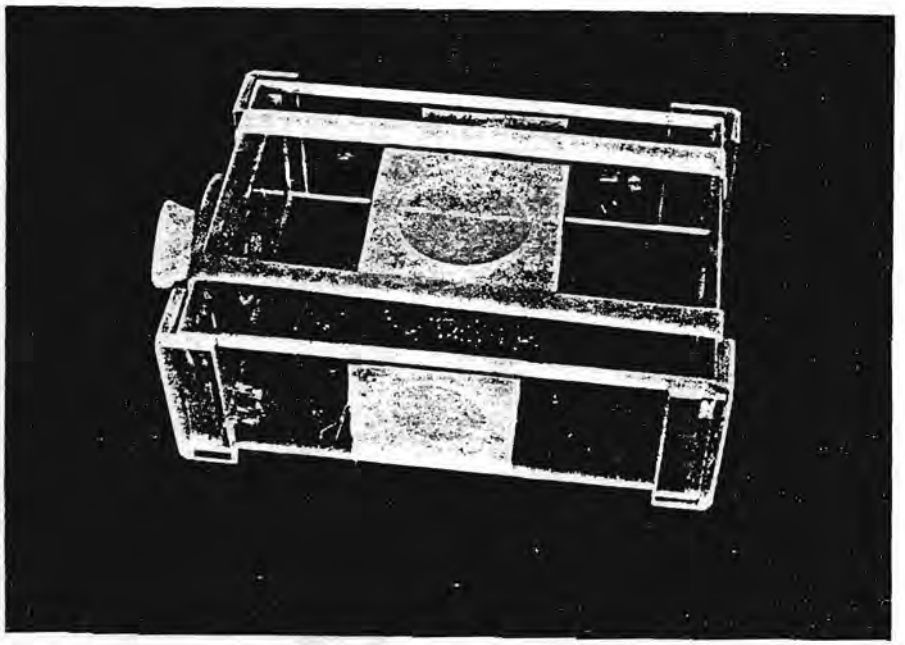
Yield of Edovum puttleri from viable and devitalized* CPB eggs.

Egg Treatment	Percent Yield	N
Devitalized	50.0	1827
Viable	51.0	2423

NS (Chi-square)

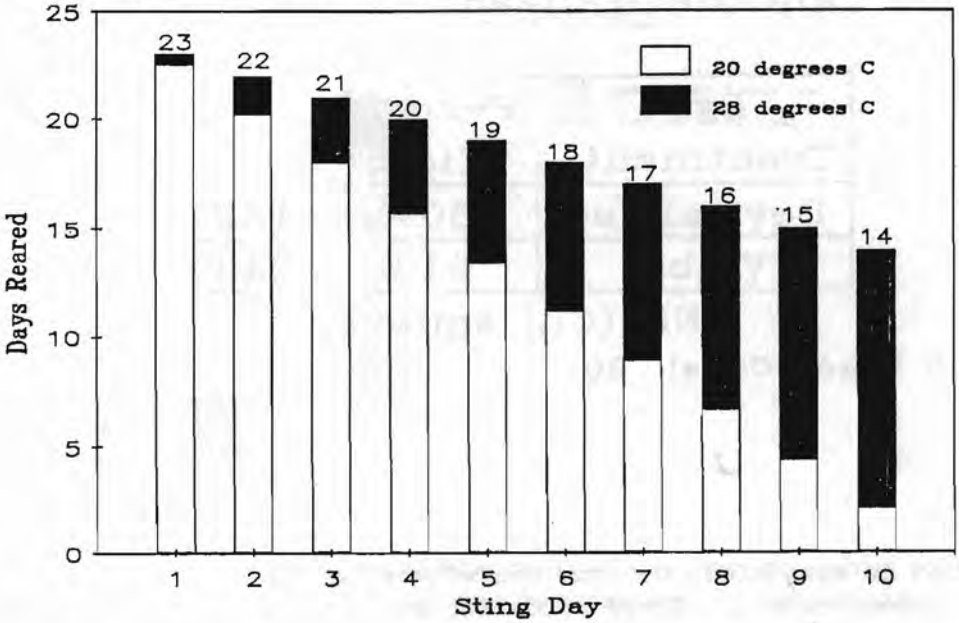
* 20 krads Cobalt 60

Eggs can then be sprinkled on cards coated with a thin layer of mucilage glue or diluted rubber cement. These cards fit into the bottom of oviposition cages. Cards can be replaced during the scotophase when wasps move off the eggs to the top of the cage, again an important reduction in handling time.



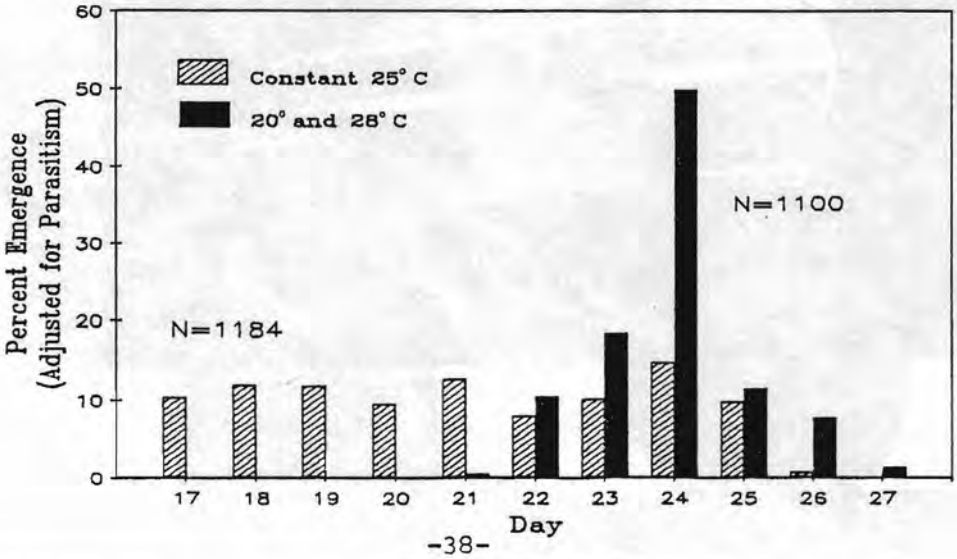
Synchronizing the emergence of E. puttleri adults can be accomplished by rearing the immature parasites under different temperature regimes. By utilizing a linear regression equation (modified from Obryki et al 1985), developmental times were varied from 24 to 14 days be exposing the developing parasites for different times at two rearing temperatures; 20 and 28 degrees C.

Rearing schedule for Edovum puttleri for synchronous emergence on day 24.



Fifty percent of the wasps reared under such a schedule emerged on, and 80 percent within one day, of the predicted date.

Emergence of Edovum puttleri from CPB eggs stung over 10 days and reared under two temperature regimes; normal 25° and for synchronous development.



Overall yields were not significantly different between the two rearing regimes.

Although these procedures are untested on a production scale, we feel that they have advantages over the present technology in reducing handling time, improved accuracy in quantifying host egg numbers, better yields, and in optimizing production efforts for release. Under these conditions we have achieved high parasitism (>80%) and low mortality (<20%).

Project Number: CPB 6.1.1
Project Title: Development of an Artificial Diet for Colorado Potato Beetle, Leptinotarsa decemlineata (Say)
Report Type: Interim
Project Leader: O. T. Forrester

The basal diet from the last progress report has been modified (Table 1). Twenty-five variations of the modified diet were evaluated for survival, rate of development and adult weight. Results of tests with plant powders, casein, antioxidants and rolled oats vs. wheat germ are given in Tables 2 through 5. As a reference, Colorado potato beetle (CPB) reared on potato plant had a survival of 82%, mean time from neonate to adult was 20 days and mean adult weight was 112 mg. CPB reared on basal diet had a survival rate of 53%, mean time to adult was 35 days and mean adult weight was 72 mg.

Table 1. Composition of diets used for CPB

Ingredient	Basal	Modified
Deionized water	550.0	650.0
Agar	10.0	10.0
Rolled Oats	40.0	40.0
Powdered Silver leaf nightshade	20.0	20.0
Casein	18.0	9.0
Lactalbumin hydrolysate	-	9.0
Sucrose	16.0	16.0
Brewers yeast hydrolysate	8.0	8.0
Wesson salt mix	4.0	-
USP XIII No. 2 salt mix	-	4.0
Vitamin pre-mix	2.5	-
Vanderzant vitamin mix	-	5.0
Beta sitosterol	0.5	0.5
Cholesterol	0.5	0.5
Methyl paraben	0.5	0.5
Sorbic acid	0.5	0.5
Raw linseed oil	1.0	-
Wheat germ oil		1.0

Host plant powders used in this test (Table 2) were silver leaf night shade (SLNS), S. elaeagnifolium, egg plant (EP), S. melongena, potato, S.tuberosum, bitter nightshade (BNS), S. dulcamara and tomato, Lycopersicon esculentum. Best results in terms of survival to adult were obtained with egg plant powder. Egg plant powder and BNS gave the highest adult weights followed by SLNS and potato. Poorest results were obtained with tomato plant powder. Diets containing potato or tomato plant powder tended to dry faster than other plant powders. Rate of development does not appear to be influenced by host plant powder.

Table 2. Effect of plant powder in modified CPB diet on survival, rate of development and adult weight.

Treatment	n	Percent survival	Mean # days to adult	Mean male weight mg.	Mean female weight mg.
Control SLNS	150	42.0	28.3	73.6	77.1
EP	150	50.0	27.9	76.1	84.5
Potato	150	42.0	28.4	66.8	81.7
BNS	150	40.7	28.9	75.0	82.9
Tomato	150	36.6	29.9	70.5	69.5

Casein performed very well as a protein source in earlier tests. Equal parts of casein and lactalbumin performed better than casein alone. This series of tests compared casein (60 mesh), casein hydrolysate, micropulverized casein (80-100 mesh) and replacement of rolled oats with wheat germ (Table 3). Micropulverized casein has some positive effect on number of days to adult. The important information obtained from this series is the increase in percent survival to adult with wheat germ, however, substituting rolled oats with wheat germ increased developmental time to adult.

Table 3. Effect of casein, micropulverized casein and casein hydrolysate on survival, rate of development and adult weight.

Treatment	n	Percent Survival	Mean # days to adult	Mean male weight mg.	Mean female weight mg.
Control	100	45	32.8	83.8	89.3
Casein hydrolysate	100	48	35.9	70.5	83.6
Micropulverized casein	100	49	30.0	78.0	90.3
Micropulverized casein + wheat germ	100	55	35.8	63.6	85.9

Further testing of micropulverized casein and replacement of rolled oats with wheat germ gave similar results, survival was higher with wheat germ and development was slower. A combination of wheat germ and rolled oats (in terms of reduced number of days to adult and survival) was superior to oats or wheat germ alone (Table 4).

Table 4. Effect of rolled oats, wheat germ and a combination of oats and wheat germ on survival, rate of development and adult weight. Micro pulverized casein was used in all test diets.

Treatment	n	Percent survival	Mean # days to adult	Mean male weight mg.	Mean female weight mg.
Rolled oats	100	30	28.6	72.8	84.1
Wheat germ	100	40	32.0	58.8	70.8
1/2 oats + 1/2 wheat germ	100	63	26.4	69.6	78.7

The use of the antioxidants alpha tocopherol and citric acid had little or no effect on rate of development, survival or adult weight. BHT had a negative effect on survival and adult weight.

Table 5. Effect of antioxidants on survival, rate of development and adult weight.

Treatment	n	Percent survival	Mean # days to adult	Mean male weight mg.	Mean female weight mg.
Control	100	42.0	29.9	68.1	74.0
Alpha tocopherol 1gm	100	38.0	29.9	69.2	76.7
Citric acid 1gm	100	38.0	29.3	71.2	80.1
BHT .5gm	100	15.0	31.0	62.2	63.9

This report is interim; results from these tests suggest that the plant powder of egg plant should replace silver leaf nightshade and that the protein source should be rolled oats and wheat germ. Further testing to increase adult weight, percent survival and reduce the number of days to adult is under way.

Project Number: GM 5.1.1
Project Title: 1987 Field Trials with Early Dimilin Applications in West Virginia
Report Period: October 1, 1986 - September 30, 1987
Report Type: Final
Project Leaders: W. H. McLane, T. Roland, B. Tanner, J. Hacker, N. Schneeberger and P. Bohne

The Animal Plant Health Inspection Service, Forest Service, W VA Department of Agriculture and Uniroyal Chemical cooperated in a study to determine the usefulness of early applications of Dimilin for gypsy moth control. Similar tests were conducted in 1986 with smaller acreage plots.

Three 5,000 acre plots were established west of Martinsburg, West Virginia. One plot was sprayed as soon as gypsy moth egg hatch was observed on one or more egg masses with a southerly exposure (Treatment 1). This treatment was started on April 20th and completed on the 22nd. There was no foliage on any of the tree species when treatment was made. A second plot was treated at time of general hatch, May 1 - May 5th (Treatment 2). At that time, 90> percent of visible egg masses had hatched out. About half of the newly hatched larvae had left the egg mass and the rest remained on the mass. Buds on white oak had just opened up and foliage on other oaks was approximately 5 percent expanded. A third plot was used as an untreated control (Treatment 3).

Two Dimilin formulations were applied, 25W and 2F. Each was treated with .031lbs. AI/gallon/acre with water as a carrier. Half of each plot received 25W and the remainder was sprayed with 2F. No sticker or other material was added to the formulations.

The material was applied with Cessna Ag truck aircraft equipped with 8004 Tee Jet flat fan nozzles. The aircraft dispersed the material at a speed of 120 mph, 50 feet over the tree tops. A pressure of 40 psi was used with a 75 foot swath. All material was applied during the morning hours.

A number of techniques were used to evaluate this study. Pre- and post-spray egg mass counts were made as well as defoliation estimates. Burlap was placed around oak trees at 25 sites within each experimental plot. Larval and pupal counts were made under each burlap at 5 time periods following treatment.

Based on total larval and pupal counts for the 5 readings, the treatment applied at general hatch time gave excellent control. The application made at first hatch gave fair results. Healthy numbers of larvae and pupae were recorded in the control plot. The first hatch treatment recorded 65 percent less total larvae than the control. The application at general hatch had 91 percent less larvae than the control plot. The differential in total pupae numbers was 59 and 98 percent respectively.

Defoliation, computed from data collected at each of 25 sites within each plot, demonstrated the effectiveness of the treatment made at general hatch. When collected, defoliation data were grouped into 20% increments. All estimates within the general hatch treatment plot were in the lower end of the 0-20% bracket. The early treatment also produced good foliage protection (27%

defoliation) when compared with 45% in the check plot.

Statistically, there was no difference between pre- and post-season egg mass counts in the 10% hatch plot treatment and the check. In the plot treated at general hatch time, post-treatment egg mass counts were statistically lower than the pre-treatment counts. In the untreated check post-treatment egg mass counts were statistically higher than in pre-treatment counts.

Based on early Dimilin treatment data from the past 2 years of studies, we recommend that Dimilin can be applied successfully starting at general gypsy moth egg hatch time. Uniroyal is presently in the process of obtaining approval for a label change. The new label will address our early treatment recommendation.

Although treatments made at first hatch time have demonstrated foliage protection and egg mass reduction, we feel additional data still need to be collected and analyzed before recommendations can be made.

This was a cooperative project and conducted by:

W. McLane, USDA, APHIS, Bldg. 1298, Otis AFB, MA 02542

T. Roland and B. Tanner, USDA, APHIS, Aircraft Operations, Moore Air Base, Edinburg, TX 78539

J. Hacker and A. Miller, W VA Dept. of Agriculture, Charleston, W VA 25305

P. Bohne, Uniroyal Chemical, Apple Hill Rd., Bennington, VT 05201

N. Schneeberger, USDA, FS, P.O. Box 4360, Morgantown, W VA 26505

Table 1. Recorded defoliation, larvae and pupae under burlaps in each treatment and control plot.

Treatment	Total live larvae under burlaps	Total pupae under burlaps	Percent defoliation
I	4,854	4,416	21
II	1,224	208	10
III	13,723	10,801	45

I - Treatment applied when 10% hatch.
II - Treatment applied when 100% hatch.
III - Control (untreated).

Table 2. Statistical analysis of pre- and post-treatment egg mass counts.

Treatment	Type	N	Mean	SD	Minimum	Maximum
I	pre	26	1,659	3,421	0	15,188
	post	26	907	782	35	2,605
II	pre	96	1,942	2,899	0	14,880
	post	96	381	583	0	3,560
III	pre	127	2,088	7,263	0	42,240
	post	127	3,627	4,316	40	33,200

I - Treatment applied when 10% hatch.
T = -1.09 PROB = 0.28
II - Treatment applied when 100% hatch.
T = 5.17 PROB = 0.00
III - Control (untreated)
T = -2.05 PROB = 0.04

Table 3. Pre- and post-treatment egg mass distribution.

	Type EM	Treatment I	Treatment II	Treatment III
Percent of sub-sampling sites with 0 EM/acre	pre	8-(2 of 26)	10-(10 of 96)	52-(66 of 127)
	post	0	20-(19 of 96)	0
Percent of sub-sampling sites with 1-50 EM/acre	pre	4-(1 of 26)	4-(4 of 96)	6-(8 of 127)
	post	8-(2 of 26)	8-(8 of 96)	1-(1 of 127)

I - Treatment applied when 10% hatch.
II - Treatment applied when 100% hatch.
III - Control (untreated).

TOTAL LIVE LARVAE FROM BANDS

	TIME					
TREATMENT	1	2	3	4	5	TOTAL
1	389	127	3342	869	127	4854
2	235	488	242	207	52	1224
3	1279	3495	5681	2189	1079	13723

TOTAL NUMBER OF PUPAE FROM BANDS

TREATMENT	TIME 1	2	3	4	5	TOTAL
1	0	300	879	3042	195	4416
2	0	28	11	125	44	208
3	0	254	2235	6810	1502	10801

WEIGHTED AVERAGE DEFOLIATION PER TREATMENT

TREATMENT 1 = 21%
TREATMENT 2 = 10%
TREATMENT 3 = 45%

NUMBER OF PLOTS WITHIN TREATMENT BLOCKS

TREATMENT	NUMBER OF PLOTS
1 (10% EGG HATCH)	26
2 (100% EGG HATCH)	96
3 (CHECK)	127

10% EGG HATCH (T1)
COMPARISON OF PRE- AND POST-TREATMENT EM COUNTS

	N	MEAN	SD	MIN	MAX
PRE	26	1659	3421	0	15188
POST	26	907	782	35	2605

T = 1.09
PROB = 0.28

100% EGG HATCH (T2)
COMPARISON OF PRE- AND POST-TREATMENT EM COUNTS

	N	MEAN	SD	MIN	MAX
PRE	96	1942	2899	0	14880
POST	96	381	583	0	3560

T = 5.17
PROB = 0.00

CHECK (T3)

COMPARISON OF PRE- AND POST-TREATMENT EM COUNTS

	N	MEAN	SD	MIN	MAX
PRE	127	2088	7263	0	42240
POST	127	3627	4316	40	33200

T = -2.05

PROB = 0.04

TREATMENT	PERCENT OF PLOTS WITH 0 EM/ACRE, PRETREATMENT	PERCENT OF PLOTS WITH 0 EM/ACRE, POST-TREATMENT
1	8 (2 OF 26)	0
2	10 (10 OF 96)	20 (19 OF 96)
3	52 (66 OF 127)	0

TREATMENT	PERCENT OF PLOTS WITH 1-50 EM/ACRE, PRETREATMENT	PERCENT OF PLOTS WITH 1-50 EM/ACRE, POST-TREATMENT
1	4 (1 OF 26)	8 (2 OF 26)
2	4 (4 OF 96)	8 (8 OF 96)
3	6 (8 OF 127)	1 (1 OF 127)

Project Number: GM 5.1.2
Project Title: 1987 Field Trials with Bacillus thuringiensis BT
Report Period: October 1, 1986 - September 30, 1987
Report Type: Final
Project Leaders: W. McLane, J. Finney and T. Roland

Three formulations of Dipel (Abbott) and a single one of SAN 415(SC-363-SANDOZ) were tested against mainly 3rd instar gypsy moth larvae near Breezewood, Pennsylvania. The main objectives of the project were to compare a treatment of Dipel 8L oil at 16BIU/96oz/acre with neat application at 16BIU/32oz/acre; compare Dipel 8L oil with Dipel 8L aqueous, compare Dipel 8L oil treatments of 16BIU/96oz/acre with 16BIU/2 gal/acre and compare the NRD 12 strain, SAN 415(SC-363) with the Dipel HD 1 strain.

Methods and Techniques

Twenty-four woodland plots were established on state forest and game lands along Bark Road in Buchanan State Forest and State Game Lands No. 65. 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 larval and egg mass counts under burlap, defoliation observations and pupal and parasite counts under burlaps.

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 80-90 percent.

Burlap was placed on 10 oak trees at random in the center 10 acres of all plots. 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. Readings continued until the start of pupation. New egg mass counts were made under burlaps at time of their removal.

At peak defoliation time (early July) a survey was conducted from the ground and by air. Total defoliation of all oak species was estimated at each prism point within each experimental plot. Aerial photographs were also taken of each spray plot and all checks.

An APHIS Cessna Ag-truck aircraft was used to apply all treatments. Diluted formulations were applied through 8004 and 8010 flat fan nozzles and neat applications were made with AU 5000 Micronair atomizers. The aircraft sprayed a 75-foot swath at 120 mph, approximately 50 feet above the target foliage when using flat fan nozzles. Micronair atomizer application was made at 115 mph. Eight Micronair atomizers were used and all applications were made at a VRU setting of 7. All blades were set at 35° and a pressure of 22 psi was

used based on numerous calibration runs. The inward most atomizer on each side was 53 inches from the Y strainer under the aircraft, with a distance of 36 inches between atomizers.

Calibration and characterization of each atomizer system was conducted at the airport before treatments started. Flat fan nozzles were calibrated in the air and Micronair atomizers while the plane was on the ground.

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 Bedford Airport.

Bond sticker was used at 2 percent by volume with all diluted mixes. Sticker was not used with neat applications. All formulations mixed and handled well. The Dipel 8L (Special-ABG-6158 Lot No. 04-022BR) flowed a lot faster during the actual neat application than at the time of calibration. This may have been do to a temperature change. SAN 415(SC-363) was very thick in nurse tanks. After spraying only 100 acres, there was a heavy build-up of material on most nozzle screens. Nozzle screens would have to be cleaned after every 200 acres if this formulation were used operationally.

All Dipel formulations contained daylo dye which made it much more visible on spray cards and foliage. This is a good feature for all non operational projects.

Because of extremely bad weather conditions at spray time, a number of plots were treated when larvae were larger than desirable.

Table 1. Treatment dates and stage of gypsy moth larvae within experimental spray plots.

Treatment		Date	Plot number	Larval stage (percent)			
				I	II	III	IV
Dipel 8L Aqueous	Neat	5/23/87	1-21-23	17	52	31	0
Dipel 8L Oil	Neat	5/23/87	7-10-20	17	52	31	0
Dipel 8L Oil (Special)	Neat 1/	5/23/87	4-9-12	17	52	31	0
SAN 415(SC-363)	96oz/A	5/24/87	13-15				
Dipel 8L Aqueous	96oz/A	5/28/87	5-16-22	0	30	62	8
Dipel 8L Oil	96oz/A	5/28/87	2-16-14	0	30	62	8
Dipel 8L Oil	2gal/A	5/28/87	3-8-11	0	30	62	8

1/ Special Dipel 8L oil formulation for neat applications

Table 2. Nozzle type and placement used for Bacillus thuringiensis applications.

Treatment		Nozzle type	Nozzle size	Angle	Number	VRU	Blade
Dipel 8L Aqueous	(Neat)	Micronair	AU-5000		8	7	35°
Dipel 8L Oil	(Neat)	Micronair	AU-5000		8	7	35°
Dipel 8L Oil (Special)	(Neat)1/	Micronair	AU-5000		8	7	35°
SAN 415	96oz/A	Flat Fan	8004	145°	36		
Dipel 8L Aqueous	96oz/A	Flat Fan	8004	90°	34		
Dipel 8L Oil	96oz/A	Flat Fan	8004	90°	32		
Dipel 8L Oil	2Gal/A	Flat Fan	8010	90°	34		

1/ Special Dipel 8L Oil formulation for neat applications.

Results

Table 3. Total number of larvae, Cotesia and pupae found under burlaps (based on 5 different readings) and percent defoliation recorded in each treatment.

Material	BIU/oz acre	Total larvae under burlap	Total pupae	Total Cotesia	Percent defoliation 1/
Dipel 8L Aqueous	16-32	932	180	273	38 (20)
Dipel 8L Oil	16-32	1,988	85	144	42 (20)
Dipel 8L Oil (sp)	16-32	1,641	160	131	42
SAN 415(SC-363)	16-96	982	75	131	35
Dipel 8L Aqueous	16-96	1,365	90	94	45
Dipel 8L Oil	16-96	1,393	175	125	35
Dipel 8L Oil	16-256	1,334	105	241	35 (15)
Check	-	1,595	165	30	65

1/ Figures in parenthesis represent average percent defoliation of the best 2 of the 3 replicates.

Table 4. Percent population change based on pre- and post-spray egg mass counts using the fixed and variable radius plot sampling techniques.

Material	BIU/oz acre	Pre-spray egg masses/acre	Post-spray egg masses/acre	Percent[2/] change
Dipel 8L Aqueous	16-NEAT	2,724	760	-61
Dipel 8L Oil	16-NEAT	3,221	2,104	+ 7
Dipel 8L Oil (SP)[1/]	16-NEAT	3,190	1,136	-42
SAN 415-SC 363	16-96	3,034	1,396	-29
Dipel 8L Aqueous	16-96	3,522	668	-66
Dipel 8L Oil	16-96	2,744	898	-54
Dipel 8L Oil	16-256	3,296	1,142	-42
Check	-	3,453	1,965	-43

1/ Special Dipel 8L Oil formulation for neat applications
2/ Corrected by Abbotts formula of 1925

Based on total numbers of larvae in 5 collections under burlap bands, a neat application of Dipel 8L Aqueous and SAN 415(SC-363) at 96 ounces per acre were the most effective treatments. Neat applications of Dipel 8L oil and Dipel 8L oil (special) ended up with more larvae under bands than did any other treatment including the check. Dipel at 2 gallons per acre was more effective than neat applications of Dipel 8L oil and gave similar results as that of Dipel 8L oil and Dipel 8L aqueous at 96 ounces per acre. The numbers of pupae found under burlaps did not correlate with larval data.

The number of Cotesia found under burlaps was in close relationship with the degree of control achieved with BT treatment. The prolonged period of time between moults by treated larvae gives Cotesia more time to attack larvae of suitable size and therefore results in its population increasing.

The number of Cotesia found under burlaps would indicate that the neat application of Dipel 8L aqueous and Dipel 8L oil applied at 2 gallons per acre were the most effective treatments. This is in some degree supported by defoliation estimates and egg mass reduction.

Pupae numbers do not support the parasite data. They show the SAN 415(SC-363), Dipel 8L oil applied neat and the Dipel 8L aqueous applied at 96 ounces to be the most effective treatments. In fact, based on pupal counts, the Dipel 8L aqueous applied neat would be the most effective treatment.

When all plots for each treatment are computed into average percent defoliation, there appears to be little differences between treatments. Neat applications appear to be as effective as treatments with dilute sprays. However, if one replicate of the Dipel 8L oil at 2 gallons per acre, Dipel 8L oil (standard) neat and Dipel 8L Aqueous neat are removed from the analysis, these treatments are by far superior to all other treatments. All other plots including the check plots had uniform defoliation in all replications.

Egg mass data indicate that the Dipel 8L aqueous formulation was the most effective, followed closely by a diluted application of Dipel 8L oil. Although Dipel 8L oil diluted and applied at 2 gallons per acre gave good foliage protection in 2 of 3 plots, egg mass reduction was no greater than with a standard Dipel 8L oil treatment of 96 ounces per acre. SAN 415(SC-363) gave only fair population reduction. Dipel 8L oil applied neat gave no population reduction.

When aircraft characterization was taking place, the neat aqueous formulation was producing droplets of approximately 100 micron VMD and oil about 200 micron VMD. The smaller droplets may have resulted in improved efficiency. All neat applications were made in the early morning hours so temperature and humidity should not have been a factor. Most dilute sprays were applied at mid-day or early afternoon and very well could have been affected by the weather conditions.

The tests do indicate that some formulations of BT can be used undiluted with as much success as diluted formulations. Increased rates of application can have a positive effect as well. However, these approaches still need to be tested extensively on large plots to confirm our findings. The aqueous formulation was more effective than the oil base material.

This study supports the need for more replication of given treatments and therefore a clearer picture of results.

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period: October 1, 1986 - September 30, 1987
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.

During 1987, Bacillus thuringiensis (Bt) and Dimilin were the insecticides of choice for gypsy moth control. A considerable amount of Dimilin treated woodlands were treated prior to larvae maturing to 2nd instar and with foliage development of less than 15 percent. Bt was applied at 12, 16 or 20 BIU/acre in a gallon or more of water. A new strain of Bt (Condor) was field tested on a limited basis.

New strains of Bt were tested in the laboratory as well as strains of Beauveria bassiana.

Laboratory tests were conducted with 3 Dimilin formulations. Oak seedlings and newly moulted 2nd instar gypsy moth larvae were used.

Table 1. Percent mortality os 2nd instar gypsy moth larvae and oak seedling defoliation following a 4 and 6 day exposure to foliage treated with Dimilin 25W and 2 separate samples of Dimilin 2F.

Formulation	lbs. AI/gal/ac	Inches rain	After 4 days mor.	After 4 days def.	After 6 days mor.
25W	.0312	-	49	77	100
	.0312	.25	73	81	96
	.0156	-	59	84	100
	.0078	-	63	63	99
2F	.0312	0	68	83	99
	.0312	.25	60	82	98
	.0156	-	56	81	98
	.0078	-	54	81	84
2F (Sample 2)	.0312	-	17	76	22
	.0312	.25	26	89	28
	.0256	-	0	88	1
	.0078	-	0	83	0
CHECK	-	-	0	80	1

Dimilin 2F was compared to Dimilin 25W when exposed to 1.0 inches of rain 2 hours after treatment. This study was replicated 20 times.

Table 2. Percent mortality of 2nd instar gypsy moth larvae 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 3. Percent mortality of 2nd instar gypsy moth larvae following exposure to oak foliage treated with 3 Dimilin formulations at .0312 lbs. AI/gal/acre.

Formulation	Inches rain	After 3 days mortality	After 7 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

At our request, Uniroyal made up special samples for "neat" applications. Two samples were tested in the laboratory and gave excellent results. Sample 1 was formulated at .0625 lbs. AI/qt. and Sample 2 at .0312 lbs. AI/qt.

Table 4. Percent mortality of 2nd instar gypsy moth larvae following exposure to oak foliage treated with neat applications of 2 Dimilin formulations.

Formulation	lbs. AI/qt/acre	Inches rain	After 4 days mortality	After 7 days mortality
Sample 1	.0625	-	48	100
	.0625	.5	29	100
	.0625	1.0	46	100
	.0625	2.0	52	100
	.0625	3.0	54	99
	.0625	5.0	39	99
Sample 2	.0312	-	68	100
	.0312	.5	52	98
	.0312	1.0	56	95
	.0312	2.0	70	97
	.0312	3.0	57	96
	.0312	5.0	62	99
CHECK	-	-	8	10
	-	5.0	0	0

Malathion ULV and Malathion CR (United-Ag-Products) were tested in the laboratory. Tests were conducted with our standard red oak seedling technique with 2nd instar, laboratory reared, gypsy moth larvae used as test insects. Each treatment was replicated 5 times with 20 larvae exposed to each plant. Tests were conducted at a temperature of 80° F and 55% RH.

Plants that were exposed to rain in our laboratory weathering chamber received 2 hours of drying time following treatment and before rain exposure. In some cases, a greater drying time was used, as noted.

Table 5. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following treatments of Malathion CR and Malathion ULV 91.

Material	Ounces per acre	Inches rain	After 1 day mor.	After 1 day def.	After 2 days mor.	After 2 days def.	After 4 days mor.	After 4 days def.
CR	8	-	30	16	71	18	90	24
ULV 91	8	-	35	18	61	27	79	31
CR	8	0.1	6	36	11	64	19	78
ULV 91	8	0.1	1	36	4	78	17	86
CR	8	0.5	0	58	0	84	0	92
ULV 91	8	0.5	0	50	0	92	0	100
CR	8	1.0	0	62	0	100	2	100
ULV 91	8	1.0	0	48	0	98	0	100
CR	16	1.0	0	48	2	92	7	99
CR	16	-	49	4	86	6	100	6
CHECK	-	-	0	52	0	84	0	94

Table 6. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following treatments of Malathion CR and Malathion ULV 91.

Material	Hours drying time	Inches rain	After 2 days mortality	After 2 days defoliation	After 4 days mortality	After 4 days defoliation
CR	2	-	80	4	96	4
ULV 91	2	-	80	2	99	2
CR	2	0.02	47	27	79	34
ULV 91	2	0.02	66	13	89	14
CR	2	0.05	29	27	55	36
ULV 91	2	0.05	33	43	48	50
CR	2	0.1	25	50	48	55
ULV 91	2	0.1	29	31	43	60
CR	24	0.1	-	-	89	22
ULV 91	24	0.1	-	-	29	80
CHECK	-	-	0	77	0	98

*All treatments made with 8 ounces per acre.

Table 7. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following treatments of Malathion CR and Malathion ULV 91 at 8 ounces per acre.

Material	Hours drying time	Inches rain	After 1 day		After 2 days		After 3 days		After 4 days	
			mor	def	mor	def	mor	def	mor	def
CR	4	-	21	12	39	14	70	14	96	16
CR	11	0.1	0	32	0	46	1	74	15	94
ULV 91	4	-	13	8	31	10	54	21	78	26
ULV 91	4	0.1	0	52	1	80	2	98	8	100
CR	8	-	14	5	24	15	38	20	69	69
CR	8	0.1	0	34	0	48	1	75	6	100
ULV 91	8	-	14	8	24	14	43	16	68	41
ULV 91	11	0.1	0	30	2	56	2	72	5	96
CR	16	-	8	23	13	42			35	92
CR	16	0.1	1	43	1	63			11	98
ULV 91	16	-	25	4	67	4			100	4
ULV 91	16	0.1	3	32	4	62			6	100
CR	20	-	7	16	32	18			64	58
CR	20	0.1	3	26	6	36			20	96
ULV 91	20	-	16	6	52	7			87	21
ULV 91	20	0.1	0	34	1	80			5	98
CHECK	4	-	0	58	0	86	0	98	14	100
CHECK	8	-	0	40	0	75	0	95	4	100
CHECK	16	-	0	48	0	86			0	100

It would appear that Malathion CR weathered no better than Malathion ULV. However, when not exposed to rainfall, Malathion CR was as active as Malathion ULV at half the dosage. This would be a great cost savings when treating substantial acreage. Both formulations weathered poorly, even when given 20 hours of drying time. It also appeared that treatments not exposed to rainfall but to extended drying time had less activity.

Laboratory tests were conducted with AC 290-230, a product of American Cyanamid.

Table 8. Percent mortality of gypsy moth larvae and seedling defoliation after exposure to seedlings treated with AC 290-230.

lbs. AI/gal acre	Inches rain	After 2 days		After 4 days		After 6 days	
		mor	def	mor	def	mor	def
1.0	-	0	7	5	7	85	7
.5	-	0	13	5	14	88	14
.5	.10	0	18	8	21	99	21
.5	.25	0	21	15	22	97	22
.25	-	0	31	6	31	94	33
.25	.10	0	21	11	21	100	21
.25	.25	0	30	6	31	97	31
.125	-	0	35	10	41	94	42
.125	.10	0	31	22	37	98	40
.125	.25	0	25	24	34	95	39
.062	-	0	48	16	52	97	52
.062	-	0	29	8	32	94	32
.062	.10	0	30	31	40	100	54
.062	.25	0	30	28	40	96	45
.031	-	1	33	11	34	96	35
.031	.10	0	32	29	40	96	54
.031	.25	0	46	24	52	91	68
.015	-	0	45	5	45	92	51
.015	.10	0	40	18	43	89	49
.015	.25	0	52	25	52	61	52
.007	-	0	60	6	64	68	80
.003	-	0	72	4	82	26	-
CHECK	-	0	81	0	100	0	100
CHECK	-	1	58	1	96	1	100
CHECK	-	0	70	0	100	0	100

Data indicate that AC 290-230 is very effective against laboratory reared 2nd instar gypsy moth larvae at dosages as low as .015 lbs AI/gal/acre. When treated oak seedlings were exposed to .10 and .25 inches of rainfall, 2 hours after treatment, no loss of material occurred with dosages as low as .031 lbs. AI/gal/acre.

A series of tests were conducted with UC-84572, a Union Carbide growth regulator.

Table 9. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following exposure to seedlings treated with various amounts of UC-84572.

Pounds of AI/gal/acre	Percent mortality after 5 days	Percent mortality after 7 days	Percent defoliation after 2 days
.25	35	100	74
.125	41	100	64
.0625	31	100	66
.0312	34	100	56
.0156	35	100	70
.0078	23	85	62
.0039	12	42	66
CONTROL	0	0	68

Table 10. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following exposure to oak seedlings treated with UC-84572 and exposed to rainfall.

Pounds of AI/gal/acre	Inches rain	Percent mortality after 5 days	Percent mortality after 7 days	Percent defoliation after 4 days
.125	-	6	32	34
.125	1.0	28	84	84
.125	.5	34	56	46
.125	.25	16	34	44
.062	-	32	83	66
.062	1.0	22	93	68
.062	.5	26	67	56
.062	.25	32	73	52
.031	-	14	38	50
.031	1.0	25	73	60
.031	.5	12	44	38
.031	.25	16	28	60
CONTROL	-	1	2	42

A limited test was conducted with Mavrik, a Zoecon product.

Table 11. Percent mortality of 2nd instar gypsy moth larvae after their exposure to oak seedlings treated with various dosages of Mavrik.

Pounds of AI/gal/acre	Percent mortality after		
	1 day	3 days	5 days
.25	2	11	27
.125	4	4	4
.062	1	4	5
.031	3	3	3
.015	0	0	0
CONTROL	0	0	0

Table 12. Percent mortality of 2nd instar gypsy moth larvae after their exposure to oak seedlings treated with Mavrik and exposed to rainfall.

Pounds of AI/gal/acre	Inches rain	Percent mortality after		Percent defoliation after 6 days
		2 days	6 days	
1.0	-	0	74	1
1.0	.25	1	72	4
0.5	-	0	33	3
0.5	.25	0	44	1
0.25	-	0	90	1
0.25	.25	0	1	1
0.12	-	0	2	2
0.12	.25	1	30	1
.06	-	0	29	5
.06	.25	0	21	6
CONTROL	-	0	0	100

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

Table 13. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with various Bt strains at 8 BIU/gal/acre.

Strain number	Percent mortality after 3 days	Percent mortality after 4 days	Percent defoliation after 3 days
3186-17	8	11	50
3186-18	14	25	47
3186-19	6	10	52
3186-20	6	13	48
3186-21	18	33	39
3186-22	10	21	32
307	29	55	28
309	23	44	39
316	20	36	31
321	25	48	39
323	40	67	19
Thuricide 48LV	14	31	19
Control	0	0	92

Table 13 represents the average of 2 tests.

A Bt formulation prepared by Jack Henderson was tested in the laboratory.

Table 14. Percent mortality of 2nd instar gypsy moth larvae and percent defoliation of red oak seedlings following exposure to Bacillus thuringiensis.

Formulation	BIU/gal	Inches rain	After 4 days mortality	After 4 days defoliation	After 6 days mortality	After 6 days defoliation
Henderson	2	-	28	22	50	-
Henderson	2	.1	0	98	4	-
Henderson	2	.25	0	97	2	-
Henderson	2	.5	0	90	4	0
Dipel-6222	2	-	2	96	7	-
Dipel-6222	2	.1	1	89	3	-
Dipel-6222	2	.25	2	80	3	-
Dipel-6222	2	.5	0	83	2	-
Dipel-6222	12	-	7	67	19	-
Dipel-6222	12	.1	8	51	21	-
Dipel-6222	12	.25	3	95	11	-
Dipel-6222	12	.5	3	60	16	-
Control	-	-	0	100	0	-

Two new strains from Mycogen were tested in the laboratory.

Table 15. 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
MTX-5411	8 BIU/96 oz	2	6	27	8
MTX-5411	12 BIU/96 oz	3	5	28	5
MTX-7275	3 BIU/gallon	0	20	32	30
MTX-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

A series of weathering tests with Dipel and 1545 sticker were conducted in the laboratory. Sticker was mixed with Bt and water at a rate of 1-2-3-4-5-6-8-10-15 percent of total mix by volume. Spray was applied to red oak seedlings and exposed to 2nd instar gypsy moth larvae. Each treatment was replicated 5 times with untreated plants used as checks. Treated plants were allowed to dry for 2 hours before being exposed to rain from raindrop nozzles. Plants were held at 80° F and 50% RH for the duration of the tests. All mortality and defoliation readings were made following a 4-day exposure of test insects to treated seedlings. In all treatments, Bt was applied at 16 BIU/96 ounces/acre.

Table 16. 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 17. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4-day exposure to oak seedlings treated with Bt and 2% 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 18. 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

Five tank mix samples of Bt were tested for the USDA, FS as part of their 1987 spray project at the Allegheny National Forest.

Table 19. Mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure to red oak seedlings treated with Bacillus thuringiensis.

Material	Dosage/rate BIU/gal/acre	Percent mortality	Percent defoliation
Tank #1	16	70	22
Tank #2	16	85	12
Tank #3	16	70	24
Lot #4	16	40	44
Tank #5	16	54	29
Dipel 8L Standard	16	51	36
Thuricide 48LV Standard	16	89	2
CHECK	-	1	98

Three Bt samples were tested for the California Department of Food and Agriculture.

Table 20. Percent mortality of 2nd instar gypsy moths larvae and seedling defoliation after exposure to seedlings treated with Bacillus thuringiensis.

	12 BIU/gal/acre				16 BIU/gal/acre				20 BIU/gal/acre			
	4 1/		6 1/		4 1/		6 1/		4 1/		6 1/	
	Mor	Def	Mor	Def	Mor	Def	Mor	Def	Mor	Def	Mor	Def
Thuricide 32LV (STD)	43	20	92	24	48	12	91	14	52	9	90	21
Dipel 8L (STD)	25	30	59	37	37	30	86	39	40	23	69	31
Cal. 51201-A1	44	9	84	20	47	6	94	10	61	6	94	8
Cal. 51201-A2	51	9	81	10	63	4	90	5	72	7	93	10
Cal. 50611	52	29	89	31	51	7	89	7	62	5	91	6
CHECK	0	96	0	100								

1/ Days of gypsy moth exposure to treated seedlings.

Three samples of Bt were tested for the National Park Service, Capitol Region.

Table 21. Percent mortality of gypsy moth larvae and seedling defoliation following a 5 and 6 day exposure to seedlings treated with samples of Dipel 8L (oil).

	12 BIU/96 oz./acre				16 BIU/96 oz./acre			
	5 days		6 days		5 days		6 days	
Sample	M	D	M	D	M	D	M	D
#1	21	25	32	40	50	17	70	19
#2	14	48	27	48	54	29	61	41
#3	4	60	11	81	2	66	12	74
Dipel 8L (STD)	41	39	52	54	39	22	51	25
CHECK	0	100			0	100		

Dipel samples from the 1987 experimental field work in Pennsylvania were tested.

Table 22. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following an exposure to seedlings treated with Bt.

	Dosage	Percent mortality		Percent defoliation	
Formulation	BIU/gal/acre	4 days	5 days	4 days	5 days
Dipel 8L Field	8	10	42	52	61
Dipel 8L Field	12	26	51	29	38
Dipel 8L Field	16	35	58	29	40
Dipel 8L (SP) Field	8	2	16	87	94
Dipel 8L (SP) Field	12	4	22	78	88
Dipel 8L (SP) Field	16	29	47	39	46
Dipel 8AF Field	8	17	44	63	69
Dipel 8AF Field	12	28	69	35	37
Dipel 8AF Field	16	49	85	22	24
Dipel 8L Lab	8	11	27	44	58
Dipel 8L Lab	12	21	44	36	48
Dipel 8L Lab	16	27	49	26	30
Control	-	0	2	80	95

Javelin, SAN 415(SC363) and Dipel 8L were tested at various dosages against 2nd and 3rd instar gypsy moth larvae.

Table 23. Percent mortality of gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with various Bt formulations.

Formulation	Dosage BIU/96oz/acre	Percent mortality II instar	Percent mortality III instar	Percent defoliation II instar	Percent defoliation III instar
Javelin	4	47	56	16	21
Javelin	8	53	78	12	8
Javelin	12	90	90	3	6
SAN 415(SC363)	4	70	72	11	26
SAN 415(SC363)	8	88	80	3	10
SAN 415(SC363)	12	89	80	4	9
Dipel 8L	4	12	5	41	100
Dipel 8L	8	25	13	41	94
Dipel 8L	12	37	42	14	35
Control	-	3	0	100	100

A series of tests were conducted with Bt to determine the number of droplets CM^2 needed to achieve good efficacy.

Table 24. Percent mortality of gypsy moth larvae and seedling defoliation following a 4-day exposure to oak seedlings treated with Bt at 1, 3 and 5 droplets per cm^2 at 16 BIU/96oz./acre.

Formulation	Instar	Percent mortality 1 cm^2	Percent mortality 3 cm^2	Percent mortality 5 cm^2	Percent defoliation 1 cm^2	Percent defoliation 3 cm^2	Percent defoliation 5 cm^2
Thuricide 48LV	I	27	25	63	20	30	4
Thuricide 48LV	II	55	70	58	30	45	15
Thuricide 48LV	III	48	-	75	27	-	22
Thuricide 48LV	IV	60	47	53	7	27	10
Dipel 8L	I	10	33	73	27	23	2
Dipel 8L	II	50	55	97	8	35	20
Dipel 8L	III	35	-	96	80	-	25
Dipel 8L	IV	87	80	83	23	8	18
Control	I	0	0	0			55
Control	II	0	0	0			97
Control	III	0	0	0			100
Control	IV	0	0	0			100

Weathering tests were conducted with Thuricide 48LV, Dipel 8L and SAN 415(SC363).

Table 25. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with Bt at 12 BIU/96 oz./acre and exposed to rainfall.

Formulation	Inches rainfall	Percent mortality after 4 days	Percent defoliation after 4 days
SAN 415(SC363)	-	89	5
SAN 415(SC363)	.1	54	28
SAN 415(SC363)	.5	41	46
Thuricide 48LV	-	75	14
Thuricide 48LV	.1	24	31
Thuricide 48LV	.5	44	58
Dipel 8L	-	50	36
Dipel 8L	.1	12	74
Dipel 8L	.5	39	44
Control	-	0	99

A number of samples from a Western Spruce Budworm project were bioassayed.

Table 26. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with various Bt formulations used on the 1987 Western Spruce Budworm Project.

Material	Sample number	Dosage BIU/gal/acre	Percent mortality	Percent defoliation
Dipel 6L	1	12	22	42
Dipel 6L	1	16	42	36
Dipel 6L	2	12	22	36
Dipel 6L	2	16	29	36
Dipel 6L	3	12	24	34
Dipel 6L	3	16	36	36
Dipel 6L	4	12	40	38
Dipel 6L	4	16	33	15
Dipel 8L Std.	Lab	12	31	36
Dipel 8L Std.	Lab	16	47	30
Thuricide 48LV	Lab	12	71	5
Thuricide 48LV	Lab	16	55	14
Thuricide 48LV	43	12	75	4
Thuricide 48LV	62	11	82	2
Thuricide 48LV	68	11	77	2
Thuricide 48LV Std.	Lab	11	67	2
Dipel 8L Std.	Lab	11	34	27
Control	-	-	2	96

Table 27. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with various Bt formulations used on the 1987 Western Spruce Budworm project.

Material	Sample number	Dosage BIU/gal/acre	Percent mortality	Percent defoliation
Thuricide 48LV	1311467	12	87	2
Thuricide 48LV	1311467	16	83	2
Thuricide 48LV	1321467	12	84	2
Thuricide 48LV	1321467	16	79	2
Thuricide 48LV	1351467	12	75	3
Thuricide 48LV	1351467	16	70	2
Thuricide 48LV	1331467	12	81	1
Thuricide 48LV	1331467	16	89	1
Thuricide 48LV	1361467	12	62	3
Thuricide 48LV	1361467	16	76	2
Thuricide 48LV	1341467	12	81	2
Thuricide 48LV	1341467	16	89	2
Thuricide 48LV Std.	-	12	83	1
Thuricide 48LV Std.	-	16	84	1
Dipel 8L	-	12	32	32
Dipel 8L	-	16	69	26
Control	-	-	1	68

Bacillus thuringiensis formulations are adversely affected by a number of environmental factors when sprayed by aircraft onto forest foliage to control gypsy moth larvae and other insects. Rainfall can cause wash-off of material and reduced insect control. Sunlight can cause the breakdown of active material and loss of efficacy. It is, therefore, important to know the amount of exposure time needed by the target insects to generate suitable control. During this reporting period, extensive laboratory testing was conducted to identify the minimum exposure needed to give suitable kill of gypsy moth larvae.

Tender oak seedlings were sprayed in a laboratory spray chamber and exposed to various instar gypsy moth larvae for short periods of time. Larvae were then removed from the treated seedlings and reared on artificial diet for a number of days. Mortality readings were made over a period of time. Three commercial Bt formulations were tested at various dosages.

Based on the data collected, gypsy moth larvae need only short periods of exposure time to Bt treated foliage for suitable mortality to occur. Therefore, poor field results may not be due as much to wash-off and ultraviolet degradation as to poor original coverage of the target foliage.

Table 28. Percent mortality of 1st instar gypsy moth larvae following limited exposure time to oak foliage treated with Dipel 8L, Thuricide 48LV and SAN 415 at 12 BIU/96oz./acre and then reared on clean artificial diet.

Material	Exposure time	Percent mortality after 2 days	4 days	6 days	10 days	12 days	14 days
Dipel 8L	8 hours	26	37	39	47	47	49
Dipel 8L	18 hours	20	44	54	73	76	82
Dipel 8L	24 hours	19	43	56	65	72	74
Thuricide 48LV	8 hours	36	57	62	65	69	70
Thuricide 48LV	18 hours	38	69	85	93	94	95
Thuricide 48LV	24 hours	24	60	76	87	92	95
SAN 415(NRD 12)	8 hours	33	48	53	59	60	63
SAN 415(NRD 12)	18 hours	40	89	97	97	98	99
SAN 415(NRD 12)	24 hours	34	83	90	97	97	98
Control	8 hours	0	0	0	0	0	0
Control	18 hours	0	2	2	6	6	8
Control	24 hours	0	3	3	4	5	5

Table 29. Percent mortality of 2nd instar gypsy moth larvae following limited exposure time to oak foliage treated with Dipel 8L, Thuricide 48LV and SAN 415 at 12 BIU/96 oz./acre and then reared on clean artificial diet.

Material	Exposure time	Percent mortality after 2 days	4 days	6 days	10 days	12 days	14 days
Dipel 8L	8 hours	27	38	50	70	77	81
Dipel 8L	18 hours	26	75	87	96	97	99
Dipel 8L	24 hours	9	21	51	80	90	94
Thuricide 48LV	8 hours	37	54	58	68	70	73
Thuricide 48LV	18 hours	36	68	85	95	97	98
Thuricide 48LV	24 hours	11	30	49	67	76	80
SAN 415(NRD 12)	8 hours	57	70	75	85	88	91
SAN 415(NRD 12)	18 hours	32	77	93	99	100	100
SAN 415(NRD 12)	24 hours	26	56	77	89	91	96
Control	8 hours	0	0	1	1	1	1
Control	18 hours	0	1	2	3	5	10
Control	24 hours	0	0	1	1	2	2

Table 30. Percent mortality of 3rd instar gypsy moth larvae following an 8 hour exposure time to oak foliage treated with Dipel 8L, Thuricide 48LV and SAN 415 at 12 BIU/96 oz./acre and then reared on clean artificial diet.

Material	Percent mortality after 2 days	4 days	6 days	10 days	12 days	14 days
Dipel 8L	23	44	58	85	93	98
Thuricide 48LV	9	34	48	67	76	88
SAN 415(NRD 12)	32	50	58	81	95	98
Control	0	0	0	0	0	1

Table 31. Percent mortality of 2nd instar gypsy moth larvae following limited exposure time to oak foliage treated with Dipel 8L at 8 and 12 BIU/96 oz./acre and then reared on clean artificial diet.

Dosage BIU/96 oz/a	Exposure time	Percent mortality after 2 days	4 days	6 days	10 days	12 days	14 days
8	15 min.	9	32	43	53	56	57
8	30 min.	9	31	40	63	65	67
8	45 min.	6	28	43	58	64	65
8	60 min.	12	21	26	35	38	42
8	120 min.	3	17	24	34	40	45
12	15 min.	9	36	59	71	72	72
12	30 min.	11	44	55	64	66	68
12	45 min.	5	33	39	47	50	51
12	60 min.	6	25	34	38	40	42
12	120 min.	5	22	26	35	36	41
Control	15 min.	0	1	2	2	2	3
Control	30 min.	0	2	4	4	5	6
Control	45 min.	0	2	5	7	8	9
Control	60 min.	0	2	6	6	6	6
Control	120 min.	1	4	6	6	6	6

Table 32. Percent mortality of 2nd instar gypsy moth larvae following limited exposure time to oak foliage treated with Dipel 8L at 16 and 20 BIU/96 oz./acre and then reared on clean artificial diet.

Dosage BIU/96 oz/a	Exposure time	Percent mortality after 2 days	4 days	6 days	10 days	12 days	14 days
16	15 min.	17	56	80	97	99	99
16	30 min.	17	48	64	77	83	86
16	45 min.	3	26	39	47	51	53
16	60 min.	7	33	45	55	58	62
16	120 min.	17	52	68	81	83	86
20	15 min.	19	68	84	92	96	98
20	30 min.	15	61	78	91	94	95
20	45 min.	17	58	67	84	86	86
20	60 min.	8	47	64	86	91	96
20	120 min.	20	67	78	87	90	91
Control	15 min.	0	1	2	2	2	3
Control	30 min.	0	2	4	4	5	6
Control	45 min.	0	2	6	6	6	6
Control	120 min.	1	4	6	6	6	6

Table 33. Percent mortality of 3rd instar gypsy moth larvae following limited exposure time to oak foliage treated with Dipel 8L at 8 and 12 BIU/96 oz./acre and then reared on clean artificial diet.

Dosage BIU/96 oz/a	Exposure time	Percent mortality after 2 days	4 days	6 days	10 days	12 days	14 days
8	15 min.	0	18	29	49	66	75
8	30 min.	0	1	5	8	18	21
8	45 min.	0	6	9	35	46	58
8	60 min.	0	1	1	5	-	-
8	120 min.	1	6	9	37	-	-
12	15 min.	1	9	15	32	46	69
12	30 min.	0	3	3	5	6	13
12	45 min.	1	10	18	41	54	57
12	60 min.	0	20	40	61	-	-
12	120 min.	3	18	39	69	-	-
Control	15 min.	0	1	3	3	3	17
Control	30 min.	0	0	0	0	0	0
Control	45 min.	0	0	0	0	0	0
Control	60 min.	0	1	1	1	-	-
Control	120 min.	0	0	0	0	-	-

Table 34. Percent mortality of 3rd instar gypsy moth larvae following limited exposure time to oak foliage treated with Dipel 8L at 16 and 20 BIU/96 oz./acre and then reared on clean artificial diet.

Dosage BIU/96 oz/a	Exposure time	Percent mortality after 2 days	4 days	6 days	10 days	12 days	14 days
16	15 min.	4	42	59	83	92	99
16	30 min.	0	30	46	67	76	87
16	45 min.	2	11	15	47	52	64
16	60 min.	0	20	44	83	-	-
16	120 min.	4	29	48	86	-	-
20	15 min.	2	50	65	93	94	99
20	30 min.	1	38	62	84	86	91
20	45 min.	2	15	26	83	86	94
20	60 min.	5	28	66	95	-	-
20	120 min.	3	18	47	84	-	-
Control	15 min.	0	1	3	3	3	17
Control	30 min.	0	0	0	0	0	0
Control	45 min.	0	0	0	0	0	0
Control	60 min.	0	1	1	1	-	-
Control	120 min.	0	0	0	0	-	-

Table 35. Percent mortality of 4th instar gypsy moth larvae following limited exposure time to oak foliage treated with Dipel 8L at 16 and 20 BIU/96 oz./acre and then reared on clean artificial diet.

Dosage BIU/96 oz/a	Exposure time	Percent mortality after 2 days	4 days	6 days	10 days	12 days	14 days
16	15 min.	0	9	24	44	51	61
16	30 min.	1	30	59	86	98	100
16	45 min.	1	17	34	59	67	76
16	60 min.	0	6	27	51	64	68
16	120 min.	0	8	26	49	59	68
16	180 min.	1	10	59	81	85	95
16	240 min.	0	17	35	71	81	96
20	15 min.	0	17	51	75	84	88
20	30 min.	2	27	62	87	98	100
20	45 min.	2	35	75	95	97	99
20	60 min.	1	40	69	71	79	80
20	120 min.	1	40	79	100		
20	180 min.	1	53	92	100		
20	240 min.	0	40	80	100		
Control	15 min.	0	0	2	5	6	6
Control	30 min.	0	1	2	2	2	2
Control	45 min.	0	0	0	0	0	1
Control	60 min.	0	0	0	1	1	1
Control	120 min.	0	0	0	0	0	0
Control	180 min.	0	0	0	0	0	0
Control	240 min.	0	0	0	0	0	0

In all previous exposure tests, larvae were exposed to treated foliage within 4 hours after seedlings were treated. A test was conducted where treated seedlings were held for 24 hours after treatment before larvae were exposed to the treated surface for short periods of time.

Table 36. Percent mortality of 2nd instar gypsy moth larvae following limited exposure time to oak foliage treated 24 hours previously with Dipel 8L at 12 BIU/192 oz./acre and then reared on clean artificial diet.

Exposure time	2 days	4 days	6 days	10 days	12 days	14 days
15 minutes	17	38	45	60	67	72
30 minutes	2	38	47	61	71	75
45 minutes	6	26	35	56	61	65
60 minutes	6	18	39	41	45	48
Left on plants	11	33	66			
Control 15 min.	0	1	1	2	3	4
Control 30 min.	0	1	3	3	5	5
Control 45 min.	0	2	3	6	7	8
Control 60 min.	0	0	0	0	1	1

Cedar oil was sprayed and brushed onto tender oak seedlings and exposed to 2nd instar gypsy moth larvae. No larvae mortality occurred when exposed to seedlings that were sprayed with Cedar oil in a spray chamber. Fifty percent of the larvae died when exposed to seedlings treated by brush with the undiluted Cedar oil concentrate. However, when brushed onto foliage, heavy phytotoxicity occurred. Phytotoxicity also occurred when plants were sprayed in a laboratory spray chamber.

Project Number: GM 5.2.1
Project Title: Comparison of 1987 Disparlure Dispensers with Various Concentrations of Lure.
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leader: E. C. Paszek and V. C. Mastro

The standard 1987 Hercon 500ug pheromone dispenser, Lot D0117, which is used to survey large areas for gypsy moth infestation, was compared in Milk Carton Traps freshly baited with Hercon dispensers of the following concentrations of lure: 63, 125, 250, 1000, 2000, 3000, 4000 and 5000ug. Ten, 100 and 1000ug concentrations of Meyer-Swartz, USDA Standard lure on cotton wicks were used as controls. Eight replicates of these treatments were bioassayed in a grid arrangement, 50m apart in 4 blocks in the Crane Wildlife Management Area of Falmouth. Traps were read 4 times and after each daily reading, they were randomized in a new block.

Table 1. Disparlure dispenser comparison 1987. Mean[1/] numbers of males captured per day per trap when traps were baited with various concentrations of lure.

Dispenser type	Dispenser size - cm	ug lure/ dispenser	N=	X	S. E.
1/8 Hercon	4x3.1	63	32	22.53a	3.14
1/2 Hercon	4x12.4	250	32	15.66ab	2.38
Cotton Wick	(Meyer-Swartz)	10	32	14.69 bc	2.72
1 Hercon	4x25	500	31	14.84 bc	2.66
2 Hercon	2(4x25)	1000	32	18.91 bc	3.96
1/4 Hercon	4x6.2	125	29	17.07 bcd	4.73
4 Hercon	4(4x25)	2000	32	17.66 bcd	5.19
Cotton Wick	(Meyer-Swartz)	100	32	13.69 bcde	2.87
10 Hercon	10(4x25)	5000	32	10.88 cde	3.13
8 Hercon	8(4x25)	4000	32	10.00 de	3.11
6 Hercon	6(4x25)	3000	32	8.63 e	2.17
Cotton Wick	(Meyer-Swartz)	1000	32	9.22 e	2.48
Blank		0	32	0.59 f	0.20

1/ Analysis performed on transformed data log (males +1), actual mean numbers of males captured per trap per day is presented. A general liner model analysis was performed on the data using the readings as the blocking variable and collapsing the replicates within a day. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level of significance. In all, 8 replicates of each treatment were placed within a block and read and randomized 4 times.

Results of this lure comparison are presented in Table 1. Traps with freshly baited Hercon pheromone dispensers in concentrations from 63ug to 2000ug captured more moths than traps with 3000 to 5000ug concentrations. the traps baited with 10ug cotton wick Meyer-Swartz control captured more moths than the 100ug cotton wick. The 1000ug cotton wick captured the smaller number of moths. High concentration of lure in freshly baited traps with Hercon Dispensers and cotton wicks capture fewer moths than traps baited with low concentrations of lure. High concentrations of lure act as a repellant to gypsy moths.

Project Number: GM 5.2.4
Project Title: Sterile F_1 Demonstration Project - Darke Co., Ohio
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leaders: V. C. Mastro, C. P. Schwalbe and A. Pellegrini-Toole
Cooperators: R. Barth, K. Roach and D. Gerold

Details of this project were presented in the 1985 (pp 108-111) and 1986 (pp 71-73) annual reports. To summarize the results to date, a single release of F_1 egg masses was made in 1985. Sampling by standard evaluation techniques used in F_1 release sites produced conflicting sterile to fertile ratios. But sampling of immature males indicated that overflooding rates were greater than 100 F_1:1 wild. Based on these data, the site was only monitored with traps (36/sq. mi.) in 1986 and 1987. Respectively, in these two years 2 and 1 males were captured at the same trap location. Although this is a marked reduction from the number of males captured in 1984 (the year prior to treatment), the possibility of a residual population exists. In 1988 to determine if there is a viable population an area of approximately 20 acres will be mass trapped at a rate of 9 traps/acre. Surrounding this will be a standard 36/sq. mi. trapping grid.

Project Number: GM 5.2.3
Project Title: Sterile F_1 Demonstration Project - Bellingham, WA
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leaders: V. C. Mastro, C. P. Schwalbe, A. Pellegrini-Toole and P. Stevenson
Project Cooperators: Eric Lagasa, Washington State Department of Agriculture

This project was initiated in 1985 when approximately 34,000 F_1 egg masses were released. In 1986 an additional release of 12,769 F_1 egg masses was made. Table 1 summarizes the results of monitoring both the 1985 and 1986 releases. The results in 1986 strongly suggested that the probability of eradication was high, therefore, in 1987 no F_1 release was made and the area was trapped at the rate of 36 delta traps/sq. mi. No males were trapped in 1987. The same area will be trapped at the same rate in 1988 to confirm eradication.

Expected and Observed Results of Releasing F_1 Progeny of Irradiated Males (10 krad) in an Isolated Gypsy Moth Population in Bellingham, WA.

Method of Estimation	1984	1985	1986	1987
Expected wild egg mass density	--	400	8	--
No. of F_1 egg masses released[1/]	0	13,600	5,108	0
Expected male overflooding ratio	--	34:1	640:1	--
Observed male ratio:				
Chromosome analysis	--	32:1	5:0	--
Mating evaluation	--	80:1	24:1	--
Monitor female fertilization	--	18:1	116:1	--
Observed female ratio:				
Mating evaluation	--	27:1	13:1	--
Field collected egg masses	--	26:1	no data	--
No. of males trapped	82	869	244	0
Estimated male population	400	9,549	2,681	0

1/ No. of egg masses presented in wild egg mass equivalents, actual numbers respectively, for 1985 and 1986 were 34,000 and 12,769 F_1 egg masses.

Project Number: GM 6.2.1
Project Title: Sterile F_1 Demonstration Project - Southern Shores, Dare Co., North Carolina
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leaders: V. C. Mastro, A. Pellegrini-Toole and C. P. Schwalbe
Cooperators: B. Wescott, H. Singletary, K. Wilson, S. Smitherman and T. ODell

The objectives of this project and preliminary data from activities in 1986 were described in the last annual report (pp 75-87). This report will summarize activities in 1986 and present partial data from work conducted in 1987. Based on results of the 1986 F_1 release, a second release was made in 1987. Similar to the release made in Gates Co., because of the uncertainties of when to expect native hatch, egg masses were distributed on two dates. On March 25 (JD 84) and April 13 (JD 103) respectively, 47,182 and 43,345 egg masses were distributed in the Southern Shores site. The early release followed the pattern of distribution used in 1986 while the second release distributed egg masses only in areas where in 1986 wild insects were found through sampling. Monitoring techniques used in 1987 were similar to those used in 1987 including egg hatch synchrony, larval and pupal sampling, placement of monitor females, male trapping and post season egg mass evaluation.

Table 2 presents the final results of the 1986 field release and preliminary results of the 1987 releases. Generally, the overflooding ratios projected by the different monitoring techniques agree, however, overflooding ratios projected by monitor females and post season egg mass data are significantly different (5% level). As in other sites, the ratio established using monitor female data is lower than ratios arrived at by sampling immature males. Prior to release at this site in 1986, we estimated that the wild egg mass population was approximately 175-200. At this density the 1986 release should have achieved a 100 F_1:1 wild ratio in adult males. Clearly, this ratio was not achieved. In the last annual report (GM 6.2.1, pp. 75-87) the possible reasons for not achieving this ratio were explored. Post season egg mass data presented for the first time in this report projects a very high overflooding rate. Again, the discrepancy between this ratio and others is unexplained.

In 1987 the split release strategy produced two distinct peaks of F_1 hatch (figure 1). However, neither group was in perfect synchrony with "wild" hatch. No native wild egg mass within the site could be located for synchrony of hatch comparisons, therefore, five wild egg masses from an infestation in Currituck Co., North Carolina, were moved into the site the week before the first F_1 release. It is not known if these eggs hatched with the same periodicity as on site wild egg masses.

Between lots of F_1 egg masses there was a wide range of initiation of hatch and peak hatch. Egg masses (code 26-29) from a production which were held under a normal diapausing temperature of 7°C for 37 days and then transferred to 5°C for the remaining 145 days (total=182 days) prior to release (JD 84) displayed peak hatch approximately 14 days before peak wild hatch. A lot of egg masses (code 30) from the same production date, but released on JD 103, initiated hatch at the same time as wild egg masses (figure 2) but peak hatch occurred five days later. The viability of these and all production lots of egg masses is presented in Table 1. In the case of the egg masses from lot code 30, held for an additional 19 days at 5°, hatch was apparently no lower than for egg masses produced on the same date but released earlier (lot codes 26, 27, 29).

Similarly, egg masses from production lot 217T were released on the two dates. Again, neither group hatched in perfect synchrony with the feral egg masses (figure 3) and apparently there was no significant reduction in egg hatch associated with holding the second lot (141) an additional 19 days at 7°C. Perhaps, surprisingly, neither of these groups of eggs displayed a very sharp hatch peak given the optimum chilling period. In a third example, the egg masses released on Julian Date 103 (second release) were in better hatch synchrony with wild egg masses than the first release (figure 4). Percentage hatch, however, for the eggs released second (lot 161) was lower than those released earlier (lots 159, 160, Table 1). Generally hatch was lower than expected from most lots of egg masses, yielding fewer F_1 neonates than expected. Overall, this resulted in approximately a 22% decrease in the number of neonates available to establish and develop. If there were no additional discrepancies between actual and theoretical survival rates, this alone would reduce overflooding rates by nearly a quarter.

Table 1. Hatch from samples of production lots of F_1 egg masses released in Southern Shores.

Production Codes	Production lot	Diapause holding condition days	temp C	Release date	Percent of total eggs hatched
26, 27, 29	192T	37/145	7°/5°	84	20.7
30	192T	37/165	7°/5°	103	24.8
136,137,138	216T	159	7°	84	26.6
141	217T	179	7°	103	38.9
139,140,142	217T	159	7°	84	37.2
151,152	218T	155	7°	84	32.3
153,154,155	218B	175	7°	103	27.4
156,157,158	219B	155	7°	84	36.2
159,160	220T	155	7°	84	36.5
161	220T	175	7°	103	22.5
186,187,188	226T	168	7°	103	28.6
196,197	230T	165	7°	103	31.5
211,212,213	232B	141	7°	84	37.6
214,215,216	233T	141	7°	84	29.7
230,231,232	238B	157	7°	103	34.3
234,235	239B	157	7°	103	35.8
272,273	247B	147	7°	103	23.9
276	248B	146	7°	103	21.6
277	248B	126	7°	84	30.4
283,284	252T	143	7°	103	47.8
285,286	253B	143	7°	103	32.0
Mean of all F_1s					31.3
Wild					79.3

Table 2. A comparison of evaluation techniques used for estimating overflooding ratios in Southern Shores, Dare Co., North Carolina in 1986 and 1987.

Techniques	1986 F_1:wild ratio	1986 Percent Fertile	1987 F_1:wild ratio	1987 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	Incomplete	
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.

Overflooding estimates established in 1987, using data obtained from males collected as immatures, indicate high overflooding rates were achieved. Although the ratio achieved using data from chromosome analysis is larger than the ratio established from mating data, they are not significantly different (Chi Square, 5% level). The ratio established using monitor female data is significantly different from ratios established using male data. This large difference is unexplained, but this difference was noted in all sites where F_1 females were placed as monitors in 1987.

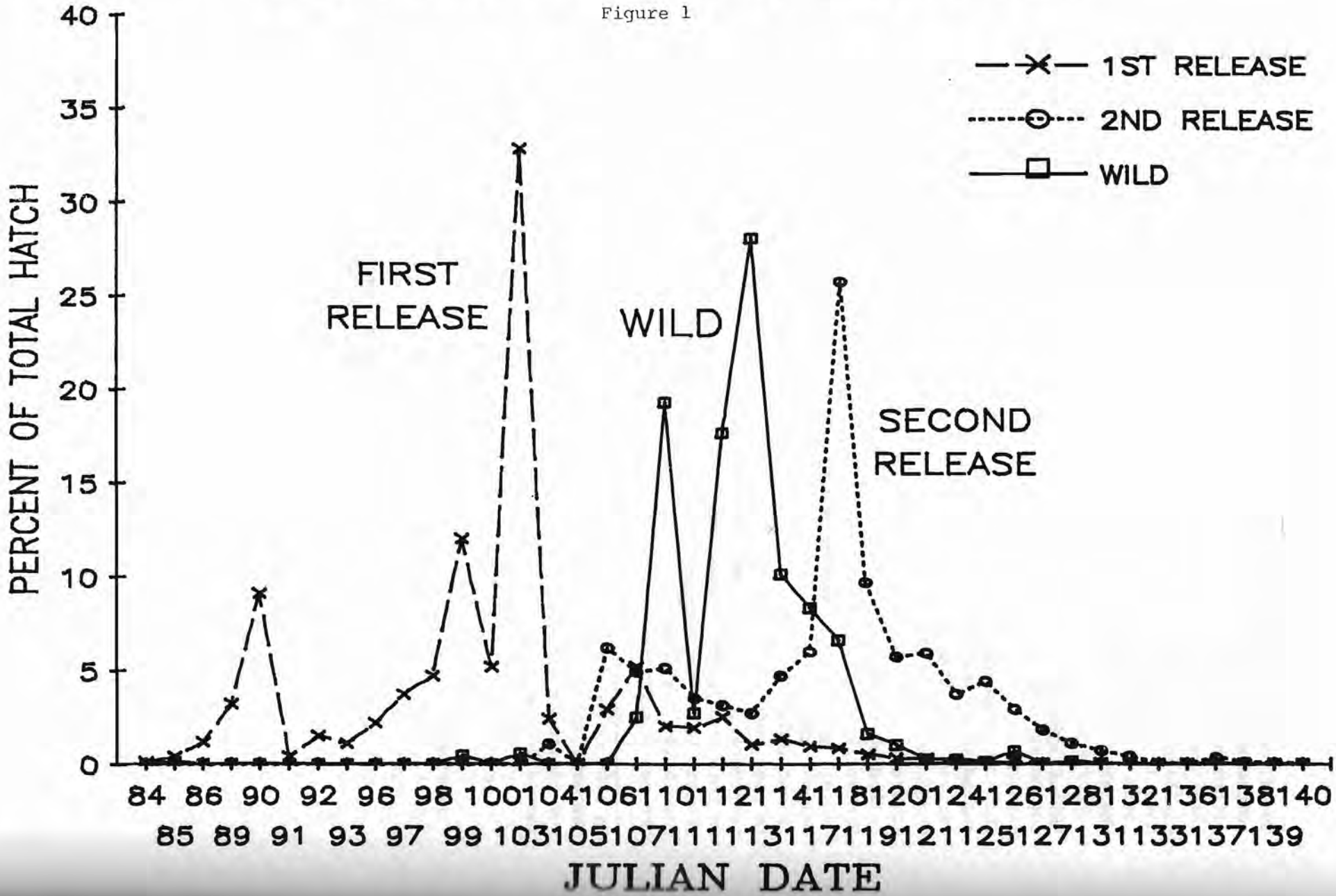
Post season egg mass data suggest that only about 2% of the field collected egg masses are the result of a wild x wild mating. The analysis of these data, however, suggests that the male mating ratio was 1 F_1:1.5 wild males. This ratio is even lower (significant at the 5% level) than the ratio suggested by monitor female data. The ratio of F_1 females to wild females derived from these data is high 33.5 F_1:1 wild (n=69). The dissimilarity in these ratios is unexplained. Sample size in some cases may be too small for accurate estimates. Also, the analysis of egg mass data generated from field collected insects relies on means which have a large amount of variance. Presently, we are attempting to improve our estimates of embryonation and hatch for the four possible mating types. Another possibility is that F_1 males simply are not competitive in the adult stage and either are not mating or not inducing a female to lay an infertile egg mass. Competitiveness tests in past years' studies, however, have never demonstrated that F_1 males were non-competitive to the degree suggested by the disparity in the ratios found in this study. This does not preclude the possibility that in the past several years the laboratory NJ strain has deteriorated in some way. Another possibility is that one or all of our sampling techniques is/are biased and we are not drawing these samples from the same "population".

In 1988 no F_1 eggs will be released in Southern Shores. However, the core of the originally infested area will be trapped at 25 traps/ha (ca. 3 ha) while the remainder will be trapped at 36 traps/sq. mi. This trapping program should define the degree of suppresion achieved.

SOUTH SHORES HATCH SYNCRONY 1987

COMBINED QC CODES FOR
FIRST RELEASE, SECOND RELEASE & WILD

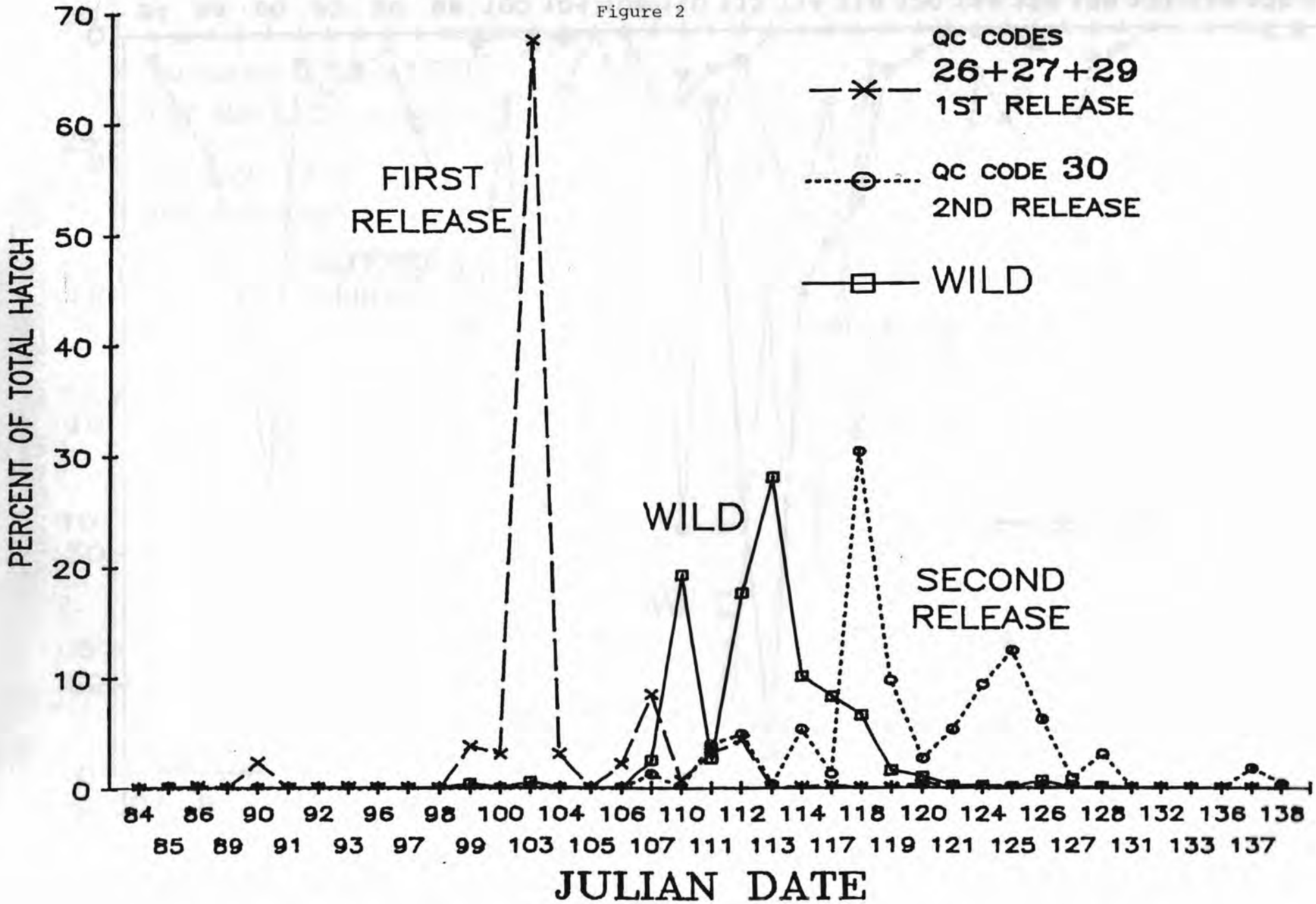
Figure 1



SOUTH SHORES HATCH SYNCRONY 1987

PRODUCTION QC CODES 26+27+29; 30

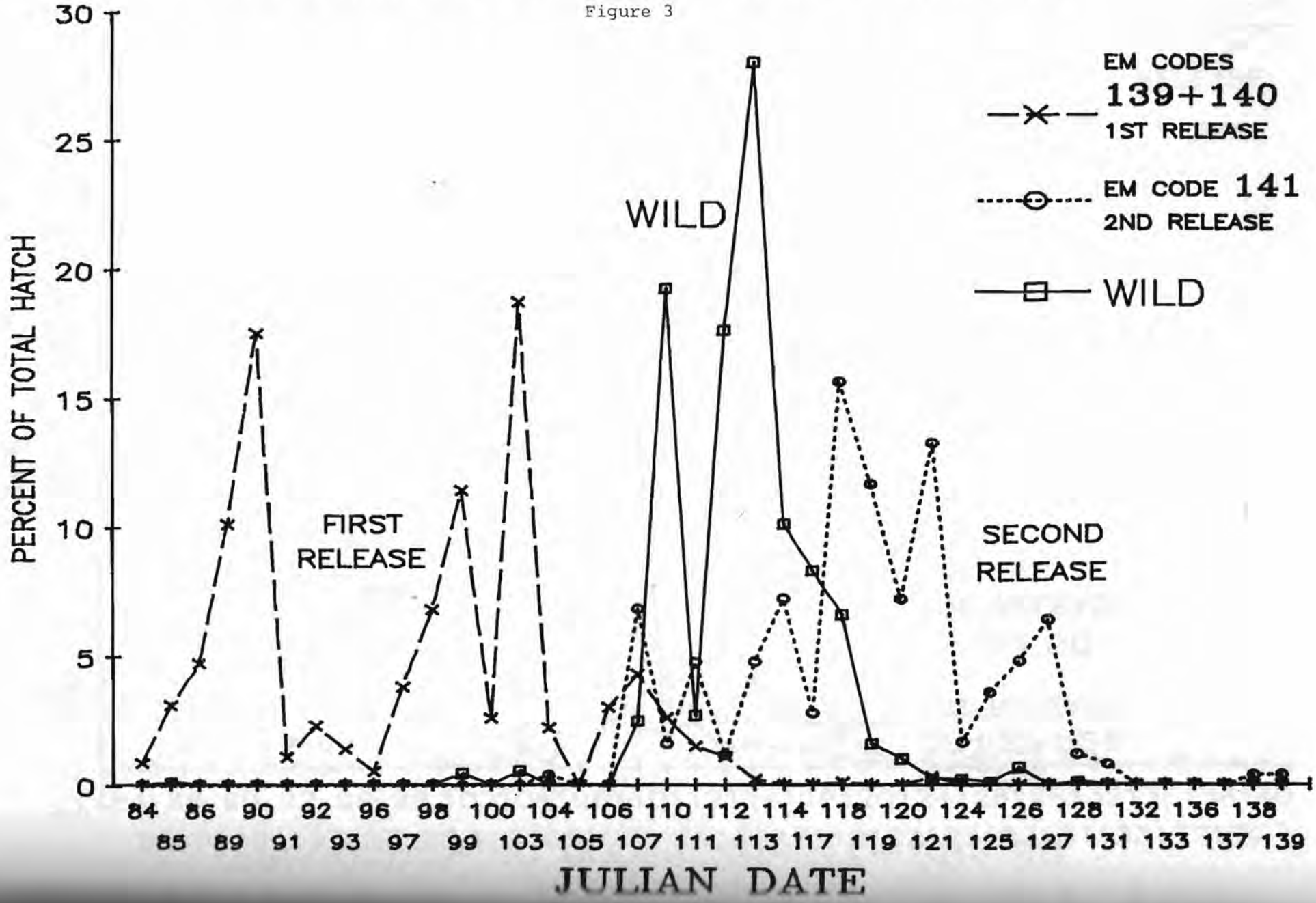
Figure 2



SOUTH SHORES HATCH SYNCRONY 1987

PRODUCTION QC CODES 139+140; 141

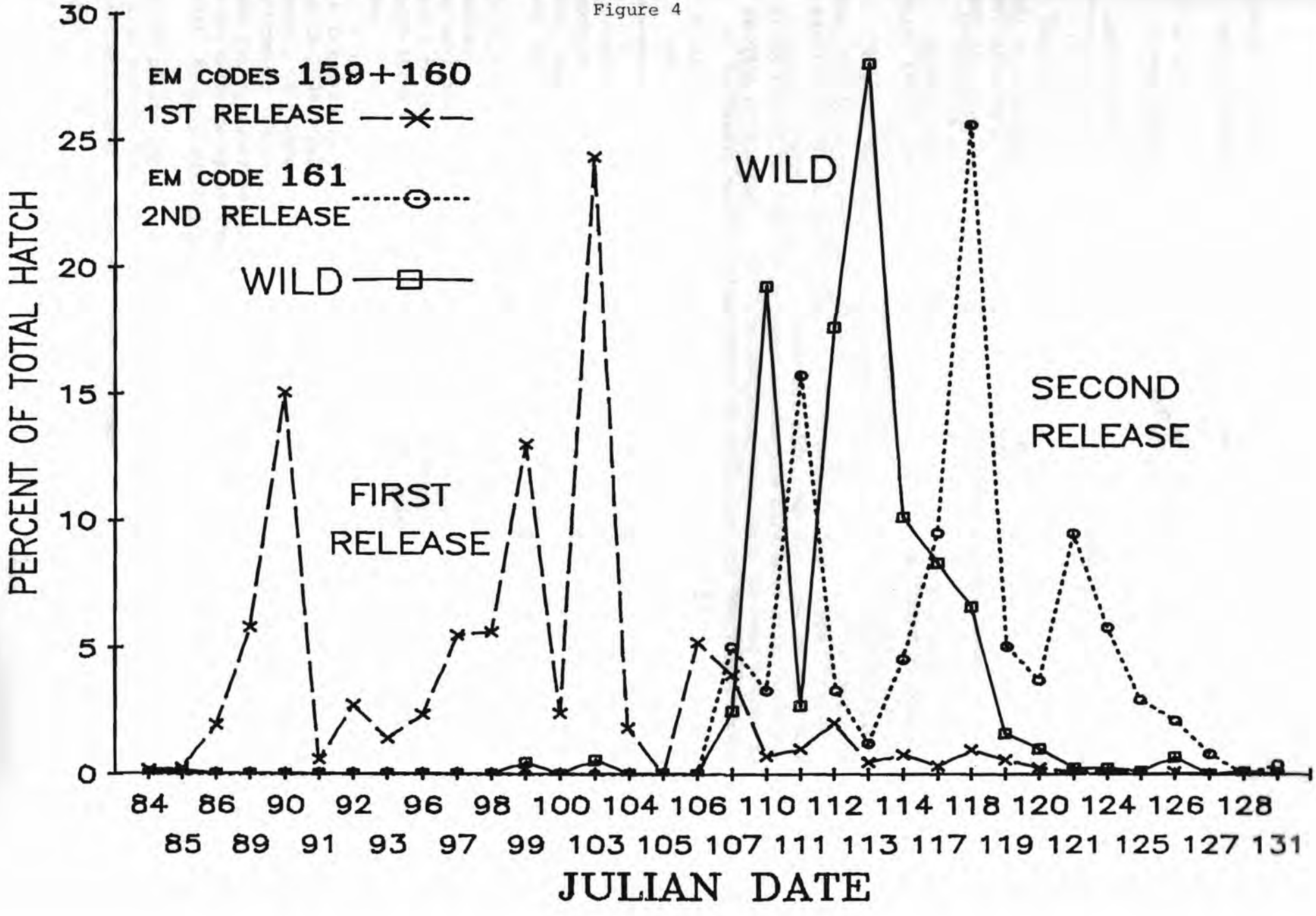
Figure 3



SOUTH SHORES HATCH SYNCHRONY 1987

PRODUCTION QC CODES 159+160; 161

Figure 4



Project Number:	GM 6.2.2
Project Title:	Sterile Male F_1 Demonstration Project - Allen Co., Ohio
Report Period:	October 1, 1986 - September 30, 1987
Report Type:	Interim
Project Leaders:	V. C. Mastro, C. P. Schwalbe, A. Pellegrini-Toole and D. Gerold
Cooperators:	D. Barth, K. Roach, Ohio Dept. of Agri., B. Smith, USDA, APHIS, PPQ

Release of F_1 egg masses were made in this isolated infestation in 1986 and 1987. Preliminary data from 1986 was presented in the last annual report (pp. 88-93). This report will finalize the 1986 data and report all available 1987 data. Table 1 summarizes the results of monitoring the 1986 release. At the conclusion of the 1986 season, we estimated pessimistically that 67 wild females had successfully mated with wild males (see GM 6.2.2, last annual report). Based on this, we released an additional 19,737 F_1 egg masses which, in theory, should have provided an adult male overflooding rate of approximately 118 F_1:1 wild. Hatch of F_1 egg masses distributed in 1987, however, was poor averaging only 18.2% or about half of the expected 40% hatch rate. Hatch of F_1 egg masses started on JD 103 and peaked on JD 111 (Figure 1). Wild egg masses were not available for hatch synchrony comparison. However, in 1986 hatch from wild egg masses began on JD 107 and peaked on JD 110, and a second peak of wild hatch occurred on JD 117. Monitoring through the 1987 season provided the estimates of overflooding ratios presented in Table 1.

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

Monitoring technique	1986	1987
No. of males trapped	389	233
No. 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)	data incomplete
Field collected post season egg masses	4:1(n=5)	data incomplete

Male ratios derived from chromosome analysis and mating evaluation were, respectively, one third to one quarter of the expected overflooding ratio. Again, the F_1:wild ratio from monitor female data (as in other sites) was much lower than other male ratios.

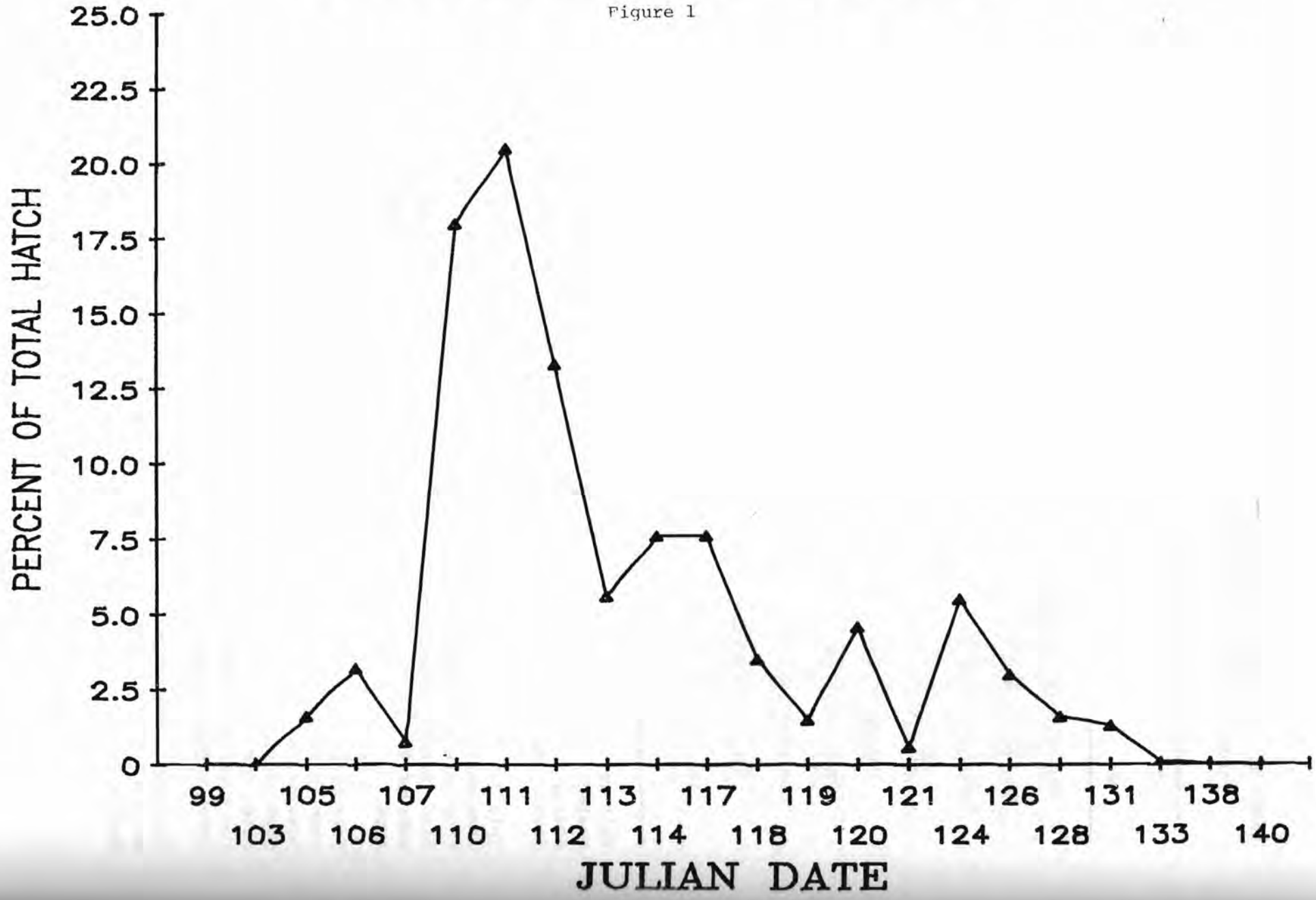
This 4.7 F_1:1 wild ratio for monitor female mating was based on only 63 females. In addition, 59 females produced unembryonated egg masses after they were retrieved from the field, and 274 failed to produce an egg mass. Also, nineteen monitor females produced egg masses with degrees of embryonation which could not statistically be separated from either mating type.

Male trapping in 1987 indicated that the overall survival of insects was lower than in 1986. Nearly equal numbers of F_1s were released in both years, however, only roughly 60% of the total number of males captured in 1986 were captured in 1987. One possible reason for this poor survival (e.g. low hatch rates for F_1 eggs) has already been mentioned. Generally, one would expect low survival rates at this site because of poor host quality (mainly black locust, Robinia pseudoacacia).

Because of the uncertainty at this point about the impact of the 1987 release, we plan to mass trap (9 traps/acre) the core of the infestation and delimitation trap (36/sq. mi.) the surrounding 4 square miles.

ALLEN COUNTY HATCH SYNCHRONY 1987
COMBINED QC CODES

Figure 1



Project Number: GM 6.2.3
Project Title: Sterile F_1 Demonstration Project, Manor, WA
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leaders: V. C. Mastro, A. Pellegrini-Toole and C. P. Schwalbe
Cooperators: E. Lagasa, Washington State Department of Agriculture

Preliminary results of this project are presented in the last Annual Report (GM 6.2.3, pp 94-96). Ratios established using data from male collections (chromosome analysis and mating evaluation) indicated that the F_1:wild male ratio was approximately 30:1. Using this estimate of overflooding rate and two estimates of wild female densities, we calculated that there were 1-7 successful wild x wild matings in 1987. The remainder of 1986 data are presented in Table 1. The sterile to fertile ratio derived from females collected as immatures and mated to normal laboratory males (23.5:1) is within expected values. Evaluation of post-season egg masses, however, provides an overflooding ratio which is much lower than expected. Indeed, 4 of the available 7 post season egg masses were judged to be fertile matings. The reason for these conflicting estimates is unknown. However, a possible explanation is sampling. All 4 fertile egg masses were collected from a single isolated oak tree on the edge of the treated area, while no other life stages were sampled from this tree. In essence,the samples were drawn from different populations (see 6.2.1 for more discussion).

Table 1. A comparison of evaluation techniques used for estimating overflooding ratios in Manor, WA 1986.

Monitoring Techniques	n	F_1:wild ratios	Percent fertile
Observed male ratios:			
Chromosome analysis	86	86:0	0
Mating evaluation	96	31:1	3.1
Monitor female	199	199:0	0
Observed female ratios:			
Mating evaluations	49	23.5:1	4.1
Field collected post season egg mass evaluation	7 1/	0.75:1 2/	57.1
Trapping (numbers captured)	528		

1/ Six of these egg masses were collected from a single isolated oak tree (*Quercus garryana*)

2/ The seven field collected egg masses were separated into the following mating types: 4 (fertile male x fertile female), 1 (sterile F_1 male x unknown female), 1 (unknown male x sterile F_1 female)

In 1987, an additional 43,705 F_1 egg masses were released on March 25 at the Manor site. Hatch of neonates from F_1 egg masses occurred over a long period, however, peak hatch occurred on JD 104 followed by a second peak on JD 112, and 113. These different peak hatch periods can be explained by comparing hatch rates of egg masses from different production lots (i.e., egg masses which experienced various lengths of chill). No wild egg masses were available to compare hatch synchrony. However, in 1986 peak wild hatch occurred on JD 111 (see GM:6.2.3 last Annual Report) after an initial peak on JD 93.

Larval and pupal collections provided a total of 202 immature males which could be typed for strain. Chromosome analysis was used to type 34 males, all as F_1s. Of the 168 males typed using mating evaluation, 167 were F_1s while 1 was wild. Ratios derived from these evaluation techniques (Table 2) suggest an F_1:wild ratio of greater than 100:1. As in other sites, the mating ratio established using monitor female data is not in agreement with the male ratio. The 5.9:1 F_1:wild ratio reported in Table 2 only includes females ovipositing before they were retrieved from the field. If unembryonated egg masses deposited by females after they were retrieved from the field are included, the ratio would be 33.7 F_1:1 wild (n=243) or approximately 2.9% fertile.

Evaluation of egg masses from field collected immature females is incomplete at this point. No egg masses could be found in the field at the completion of the season for evaluation.

Table 2. A comparison of evaluation techniques used for estimating overflooding ratios in Manor, WA 1987.

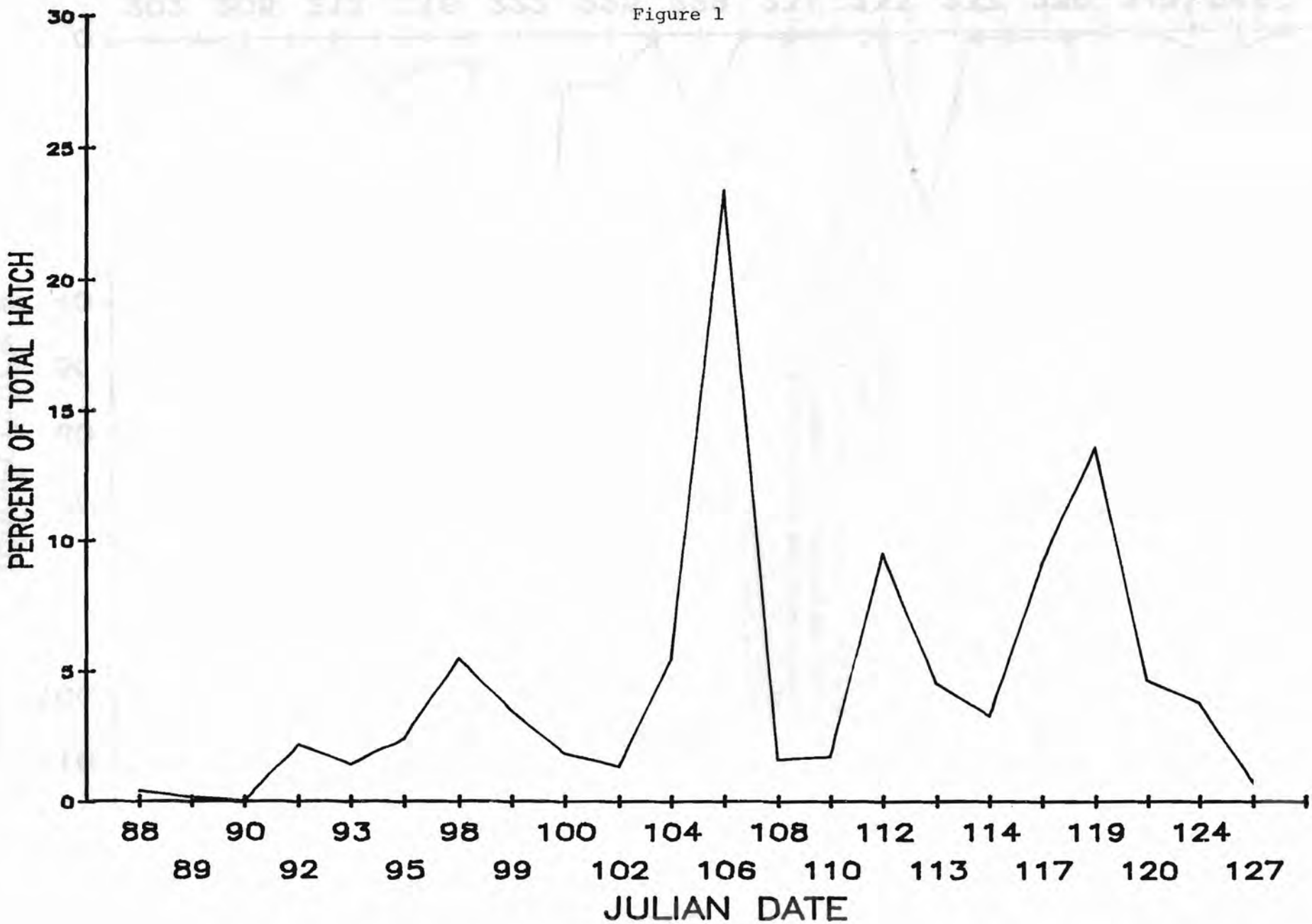
Monitoring Techniques	n	F_1:wild ratios	Percent fertile
Observed ratios:			
Chromosome analysis	34	34:0	0.0
Mating evaluations	168	167:1	0.6
Monitor females	48 1/	5.9:1	14.6
Observed female ratios:			
Mating evaluation	data incomplete		
Field collected post season egg mass evaluation	no egg masses could be located		
Trapping (number captured)	205		

1/ An additional 195 females produced unembryonated egg masses after retrieval from the field, 368 failed to produce an egg mass and 3 produced egg masses with degrees of embryonation which could not be statistically separated from either of the two possible mating types.

Trapping in 1987 was conducted at a lower trap density (36/sq mi) than in 1986 (ca. 240 traps/sq mi). The number of males captured in 1987 was lower than in 1986 (i.e. 205 vs 528). This decrease in capture may be explained simply on the basis of trap density and may indicate an actual increase in male density. Pattern of capture in 1987 indicated a sharp peak on JD 222 (Figure 2) was similar to capture in 1986. Plans for 1988 are to trap the site to determine if any residual populations exist.

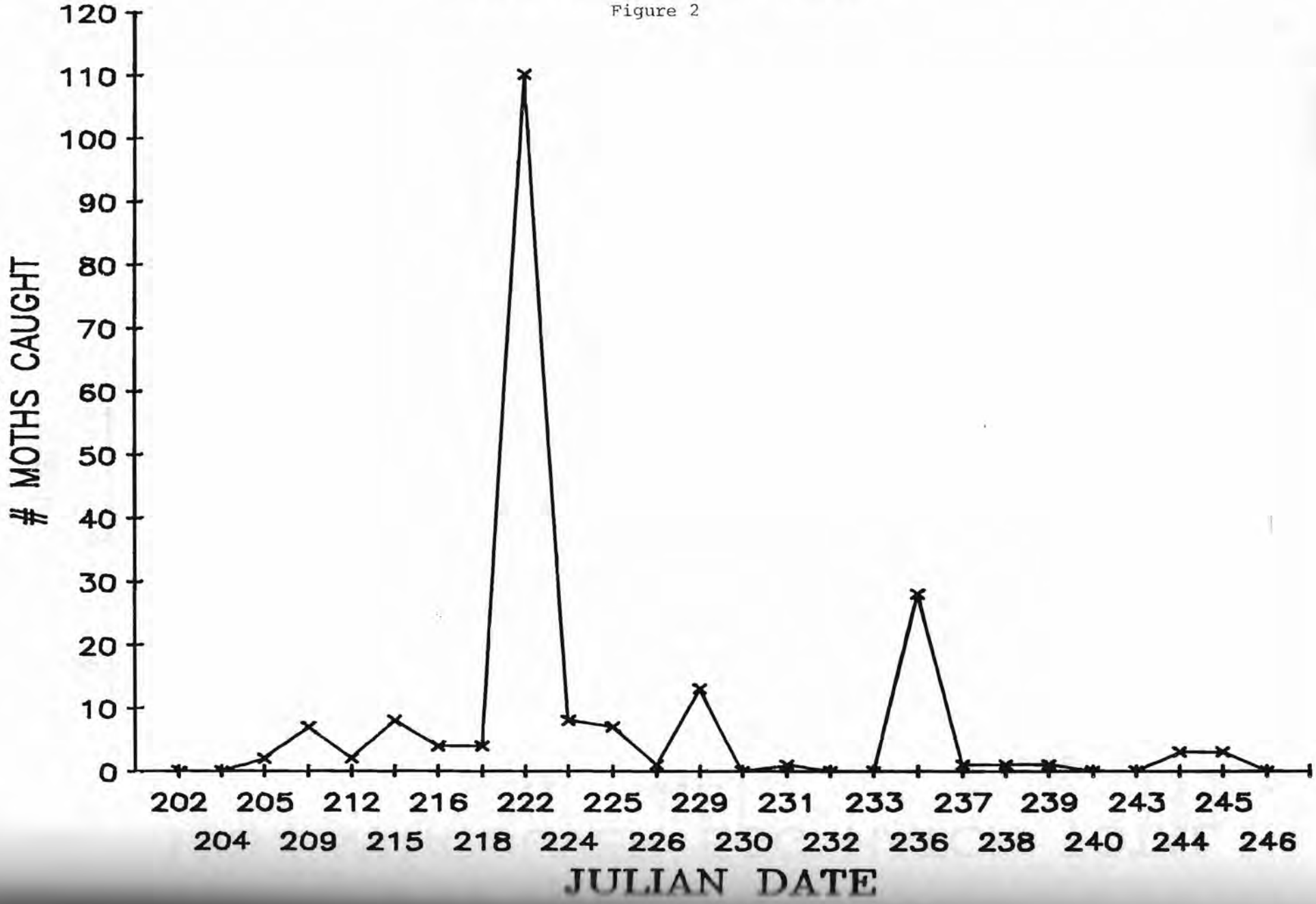
1987 VANCOUVER EGG HATCH TOTAL

Figure 1



1987 VANCOUVER TRAP SURVEY
COMBINED TRAPS

Figure 2



Project Number: GM 6.2.4
Project Title: Sterile F_1 Demonstration Project - Portland, Oregon
Report Period: October 1, 1986 - September 30, 1987
Report Type: Final
Project Leaders: V. C. Mastro, C. P. Schwalbe and A. Pellegrini-Toole
Cooperators: Alan Mudge, Oregon Department of Agriculture, Gary Smith, USDA, APHIS, PPQ

Preliminary results of this study were presented in the last report. The final results of 1986 field data are summarized in Table 1. As was detailed in the last report, the observed overflooding rates were low. The major suspected reason was the delay in F_1 egg hatch (see GM 6.2.4 1986). All the observed ratios noted in Table 1 indicate the actual mating ratio in the field was probably on the order of 1:1. Also, the small numbers of males trapped in the release area indicate very low survival rates.

At the trapping density used, approximately 34 percent of all males should have been trapped providing an estimate of 1,002 males. If a 1:1 F_1:wild ratio is applied to this estimate, then equal numbers, 501 males were wild and sterile. Assuming the wild sex ratio in the field was 1:1 and wild females mated with males at a 1:1 ratio then roughly 250 females should have produced fertile egg masses providing essentially the same egg mass population density for 1987 as in 1986.

Table 1. Results of monitoring a release of F_1 sterile egg mass in Portland, Oregon, 1986.

Monitoring Technique	Ratio F_1:Wild	Percent Fertile	n
No. of males trapped	341		
Observed male ratios:			
Chromosome analysis	3.7:1	17.6	187
Mating evaluation[1/]	1.6:1	43.8	438
Monitor females[2/]	4.2:1	19.4	67
Observed female ratio:			
Mating evaluation[3/]	0.8:1	56.8	266
Field collected post season egg mass			
Overall	2.9:1	25.6	43
Male ratio	0.3:1	74.2	31
Female ratio	1.04:1	48.9	47

1/ An additional 62 pairs produced egg masses with no embryonated eggs.
2/ An additional 212 egg masses contained all unembryonated eggs and 10 pairs produced egg masses which could not be assigned statistically to either mating type.
3/ An additional 9 pairs produced egg masses with no embryonated eggs,27 pairs produced egg masses which could not be assigned statistically to either mating type and 19 pairs failed to produce an egg mass.

Because the male trapping program in 1986 yielded captures far from the release area, the decision was made to intensively trap the study site in 1987 with no further releases of F_1 egg masses. On May 21st one ground application of BT was made to three properties in the core of the infested area. The final results in 1987 were 14 males trapped within the sterile male release area (9 traps/acre) and 2 additional males trapped in a delimitation grid (49 traps/sq. mi.) around the study site. The impacts of the 1986 release and subsequent treatment with BT are impossible to separate. We anticipate no further work in this project.

Project Number: GM 7.2.1
Project Title: Comparative Location Patterns of Late-stadium Gypsy Moths
Report Period: June 1 - September 30, 1987
Report Type: Interim
Project Leader: R.W. Hansen

METHODS

Test arena

A 6.5-m tall "red" oak (Quercus sp.) and a 5.5-m tall white oak (Quercus alba) were selected for study. The two adjacent trees were enclosed in a scaffolding system that permitted access to the entire tree. Understory vegetation >0.5 m in height and most large woody debris were removed from an area roughly 2 m around each bole.

Released larvae

Gypsy moth larvae used in the earliest releases had been reared since eclosion on excised red oak branches. For later releases, larvae were transferred from artificial diet to white or red oak foliage at least ten days prior to release. Typically, fifth-stage larvae were employed in this study and most were selected soon after the fourth molt. However, a few fourth instars were occasionally included.

Larval treatments included: (a) irradiated laboratory strain (hereafter referred to as "F_1"), parent male pupae treated with 10 krad; (b) nonirradiated laboratory ("New Jersey", G_{31}) strain ("SS"); and (c) a "wild" strain ("WT"), collected as egg masses in Rhode Island. Twenty-five larvae of each type were thoroughly dusted with different fluorescent powders and released on the target tree bole (1.5 m above ground). Three groups of larvae were released on the white oak (June 29, July 7, and July 20) and two on the red oak (July 14 and July 28). Releases were made in mid- to late afternoon.

Observations were begun 12-18 hrs after release. During each observational period, the entire tree and surrounding vegetation and ground litter were examined. Marked-larval location was recorded by site (see Table 1) and height profile (successive 1 m strata) and, if possible, larval activity was noted. Temperature, relative humidity, and weather conditions were recorded at the start of each observation period.

Observations were made at irregular intervals throughout the day. Nocturnal observations employed a portable "black light" that illuminated the marked larvae. The insects from a given release could be followed for about 5 or 6 days until most had molted or were lost (dispersal, predation, etc.).

Table 1. Locations of released gypsy moth larvae.

Code	Description
	On-tree
BOL	Tree bole
LBR	Large branches (>3 cm diameter)
MBR	Medium branches (1-3 cm diameter)
TWG	Twigs (<1 cm diameter)
LVS	Leaves
	Off-tree
LI	Leaf litter
WO	Woody debris (e.g. branch litter)
UN	Understory vegetation

RESULTS

Observational periods were divided into "day" (0500-2100 hrs) and "night" (2100-0500) periods, and data for each were pooled by host. Treatment differences in larval location and height distribution patterns were analyzed using the Chi-square statistic. The null hypothesis in each case was that larval height or location was independent of larval treatment.

Larval treatment had no effect on height distribution on white oak during the day (X^2=9.52; P>0.25) or night (X^2=13.24; P>0.10) (see Figs. 1 and 2). On this host, the largest group of larvae was recovered in the 1-2 m stratum. Treatment also had nonsignificant effects on diurnal and nocturnal height distribution on red oak (X^2=9.76, P>0.10 and X^2=5.78, P>0.25, respectively; see Figs. 3 and 4). Most larvae were found at >3 m height on red oak.

Larval location was compared in two ways. First, the number of larvae situated on or off the tree was compared (Table 2). Location differed between treatments only for diurnal observations on white oak. This result can largely be attributed to a greater than expected "off-tree" occurrence of F_1 larvae.

Larval location patterns were also compared using various on-tree locations listed in Table 1 (Figs. 5-8). Again, larval treatment had a significant effect only for the daytime white oak data (Fig. 5). Most of the Chi-square statistic can explained by the higher than expected off-tree, and lower than expected "on-twig", occurrence of F_1 larvae.

Table 2. Comparative location of released gypsy moth larvae.

			Number of larvae					
Host	Time	Treatment	Off-tree	(%)	On-tree	(%)	X^2	P
WO	Day	F1	25	(15.6)	135	(84.4)	7.88	<0.025
		SS	12	(7.3)	152	(92.7)		
		WT	11	(7.3)	139	(92.7)		
WO	Night	F1	1	(2.2)	45	(97.8)	0.89	>0.50
		SS	3	(6.7)	42	(93.3)		
		WT	2	(4.9)	39	(95.1)		
RO	Day	F1	19	(26.8)	52	(73.2)	1.83	>0.25
		SS	9	(17.6)	42	(82.4)		
		WT	14	(28.0)	36	(72.0)		
RO	Night	F1	7	(23.3)	23	(76.7)	0.41	>0.75
		SS	3	(15.8)	16	(84.2)		
		WT	3	(20.0)	12	(80.0)		

CONCLUSION

In general, it appears that irradiated (F_1) gypsy moth larvae exhibited distribution patterns similar to those of nonirradiated laboratory and wild strains. However, F_1 larvae were apparently more likely to exhibit diurnal off-tree movements, at least on white oak. Late-stadium gypsy moths resting in forest litter are known to experience higher predation by small mammals. This phenomenon will be the subject of continued study.

A rather surprising result is the relative infrequency of off-tree movement during the day (Figs. 5 and 7). Larvae situated in the leaf litter were difficult to find and thus this category may be underrepresented in my data. However, it is unlikely that overall treatment effects were altered.

Figure 1. DIURNAL WHITE OAK OBSERVATIONS (0500–2100 hrs EDT)

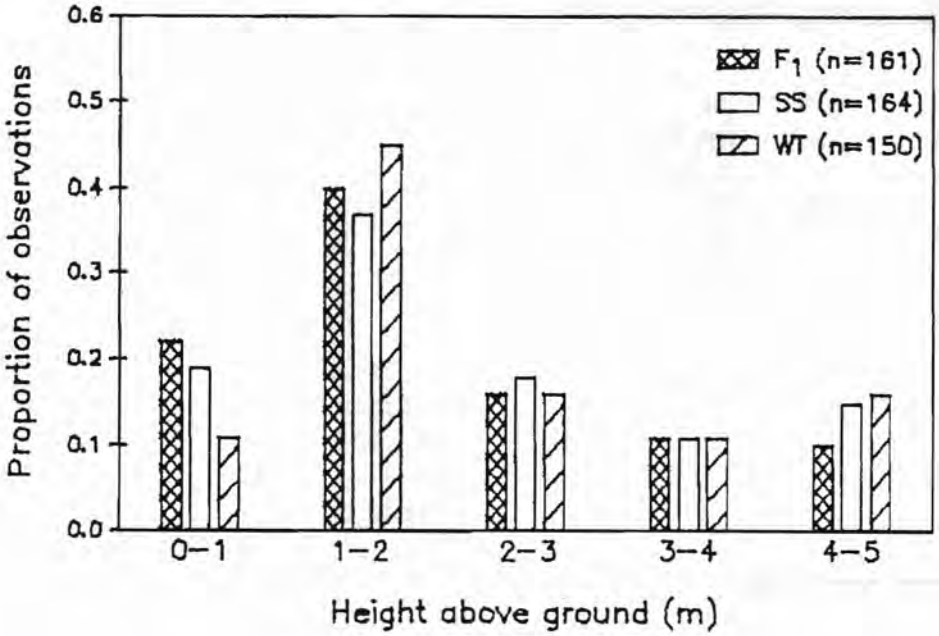


Figure 2. NOCTURNAL WHITE OAK OBSERVATIONS (2100–0500 hrs EDT)

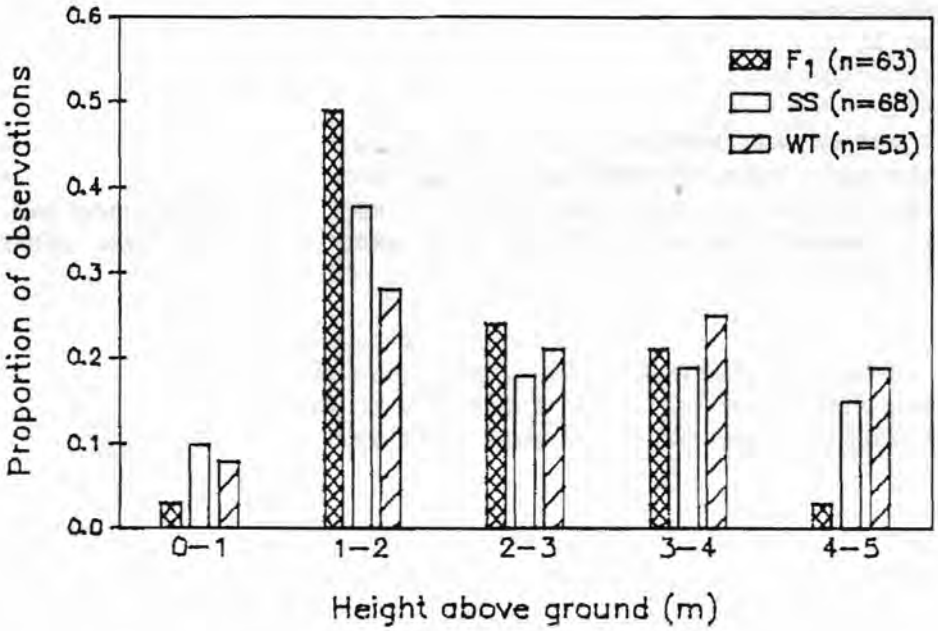


Figure 3. DIURNAL OBSERVATIONS ON RED OAK (0500–2100 hrs EDT)

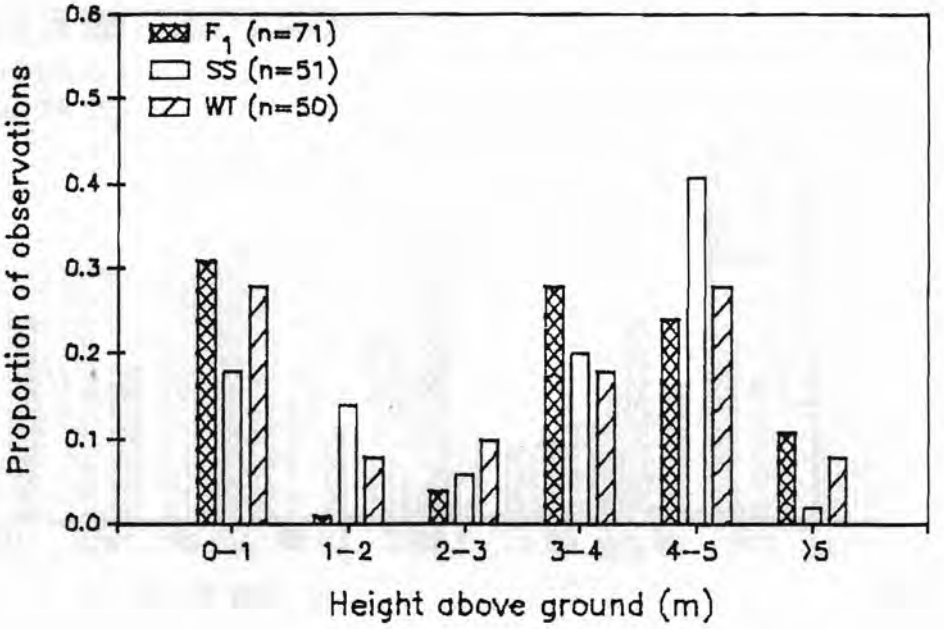


Figure 4. NOCTURNAL OBSERVATIONS ON RED OAK (2100–0500 hrs EDT)

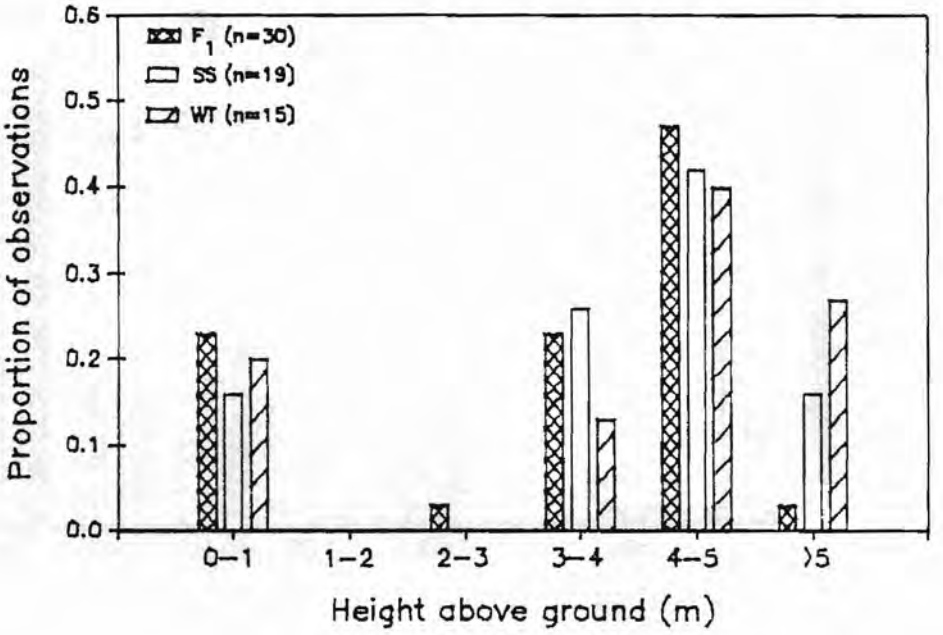


Figure 5. DIURNAL OBSERVATIONS ON WHITE OAK (0500–2100 hrs EDT)

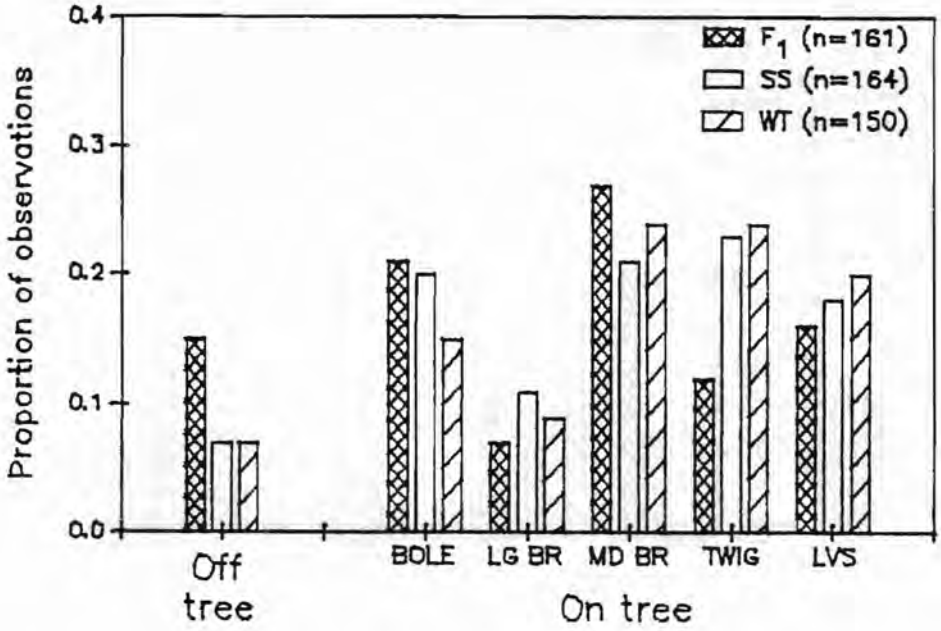


Figure 6. NOCTURNAL WHITE OAK OBSERVATIONS (2100–0500 hrs EDT)

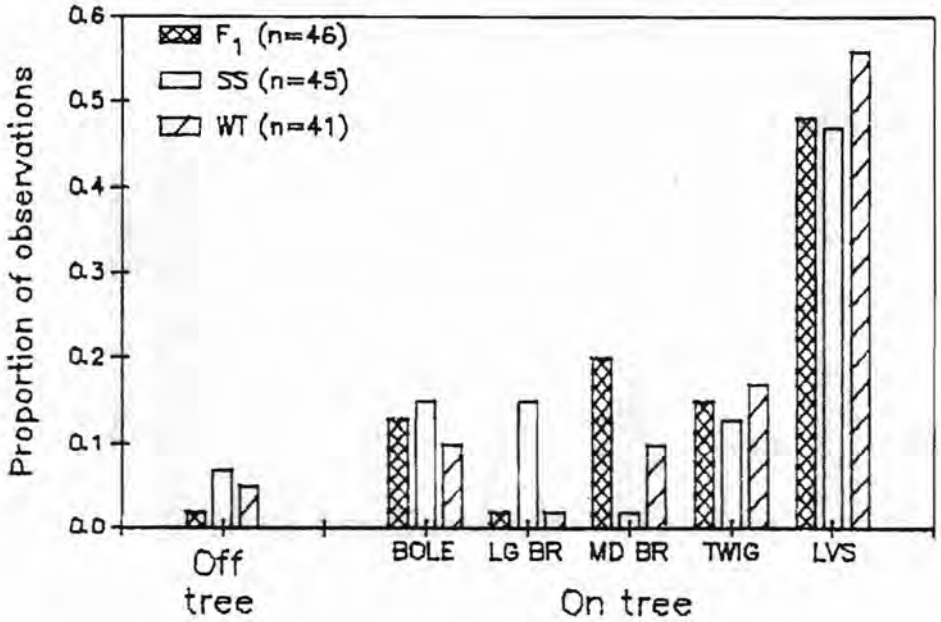


Figure 7. DIURNAL OBSERVATIONS ON RED OAK (0500–2100 hrs EDT)

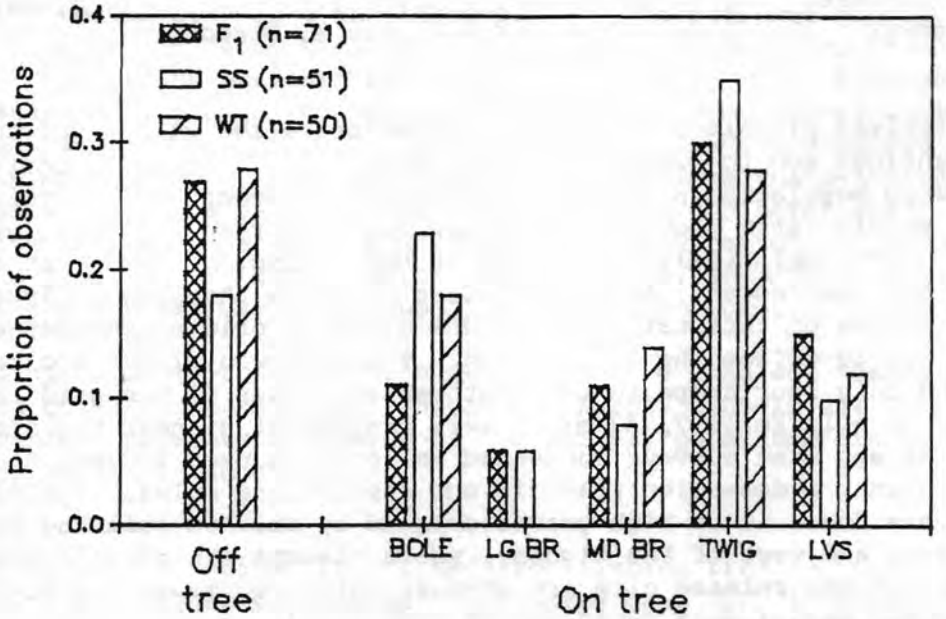
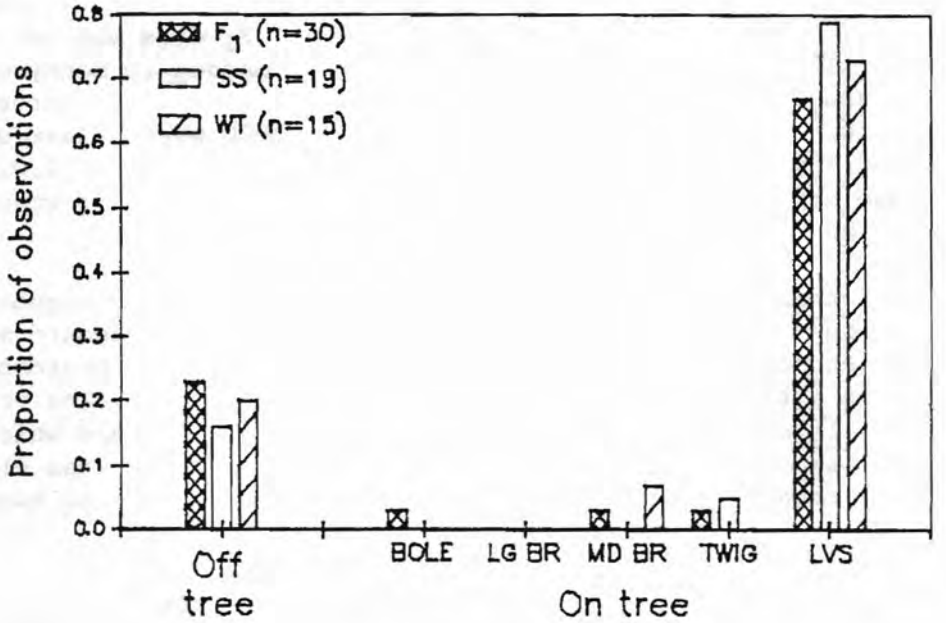


Figure 8. NOCTURNAL OBSERVATIONS ON RED OAK (2100–0500 hrs EDT)



Project Number: GM 7.2.2
Project Title: Sterile Male Demonstration Project, Gates Co., NC
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leaders: V. C. Mastro, A. Pellegrini-Toole, C. P. Schwalbe
Cooperators: H. Singletary, K. Wilson, W. Wescott

The objectives of this study were to demonstrate the utility of the F_1 egg mass technique and to compare the establishment, growth and survival of F_1s with a wild population of gypsy moth under field conditions. This site offered particularly good opportunities for comparisons between wild and F_1s because of the relatively abundant wild population. However, it also offered some unique challenges. An initial trap catch in the area in 1984 was the first evidence of infestation and a delimitation grid of pheromone traps in 1986 failed to define the infestation. The grid was placed too far to the north and only four traps on the southwestern edges of the grid captured moths (n=18). In all, in 1987, 37 males were trapped at or near the site. Subsequent egg mass surveys succeeded in locating over 100 egg masses. Further south, a detection trap did not capture any males. The site where egg masses were found is on high ground created by fill in building up a road bed. To the east and west of the highway, which bisects the release area, is swamp. Hosts within the release site are predominantly red maple and black willow, and wild egg masses were found almost exclusively on the latter. Another infestation has been found within 2 miles of this test site. Available trapping information does not clearly preclude the possibility of the two sites really being a part of a single infestation which extends throughout the swamp. This situation somewhat confounds interpretation of sterile male sampling results.

Because a good estimate of the native egg mass population could not be made from trap catches or egg mass surveys, an estimate of the projected overflooding ratio for a given number of released F_1 eggs was not made. In the spring of 1987, approximately 100,300 F_1 egg masses [progeny of irradiated males (10 krads) x normal females] were released into ca. 12 acres in the Gates Co. site. A portion of this total, ca. 57,900 were released on March 27 (J.D. 86) and the remainder 42,400 were released on April 14 (J.D. 104). This split release strategy was used because of the uncertainty of when to expect native egg hatch.

Monitoring techniques used at this site were similar to techniques used at all other pilot study sites. The only exception was that a standard monitoring grid of 36 pheromone traps/sq. mi. was not used. Instead, pheromone traps were placed on a tighter grid system (ca. 550 feet apart). The grid was laid out so that 3 lines of traps paralleled each side of the road which bisected the release site. This grid placement positioned the two lines closest to the road on high ground while the remaining four lines were in the swamp.

Results and Discussion

Early release of egg masses in Gates Co. provided better synchrony of hatch with the wild population than the later releases (Figure 1). Egg masses which were held the longest under normal chilling conditions (7°C) and released

early began to hatch immediately when very little foliage was expanded (Fig. 2). However, egg masses held for longer periods in chill (i.e. 182-185 days) (Fig. 3) under a modified chilling regime (i.e. 34-37 days at 7°C and the remaining time at 5°C) hatched in good synchrony with the wild egg mass samples. In the second release, hatch from egg masses held under the modified holding regime of 7°/5°C and released on J. D. 104, was poorly synchronized (Fig. 4) and hatch was reduced (Table 1). Generally, hatch was lower than the expected 40 percent (Table 1). Given asynchrony of hatch and lower hatch rates, possibly only 50 percent of the F_1 neonates were available to establish and develop in synchrony with wilds. An incident occurred which may have further influenced the availability of F_1s to some unknown extent. On April 16, heavy rains raised the level of the swamp to a point where all of the release site was under water. This occurred two days after the second release and the impact on F_1 survival is unknown. Also, samples could not be collected during a several day period until the water receded.

Egg mass sampling produced the following results: five wild egg mass samples produced a mean of 473 neonates/mass. Hatch was nearly 100% in all five wild egg masses. Table 2 presents a summary of all larval, pupal and adult monitoring data available to date.

Table 1. Proportions of eggs which hatched from samples from various production lots of eggs released in Gates Co., N.C. in 1987.

Production Lot QC code	Julian release date	Days in chill 7°C	Days in chill 5°C	Mean percent of total eggs which hatched
31-32	86	37	148	27.8
36-37	86	34	148	28.3
38	104	34	166	14.7
45	104	34	165	29.2
143-145	86	158	-	31.7
146-147	86	158	-	28.3
148-150	86	158	-	28.4
168-170	86	154	-	22.9
175-176	104	172	-	22.1
177-179	104	172	-	21.2
189-191	104	169	-	26.1
192-194	104	168	-	24.4
217-219	86	144	-	31.7
220-222	86	144	-	42.8
223-225	104	161	-	18.5
248-250	86	135	-	31.9
254-256	104	151	-	24.7
257-259	104	151	-	28.2
287-288	86	123	-	31.7
291	104	140	-	22.3
293	104	139	-	25.6

Table 2. Results of monitoring sterile F_1:wild overflooding ratios in Gates Co., North Carolina, 1987.

Technique	n	F_1:wild ratio	Percentage fertile
Male chromosome analysis 1/	147	147:0	0%
Male mating evaluation 1/	623	28.7:1	3.4%
F_1 monitor female egg mass evaluation	58 2/	3.3:1	27.6%
Female mating evaluation 1/ - data not available			
Post season egg mass evaluation - data not available			
Males trapped	2,534		

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 mating ratios. Of these, 223 did not lay an egg mass, 48 produced totally unembryonated egg masses after retrieval from the field and 7 produced egg masses that contained percentages of embryonated eggs not statistically different for either male mating type (i.e. F_1 male or wild male x F_1 female).

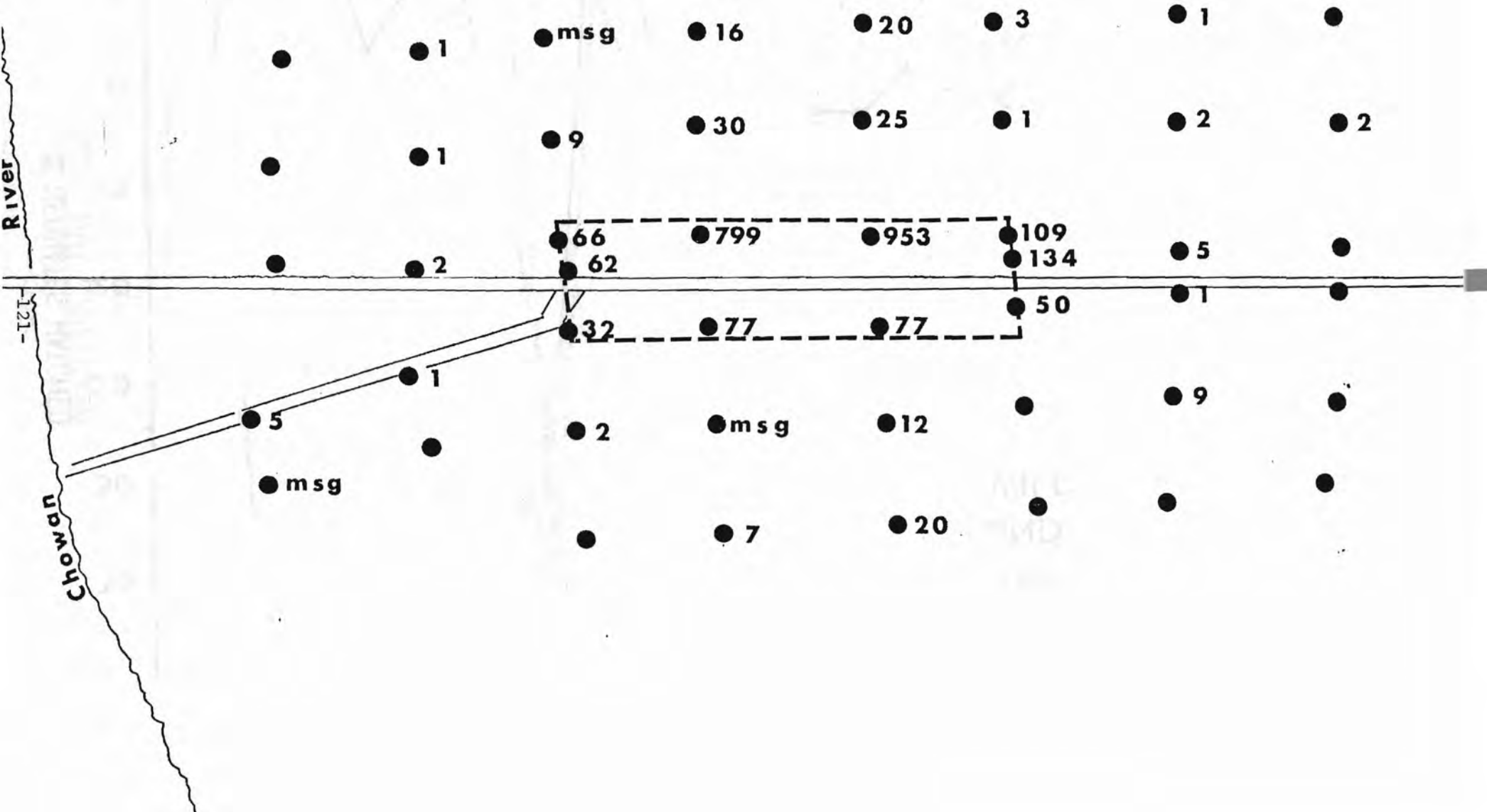
Estimates of overflooding ratios obtained from evaluations of males are clearly in conflict with mating ratios derived from monitor female data. This difference remains unexplained as does the apparent failure of monitor females to produce egg masses.

Recently, 4 egg masses have been found on a residential property several hundred feet outside of the F_1 release area. Of these, three egg masses sent to the Otis MDC displayed characteristics of wild hatch and this area will be included in the 1988 release or treated with an insecticide.

An unsuccessful post season effort was made to calibrate the trapping grid used in Gates, by releasing F_1 internally marked males. Few males were caught in traps and, in some cases, carcasses of males in rain saturated traps were so decomposed that they were impossible to count or distinguish as marked. In 1988 this exercise will be repeated with, hopefully, better results.

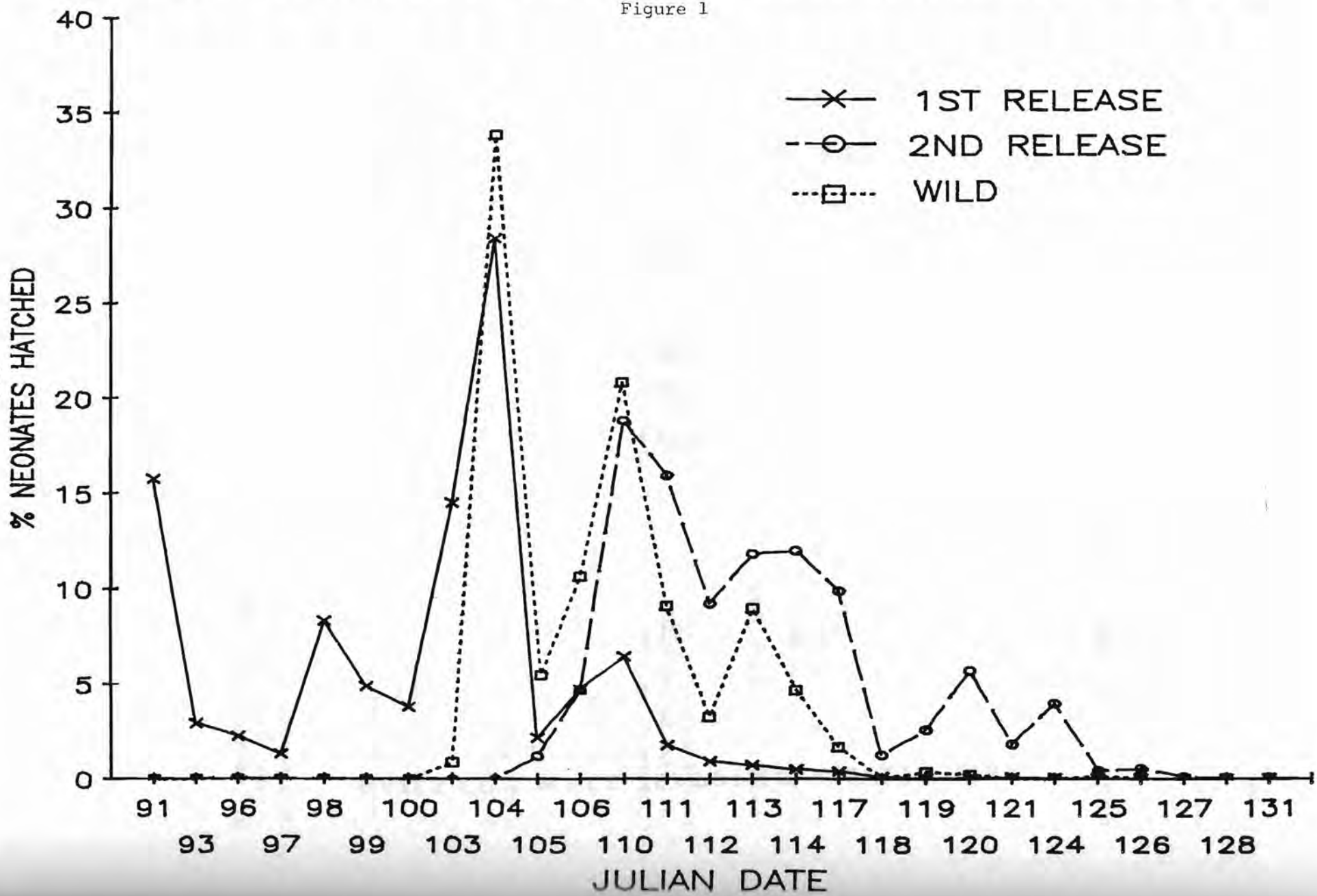
In 1988 we plan a similar release of F_1 egg masses which will hatch in synchrony with the wild population. Unfortunately, good estimates of the residual wild population are not available at this point.

GATES CO., MALE TRAPPING RESULTS 1987



GATES COUNTY HATCH SYNCHRONY 1987

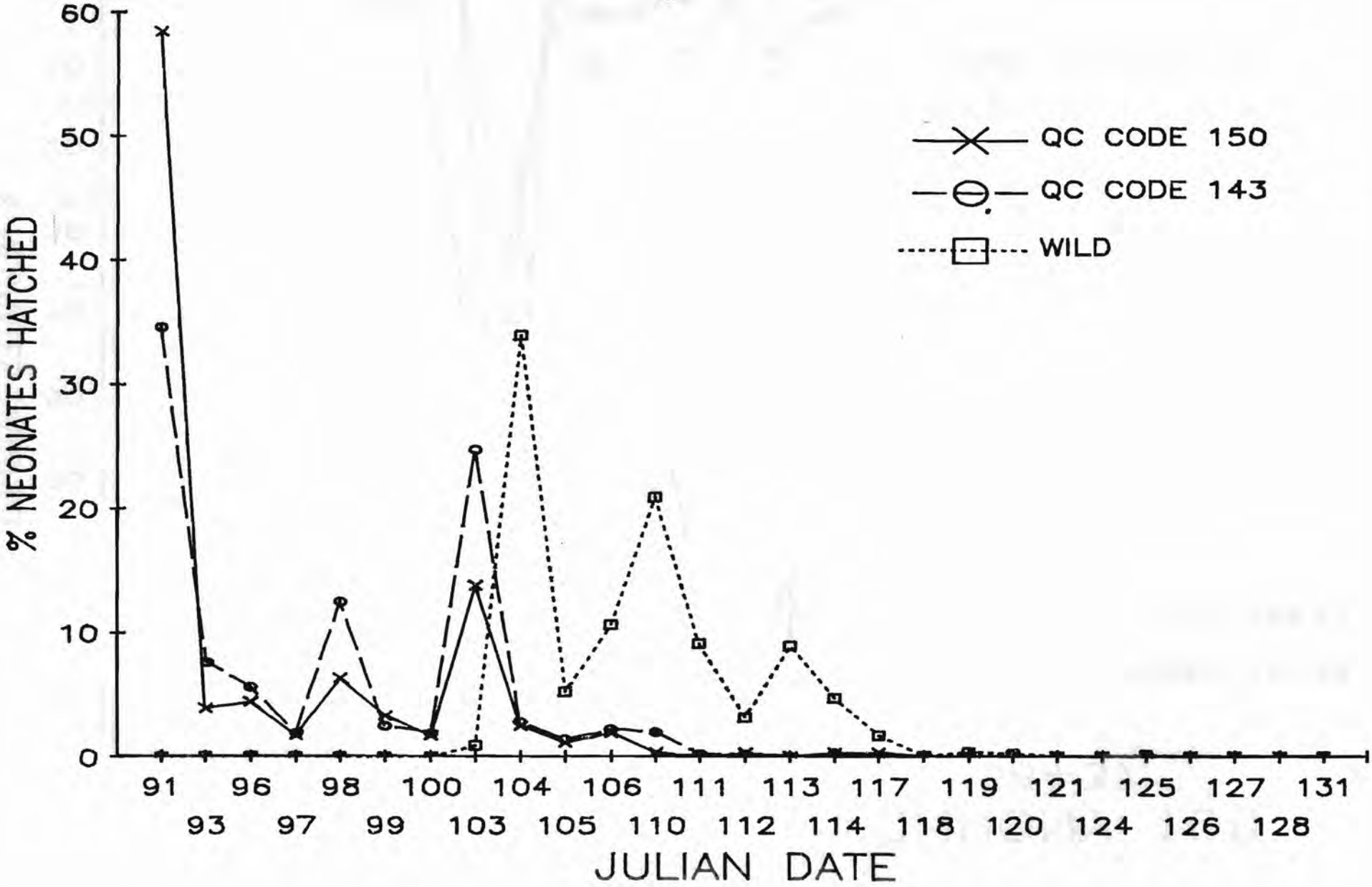
Figure 1



GATES COUNTY HATCH SYNCHRONY 1987

PRODUCTION QC CODES 150, 143

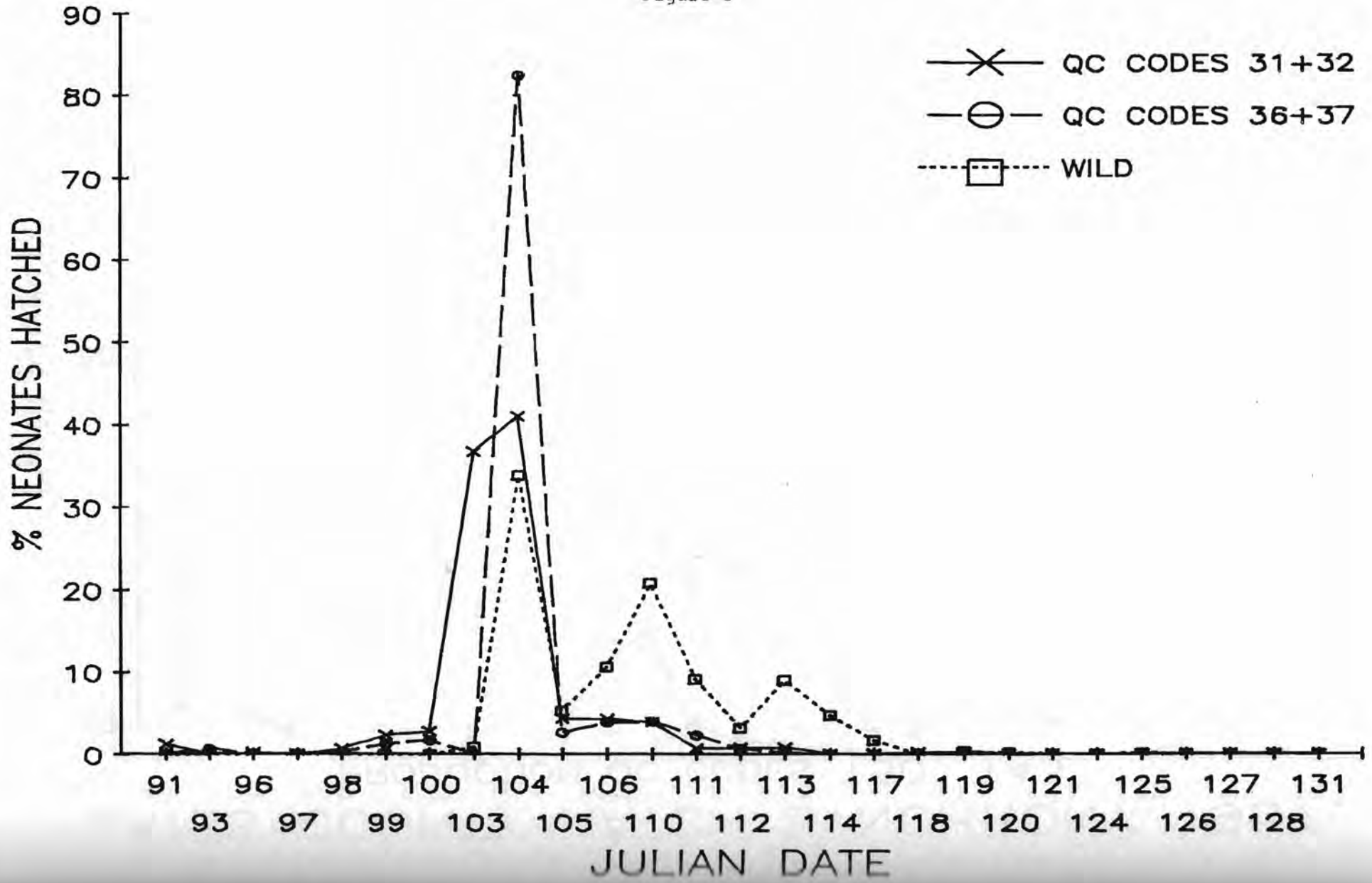
Figure 2



GATES COUNTY HATCH SYNCHRONY 1987

PRODUCTION QC CODES 31+32 & 36+37

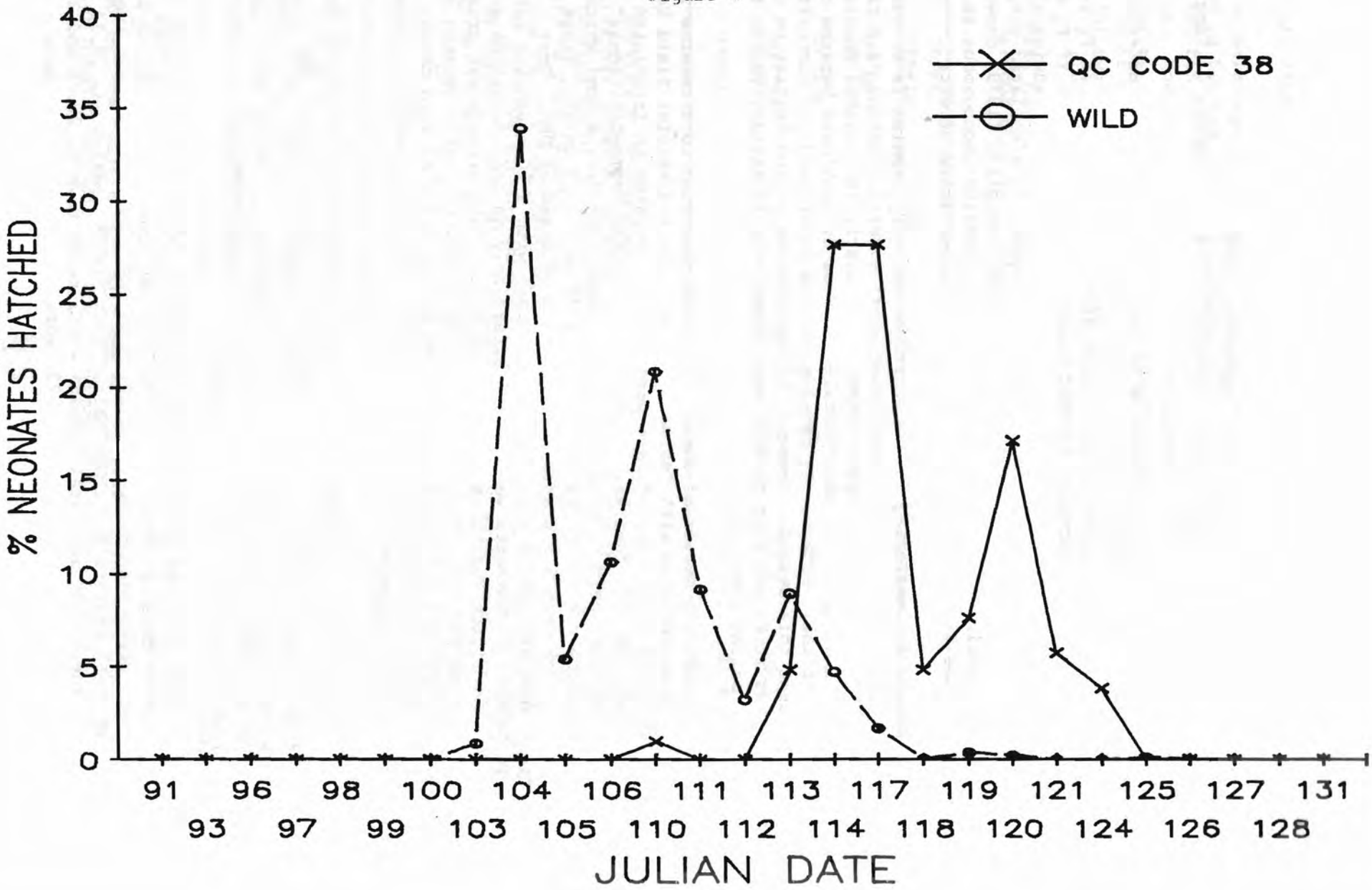
Figure 3



GATES COUNTY HATCH SYNCHRONY 1987

PRODUCTION QC CODE 38

Figure 4



Project Number: GM 7.2.3
Project Title: Competitiveness of Gypsy Moth Adults
Report Type: Interim
Report Period: October 1, 1986 - September, 1987
Project Leader: V. C. Mastro, A. Pellegrini-Toole, D. Lance, J. Tang, C. P. Schwalbe

Competitiveness of F_1 sterile males is of crucial importance in determining the potential impact of an F_1 egg release on a wild population. Theoretically, female F_1 adults could also affect the result of an F_1 release in circumstances where survival favored predominantly females or if F_1 males are largely noncompetitive. Generally, it is assumed that the advisability of releasing only one sex vs. both sexes in SIT programs is associated with the mating habits of the species considered. However, Knipling (1979) shows that, at least theoretically, the mating habits of the females (monogamous vs. polygamous) has little impact on the potential suppressive effect.

Another reason for exploring the competitiveness of F_1 females is to determine their utility for sentinels in monitoring sterile:fertile mating male ratios in field releases. In F_1 release sites, particularly in isolated populations, it is undesirable to use normal "fertile" females as sentinels because of the possibility of their escape. F_1 females, on the other hand, if competitive, do not present this hazard. However, it was unknown at the initiation of these studies if F_1 and wild females were comparable in attractiveness to males or in mating behavior.

Beginning in 1984, a series of small tests were conducted to determine if F_1 females were competitive with "normal" females. In the initial field test, F_1 females (progeny of irradiated male parent) were compared to irradiated (15 krad) females and wild females. To compare the field "competitiveness" of females, they were held in wire mesh ($6mm^2$) cages (7cm dia. x 6cm) which were suspended in the center of traps (12mm mesh, 30 cm L x 25 cm W). Traps were coated with tack trap and stapled to the boles of trees at DBH. Trap positions were arrayed in a circle (70 m radius) with a 20 m spacing between trap positions. Treatments were placed alternately on trap positions and rotated one position during each day of the test. This arrangement provided seven positions for each treatment. Traps were checked hourly between 9:00 and 16:00 hours, and all males were removed and recorded at each check. Traps were also checked the following morning at ca. 8:00 a.m., usually before any appreciable male flight had begun.

On three days, virgin, one-day-old (the day following eclosion) F_1-8K females, (progeny of males irradiated with 8 krads as 8-11 day-old pupae) were compared to laboratory females treated with 15 krads (NJ-15K) and to females which had been field collected as pupae (wild-Ashumet). On two additional days, 7/25 and 7/26, F_1 female progeny of males irradiated with 10 krads (F_1-10K) were substituted for F_1-8K females.

Mean daily male captures per female are presented in Table 1. Overall, for the first three days of testing, mean capture per female for F_1-8K, NJ-15K and wild-Ashumet) were, respectively, 10.43, 9.95 and 9.85. On the last two days of testing, mean capture for traps baited with F_1-10K females was 25.33 while capture for the NJ-15K and feral strains were, respectively, 20.86 and 23.5. The first two days of testing (7/22-7/23) were cool, suppressing male

activity and hence mating. However, on the last three test days, temperatures were warmer, enhancing male activity.

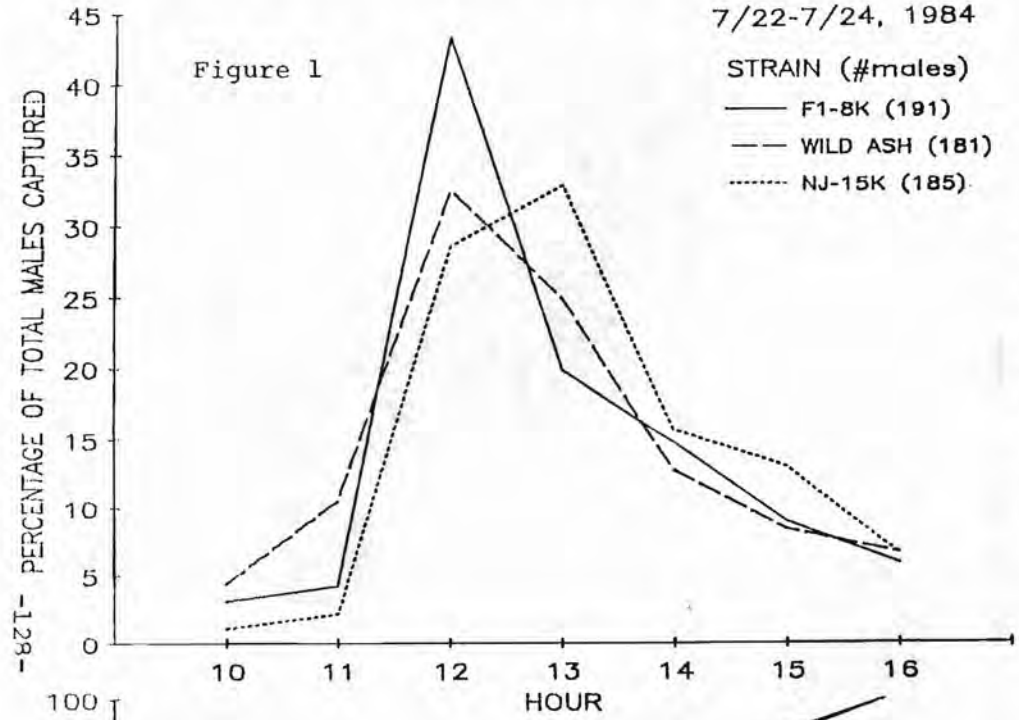
Pattern of male capture during the course of the various test days is presented in figures 1-5. On several days (7/22, 7/23, 7/25) traps baited with NJ-15K females began capturing males later than either of the other two treatments. However, there were no other consistent differences in pattern of male captures. Traps baited with F_1 females appear to begin capturing males as early as traps baited with feral females and generally periodicity of capture follows that of traps baited with wild females.

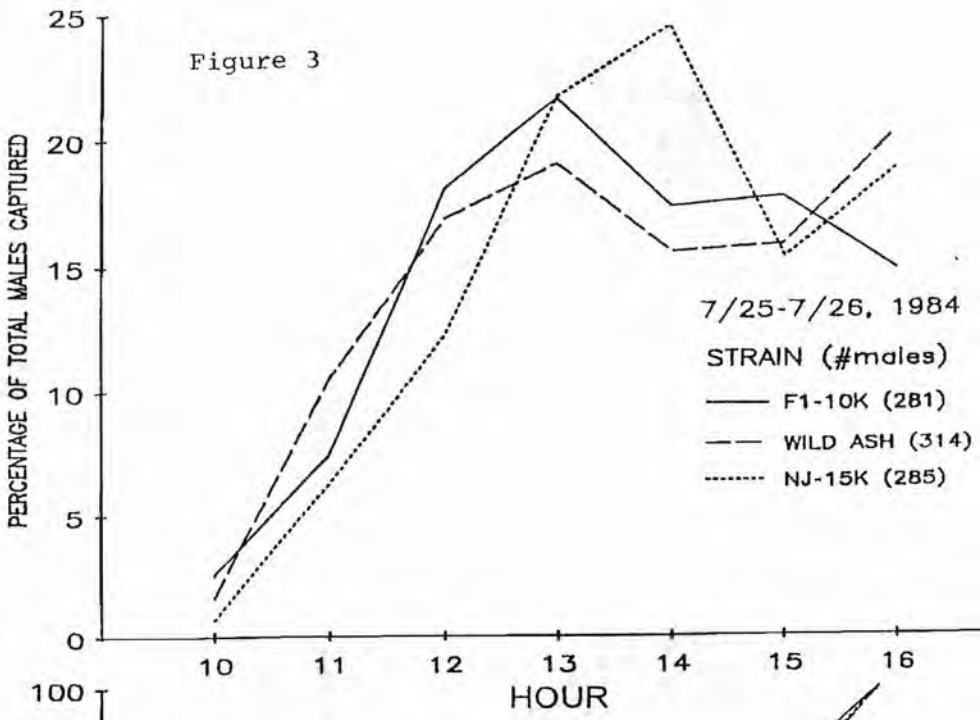
Table 1. Mean number of males captured daily per trap baited with females of various strains (1984).

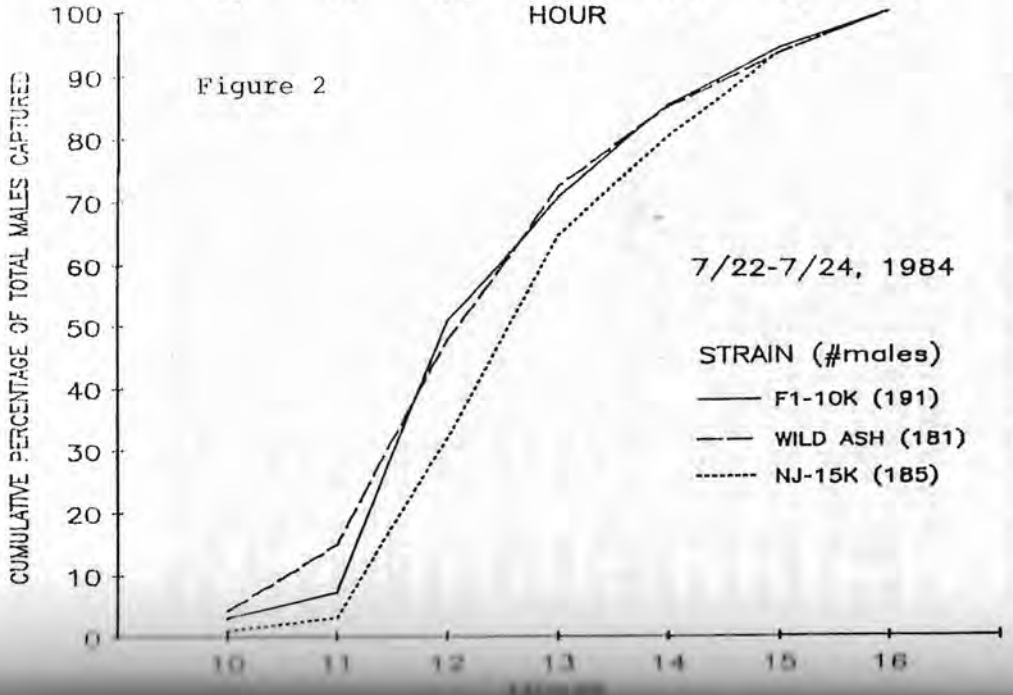
Strain Female	7/22	7/23	7/24	7/25	7/26
F_1 8K	5.0	4.3	22.0	-	-
F_1 10K	-	-	-	24.0	27.2
NJ 15K	2.9	4.4	22.3	14.9	26.9
Feral	7.7	5.1	17.0	15.4	31.6

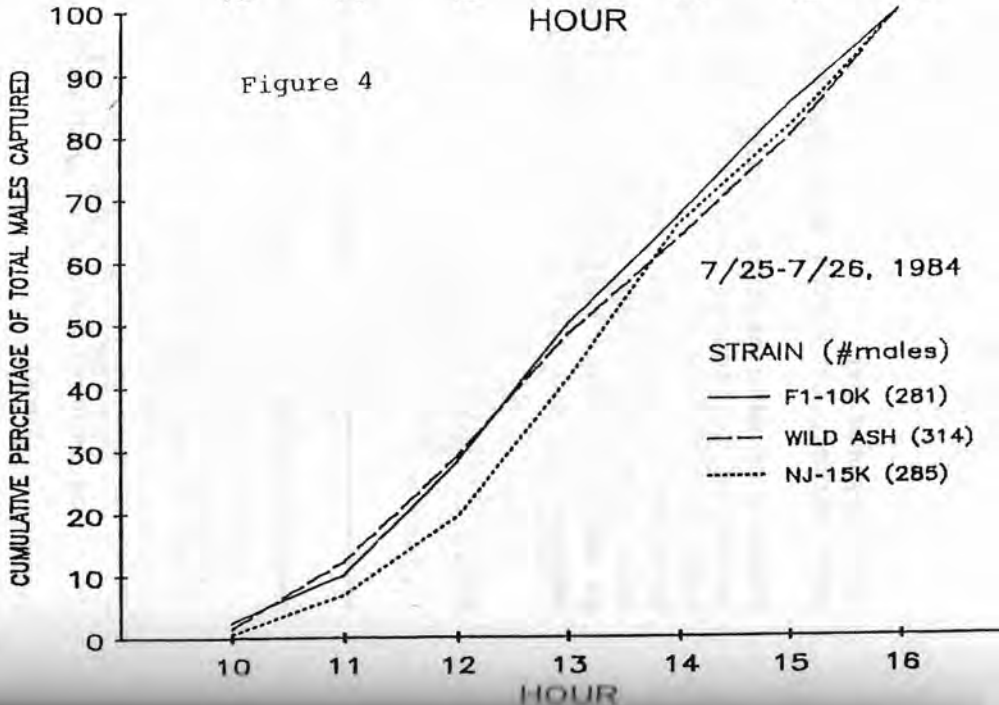
In 1985, additional studies were conducted to further explore female competitiveness. Studies in 1984 indicated that F_1 females were as attractive as wild females in luring males to traps, however, it was unknown if males would mate as readily with them. A small field test was conducted in 1985 to compare how readily females of five strains would attract and mate with wild males. Of the five strains chosen for comparison, three were F_1 progeny of irradiated males (NJ strain) which had been treated with three dosages (6, 8 and 10 krads) of radiation and untreated laboratory NJ strain females. The fourth and fifth strains were both normal untreated laboratory reared females, NJ_{F28} and $Ashumet_{P1}$ (reared from field collected egg masses). To explore the mating frequency of the various female types, sites were established on lines (approximately 40 m spacing) in a moderate density native population. At each site, a tree was chosen with a DBH of approximately 8 inches. During testing, females were placed on the bole, within the observer's reach, and allowed to establish a resting/calling behavior. Females were placed by 8:30 a.m. and were checked at intervals of no longer than 15 minutes. Females found <u>in copula</u> were replaced; however, females not calling or mating within a half hour of placement were also replaced and recorded. All females used for testing were virgin and one day-old (the day after eclosion) when placed. On each day of testing, two complete lines of females were placed and monitored and the test was repeated on three separate dates (July 24 and August 4 and 5). On the first day of testing, all mating pairs found <u>in copula</u> were collected and the females were held for oviposition. Resulting egg masses were later cleaned and examined for embryonation.

A comparison of male capture periodicity in traps baited with females of various strains.



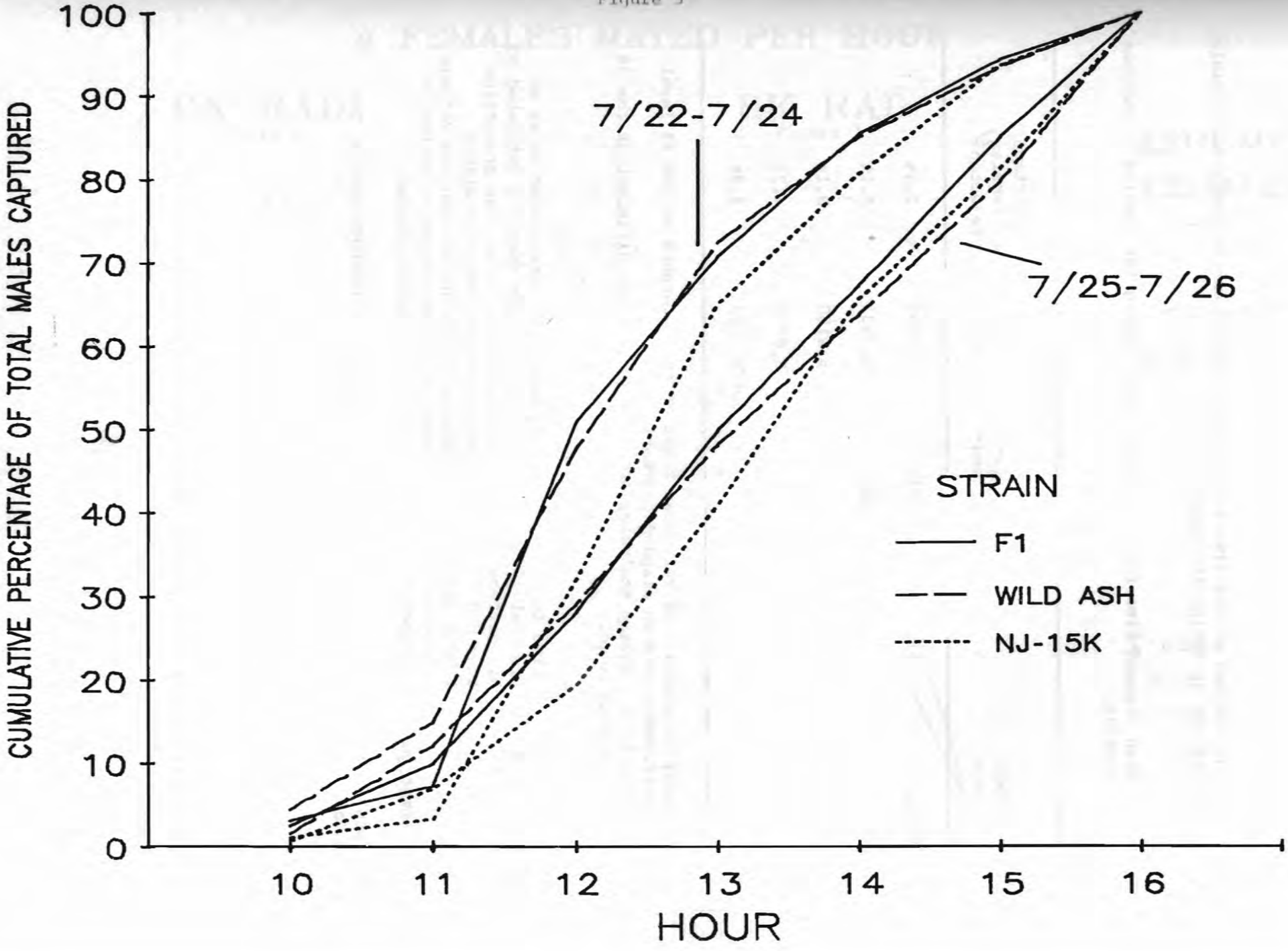






Combined 7/22-7/24 and 7/25-7/26 graphs

Figure 5



The number of females of the five strains which mated can be compared in Table 2 and the pattern of mating in Figures 6-10.

Table 2. Mean elapsed time from placement until mating for females of various strains.

Female strain	n	Mean elapsed pre-mating times 1/	S.D.	Proportion of females rejected 2/
NJ_{F28}	161	13.5031	5.7599	2.9%
$Ashumet_{P1}$	143	14.1888	5.7531	5.1%
F_1 6 krad	134	14.5970	6.2180	8.1%
F_1 8 krad	126	15.4921	5.8798	4.2%
F_1 10 krad	137	14.3431	5.7783	3.2%

1/ According to analysis of variance, there is no difference at the 5% level of significance between elapsed pre-mating times.
2/ According to chi square analysis, proportions were not different at the 5% level of significance.

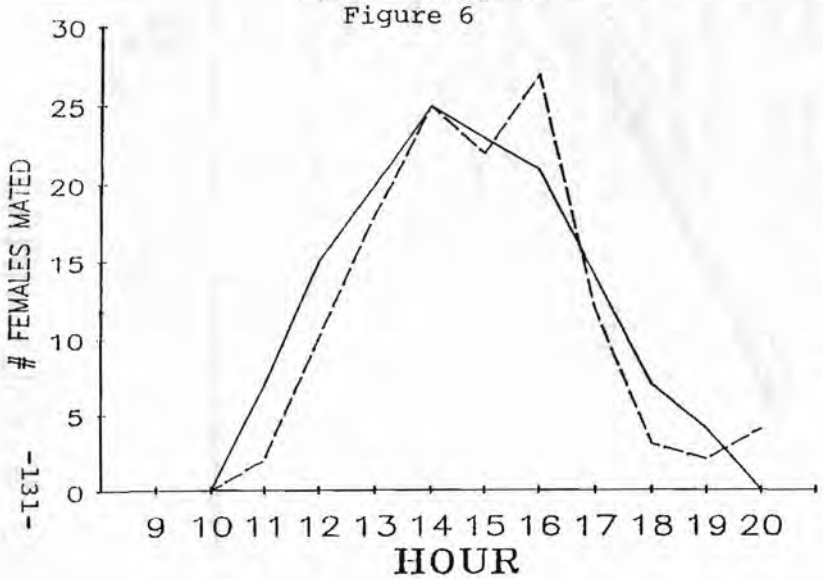
In terms of elapsed time to begin mating, F_1 progeny of irradiated males on the average took approximately one to two minutes longer than the untreated NJ females, however, this difference was not significant. Also, the proportion of females which either did not display calling/mating behavior within approximately a half hour was not significantly different between the various strains. Also, the number of females mated/hour or the frequency of female replacement generally appears to be similar for all strains tested.

The results of examination of egg masses oviposited by females used on the first day of testing is presented in Table 3.

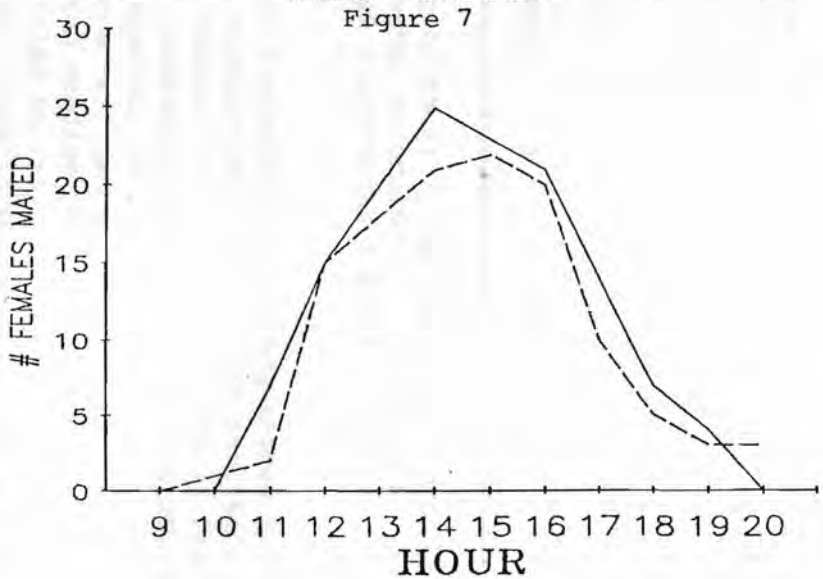
MATING PERIODICITY, FEMALE STRAINS

FEMALES MATED PER HOUR

6K RAD
Figure 6



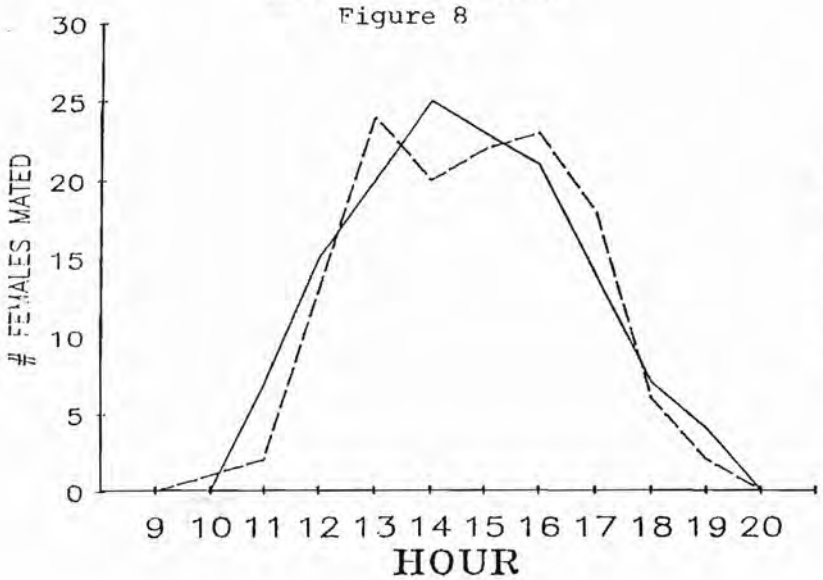
8K RAD
Figure 7



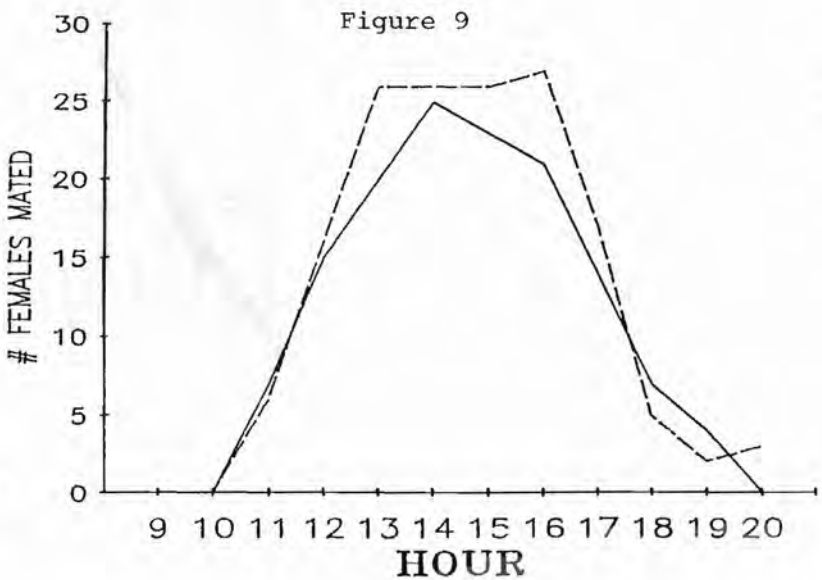
ASHUMET FEMALES

GRAPH HEADING

10K RAD
Figure 8



N.J.
Figure 9



X axis values are period ending on that hour.

FEMME FATALE 1985

% FEMALES MATED PER HOUR INTERVAL, CUMULATIVE

Figure 10

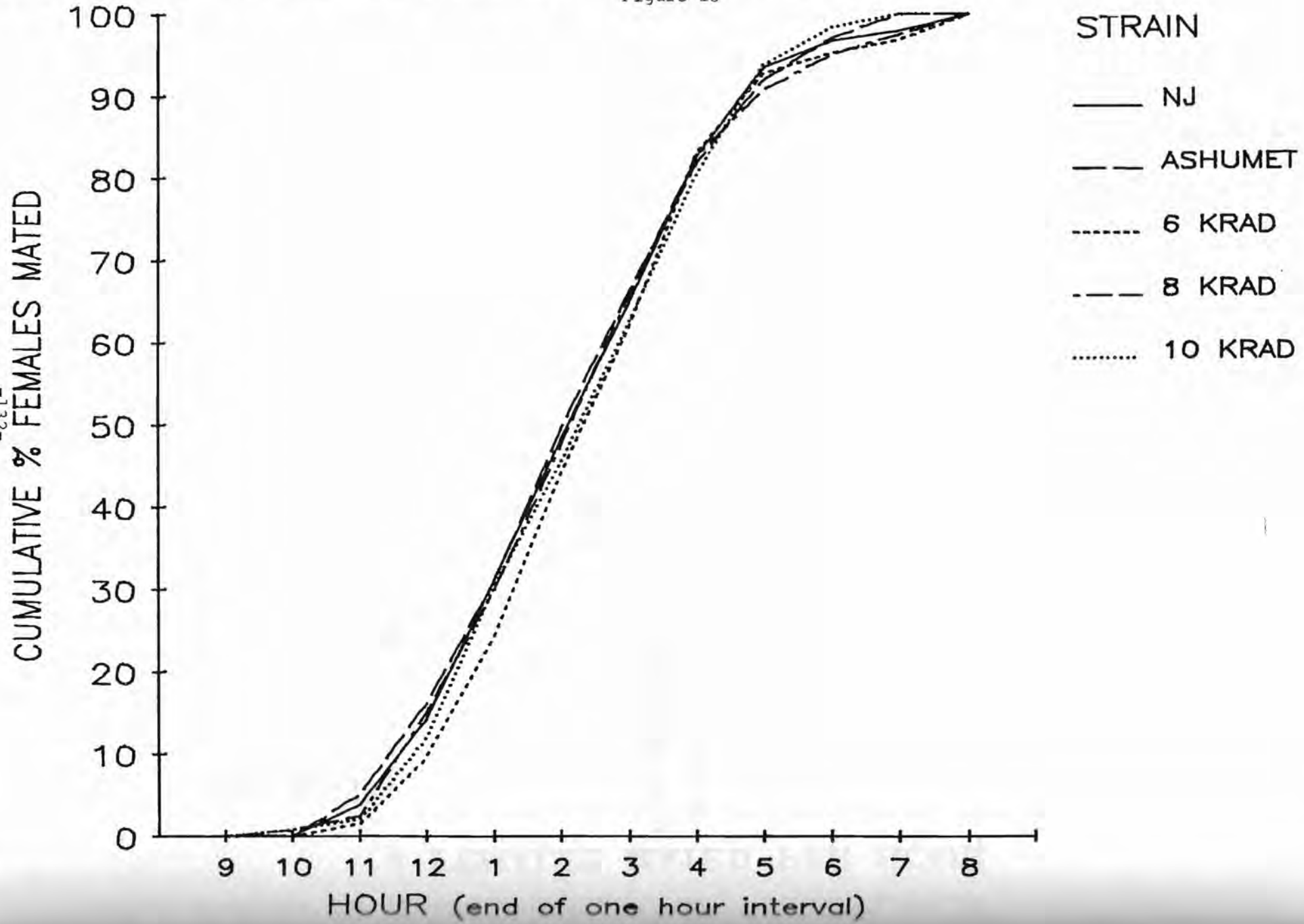


Table 3. Types of egg masses oviposited by females of various strains when mated to feral males in a field trial (July 24, 1985).

Female strain	n	Type of egg mass: Greater than 1 embryonated egg	Type of egg mass: All unembryonated	Female failing to oviposit 1/
F_1 6K	40	80.0%	0.0	20.1%
F_1 8K	47	97.9%	0.0	2.1%
F_1 10K	41	97.6%	2.4	0.0%
NJ_{F28}	49	100%	0.0	0.0%
$Ashumet_{P1}$	41	97.6%	0.0	2.4%

1/ Proportions of F_1 6K females not producing an egg mass is significantly different according to chi square at the 5% level from the proportion of normal (treatments 4 and 5) females not producing egg masses.

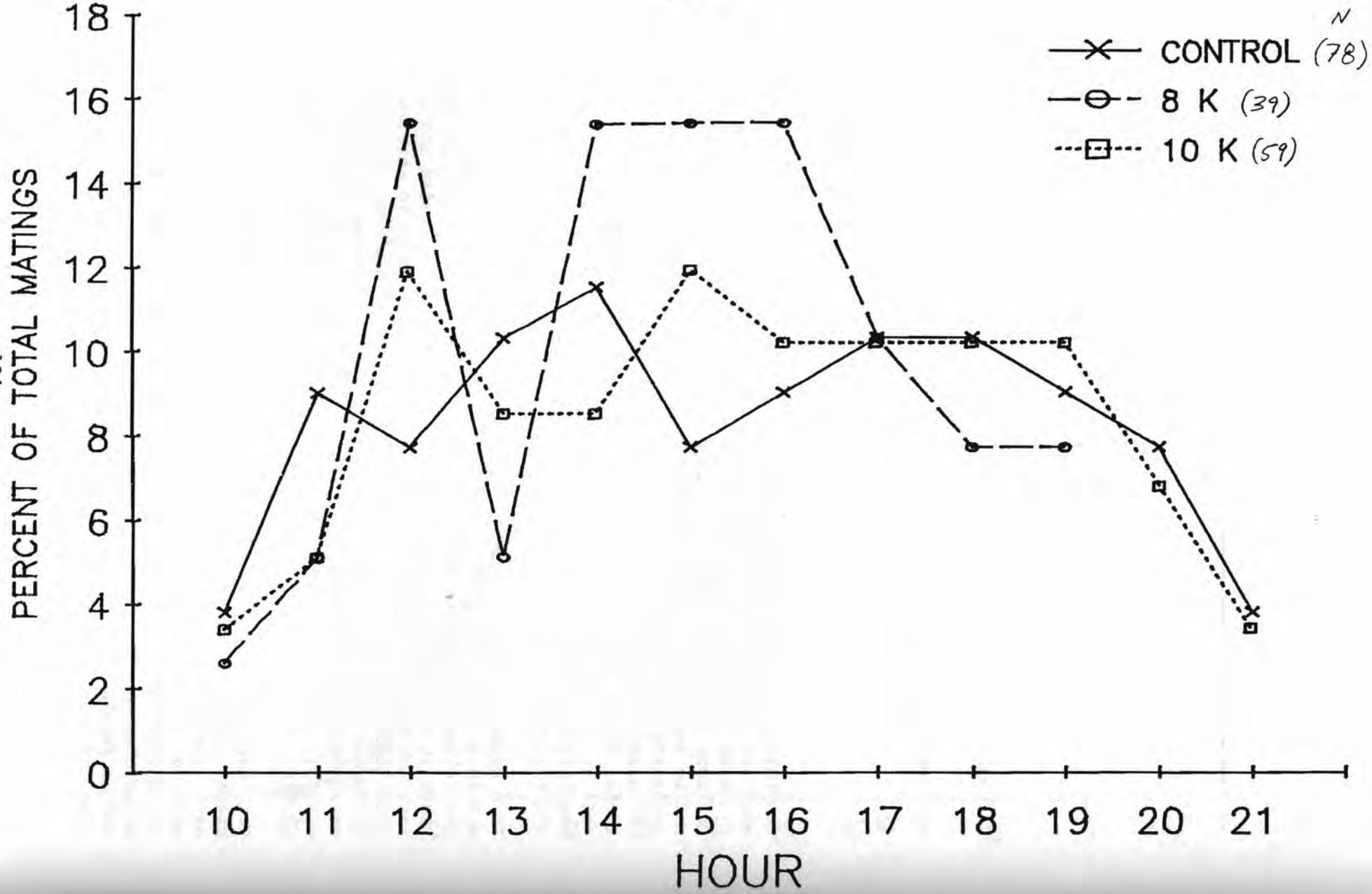
Generally, the proportion of egg masses which contained only unembryonated eggs was similar for the five strains tested. However, a higher proportion of F_1-6krad females (significant 5% level) failed to oviposit. In theory and in previous laboratory studies (dosage irradiation studies), this outcome would not be expected. A lower radiation dose delivered to a P_1 male parent should translate into less genetic damage in the F_1 progeny. In dose x age irradiation studies done in the laboratory, the proportion of F_1 females that either failed to oviposit or produced unembryonated egg masses was the same (6%) at all three levels of irradiation. The results in this field trial are unexplained. Generally, it appears that F_1 females are as competitive as other strains tested in attracting and mating with feral males.

In a separate study in 1985, females of various strains were placed on trees within an area where in the spring an F_1 10k egg mass release had been made. The release area had a native population of gypsy moths and in the adult stage it was expected that there would be a mixture of wild and F_1 males. To test the competitiveness of various strains of females to attract and mate with males of the two possible types, females were placed in the site on four different days. On three days, two types of F_1 females (8 krad and 10 krad) were compared to females of the standard NJ strain. On the final day of testing, the three strains of females which were compared were two F_1 strain (6 krad and 10 krad) and the standard NJ strain.

For testing, females were placed on trees at sites spaced approximately 50m apart. Three trees of nearly equal diameter (ca. 10 inches) were selected at each site and any native insects were removed from the tree bole. Randomly on each day of testing, a female treatment was assigned to each female site. On the day of testing, a virgin, one-day-old female of the appropriate strain was placed on a single tree at a site by 7:00 a.m. When mating occurred, a second female of the same strain was placed on an additional tree at the same site and allowed to establish a resting/calling position. Once the second

1985 SANDWICH MATING PERIODICITY
J.D. 211

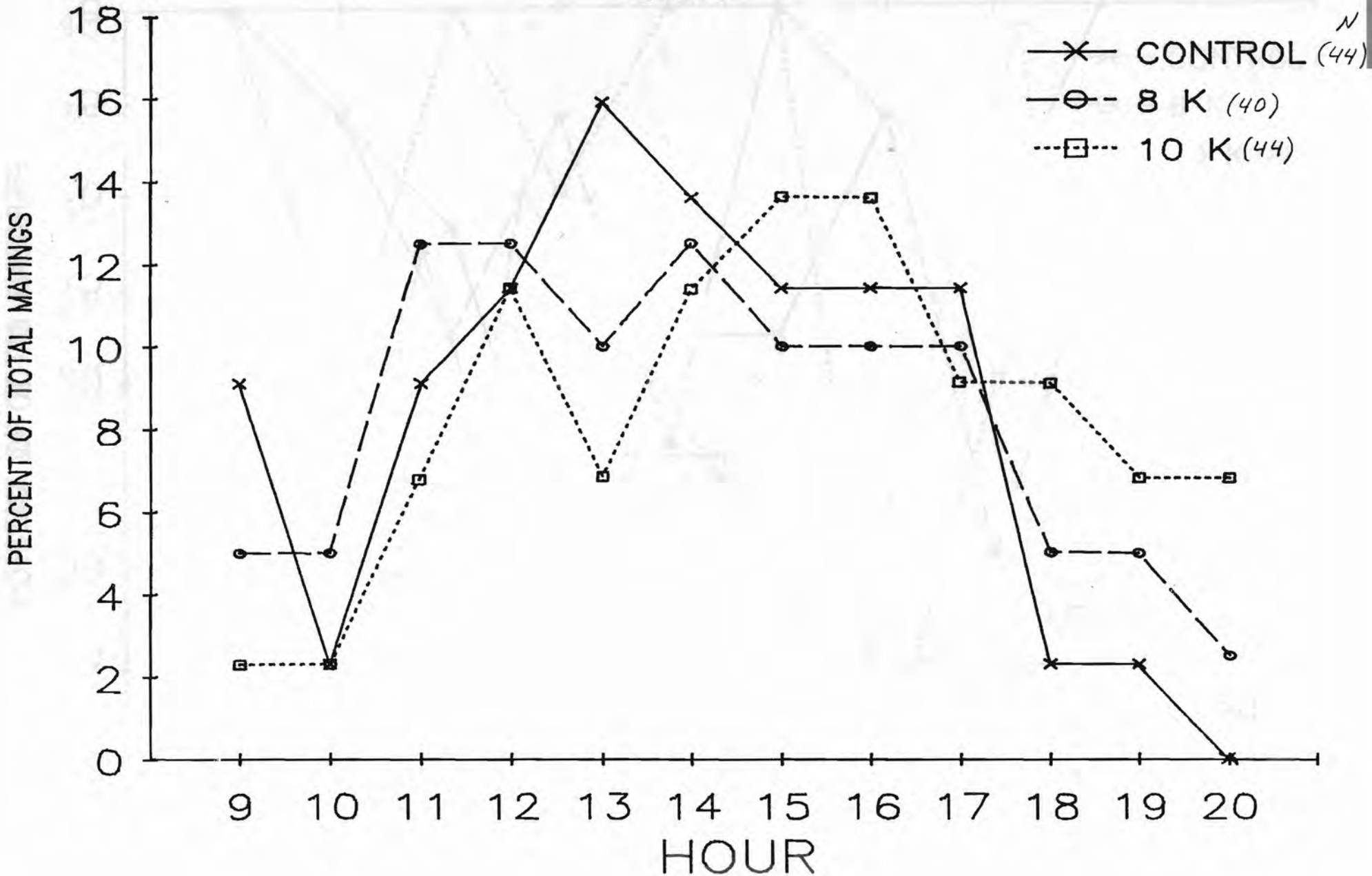
Figure 11



1985 SANDWICH MATING PERIODICITY

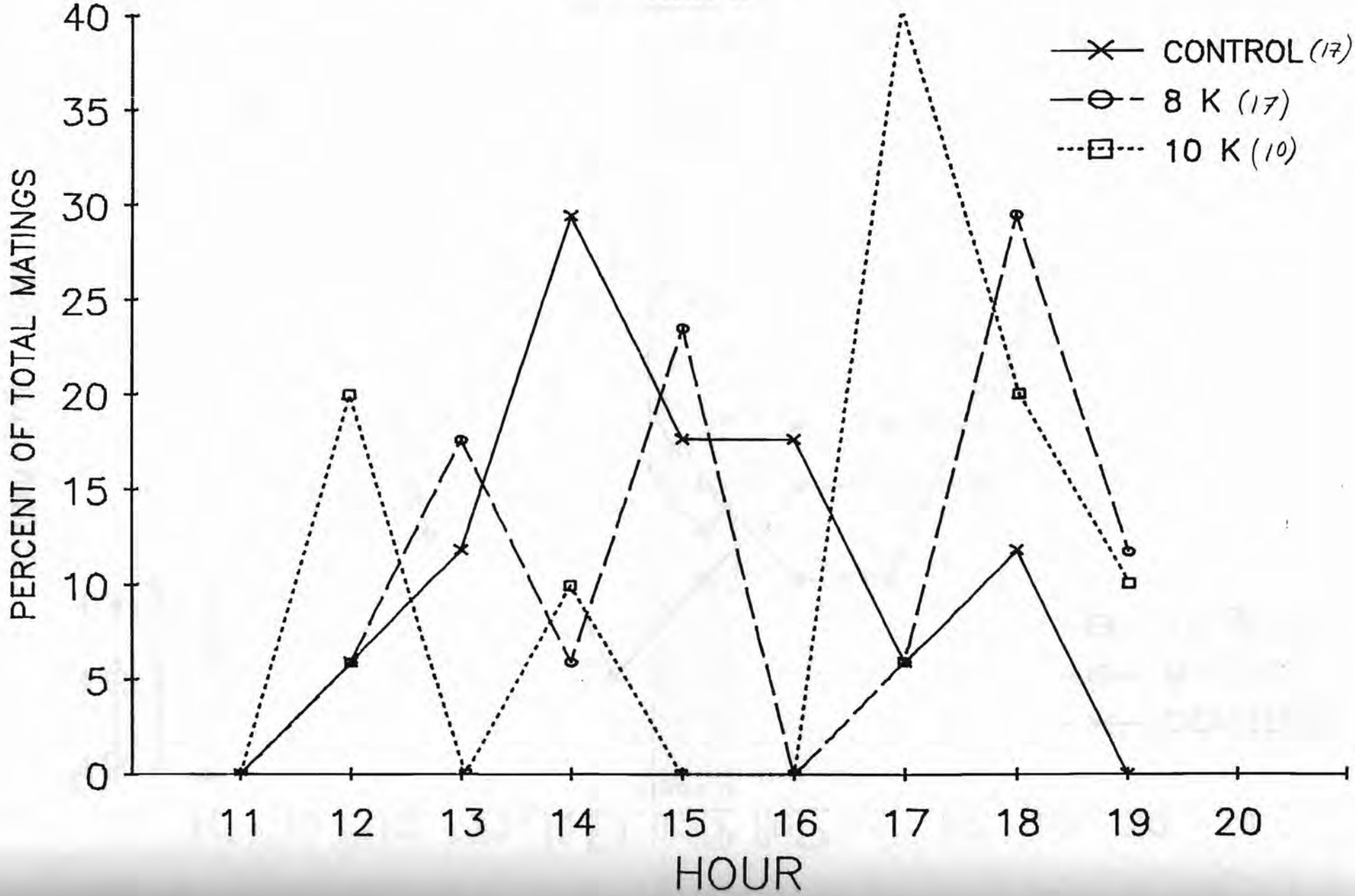
J.D. 212

Figure 12



1985 SANDWICH MATING PERIODICITY
J.D. 214

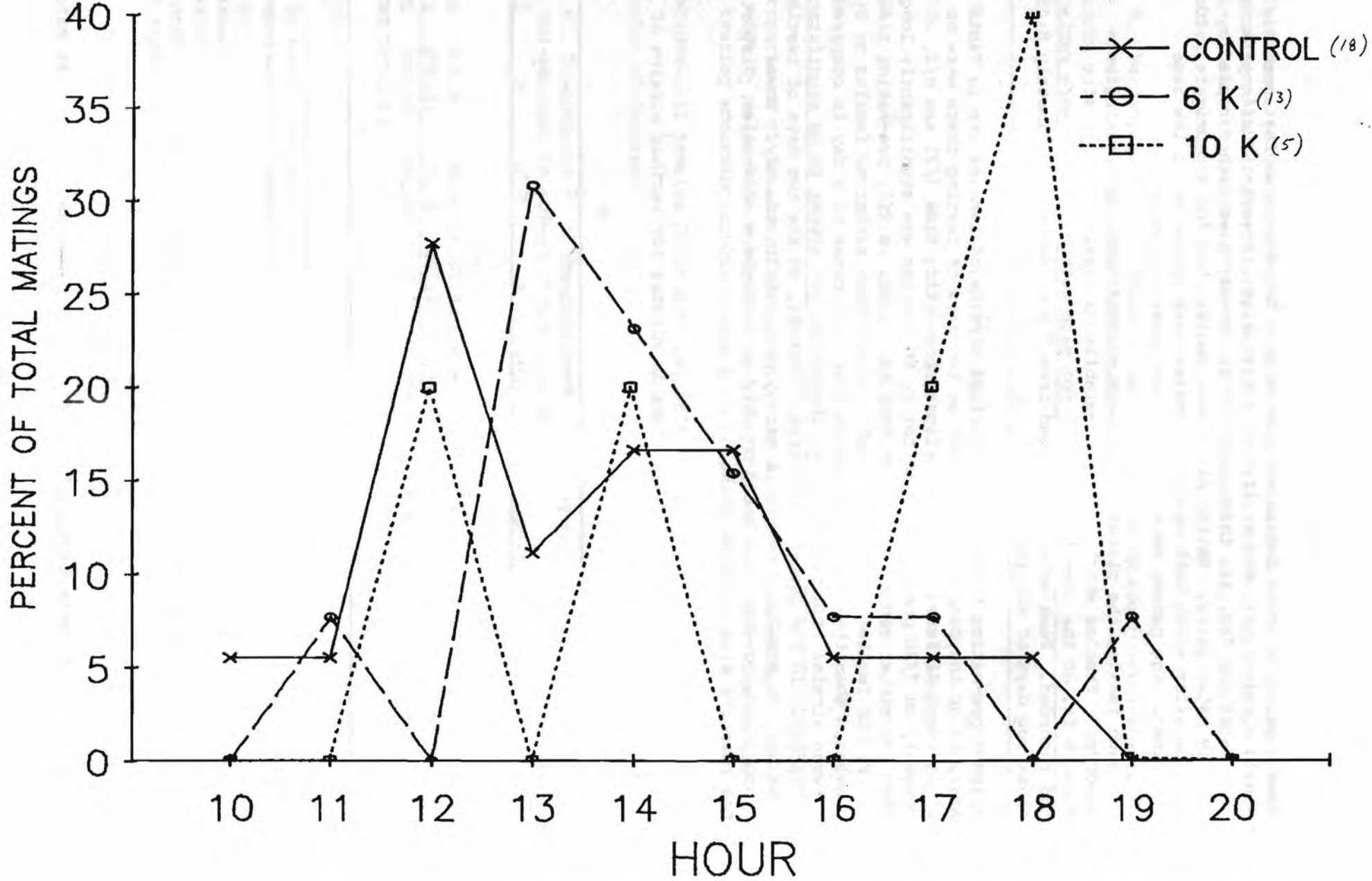
Figure 13



1985 SANDWICH MATING PERIODICITY

J.D. 219

Figure 14



female mated, a third female was placed on a third tree so that normally a single virgin female was available at each site. However, during peak mating periods of the day, all three trees of a site may have been occupied by freshly mated pairs. Mating pairs were undisturbed for approximately 20 minutes after which both male and females were collected in the same container. Egg masses were retained for later evaluation.

For each female, the placement time and elapsed time until mating were recorded. Females which would not establish a resting/calling site within the first 6 ft. of the tree bole, or did not mate within an hour, were replaced and recorded. Females were observed from 7 a.m. until approximately 8:45 p.m. (dusk) on days of observation.

Elapsed pre-mating times for the various strains of females are in Table 4. Analysis of the data indicates that on two days of testing there were no significant differences in mean elapsed pre-mating time 7/31 and 8/2. However, on 7/30 pre-mating time for F_1 8K females was significantly longer than for either control or F_1 10K females. Also, on 8/7, pre-mating times for F_1-10K females were significantly longer than either NJ females or F_1-6K females. When the pre-mating period over the course of a day is compared between strains (Figures 11 to 14) there does not appear to be significant differences in the pattern of mating. However, on the two days of testing F_1 females, 10K females displayed a mating peak late in the day. These proportions, however, are based on only small numbers of females, distorting the relative size of these peaks.

Table 4. Mean elapsed pre-mating time in minutes for various strains of females.

Date	Female strain							
	NJ		F_1-6K		F_1-8K		F_1-10K	
	x	S.D.	x	S.D.	x	S.D.	x	S.D.
7/30	7.236	8.56	-	-	15.711	20.00	9.373	13.30
7/31	13.886	12.00	-	-	21.225	20.21	16.886	14.49
8/2	41.294	34.32	-	-	47.313	43.49	30.600	29.61
8/7	43.000	55.44	69.385	47.39	-	-	140.429	142.70

Egg mass evaluation was used to determine mating type (i.e. male type) of all pairs. Table 5 presents the results of egg mass evaluation from the four days of testing. Generally, females from the various strains appeared to mate with F_1 males and wild males in the same ratios. This indicates that F_1 females can be used reliably as monitor females for assessing mating ratios in field releases. However, 10 krad F_1 females produce more egg masses which cannot be positively separated using current egg mass evaluation techniques (Table 6). This results in larger numbers of egg masses classified in the unknown category. Also equal proportions of all female types fail to oviposit.

In 1987, studies were initiated to determine the frequency of females mating

more than once. Studies included comparisons of the frequency of a second mating of females mated initially to either F_1 (10K) or fertile males. Results of this study have not been fully analyzed and will be reported in the next annual report.

Table 5. Percentage 1/ of females of various strains mating 2/ with sterile males as determined by egg mass evaluation.

Female type	Julian date 211	212	214	219
NJ Control	43.1 (65)3/	39.0 (41)	41.2 (17)	35.7 (14)
F_1 - 10K	51.2 (43)	48.3 (29)	33.3 (9)	50.0 (4)
F_1 - 8K	39.4 (33)	25.0 (32)	33.3 (9)	-
F_1 - 6K	-	-	-	54.6 (11)

1/ Proportions of females of various strains which were determined to have mated with F_1 males were not significantly different from each other within a day's testing according to Chi Square Analysis.

2/ Excluded all females failing to oviposit.

3/ Number of females.

Table 6. Proportions 1/ of females ovipositing egg masses which could be assigned to either of two male mating types.

Female strain	Proportion 2/ of egg masses which could be categorized	Proportion of females producing an egg mass
NJ (control)	91.3a	96.2a
F_1 - 10K	77.3 b	94.8a

1/ Data were pooled over all 4 days of testing.

2/ Proportions in columns not followed by the same letter are significantly different at the 5% level according to Chi Square.

Project Number: GM 7.2.4
Project Title: Disparlure Dispenser Comparison Aging Study - 1987
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leaders: E. C. Paszek and V. C. Mastro

Four new specially formulated dispensers from the Hercon Laboratories Corporation were compared with 2 Recon dispensers made by Agron, Incorporated. These dispensers were aged for 0, 2, 6 and 10 weeks in the greenhouse. The 1987 Hercon 500ug standard (Lot D0117) and the Meyer-Schwartz USDA standard (100ug on cotton wick) were used as controls. Also tested were 2 new 0-1 day-old laminate formulations Hercon 2470-74-1 (7.0mg/in^2) and Hercon L470-74.2 (5.0mg/in^2). This study was conducteded 7/29/88-8/3/87 in the Crane wildlife management area in 5 blocks of 32 treatments. The traps were read and randomized 5 times. A chemical analysis of the aged disparlure dispensers was done by Dr. B. A. Leonhardt, Research Chemist, Insect Ecology Laboratory, AEQI, Beltsville, MD.

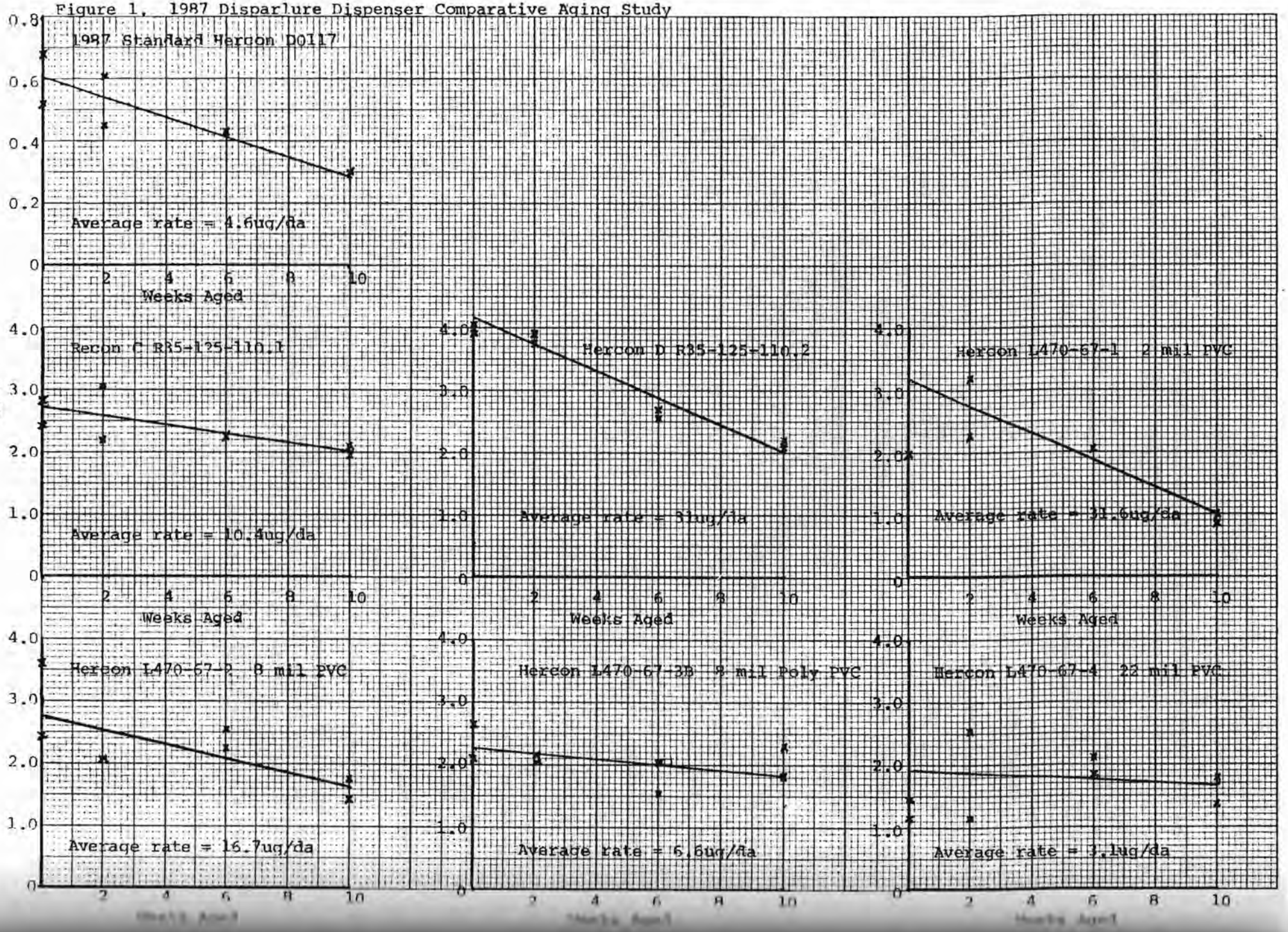
Table 1. Disparlure dispenser comparison - 1987. Mean numbers 1/ of males captured per day per trap (standard error in parenthesis).

Dispenser type	Dispenser age in weeks 0	2	6	10
Hercon D0117 (1987 Standard)	8.84ab (1.602)	10.64a (2.512)	9.76abc (1.899)	9.56abcd (1.969)
Hercon L470-67-1 (2 mil. PVC)	5.96 e (1.684)	7.08 bcde (1.77)	7.68abcde (1.460)	6.40 de (1.628)
Hercon L470-67-2 (8 mil. PVC)	6.040 e (2.465)	5.52 de (1.206)	7.40abcde (1.788)	5.54 de (1.451)
Hercon L470-67-3B (8 mil. Poly)	6.20 e (1.640)	9.76abcde (2.792)	7.64 bcde (2.065)	5.76 e (1.451)
Hercon L470-66-4 (22 mil. Poly)	5.84 bcde (1.161)	5.36 bcde (0.874)	4.45 e (1.011)	5.80 cde (1.266)
Recon G. R35-125-110.1 (2mg. 7mm)	6.12 e (2.287)	5.36 de (1.235)	5.92abcde (0.990)	3.88 e (0.686)
Recon D. R35-125-110.2 (2mg. 13.5mm)	7.64 de (2.795)	4.64 de (0.709)	4.60 e (0.775)	3.44 e (0.523)
Cotton 100 ug (Meyer-Schwartz)	3.68 e (0.152)	-	-	-
Hercon L470-74-1 (7.0 mg/in^2)	6.36 bcde (1.884)	-	-	-
Hercon L470-74-2 (5.0mg/in^2)	4.96 de (0.926)	-	-	-
Blank trap	0.16 f (0.075)	-	-	-

1/ Analysis of variance performed on log (n +1), actual values are presented. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level of significance.

2/ Five complete blocks of 32 treatments were read and randomized five times.

Figure 1. 1987 Disparlure Dispenser Comparative Aging Study



Results of the study are presented in Table 1. The 1987 standard Hercon 500ug survey dispensers aged at 0, 2, 6 and 10 week intervals captured most moths. The specially formulated Hercon dispensers, L470-67-1 (2mil-PVC), L470-67-2 (8mil-PVC), L470-67-3B (8mil-Poly) and Recon C. dispenser R35-125-110-1 (2mg-7mm) with nominally 2mg of lure in 6 week aging treatment compared favorably in 500ug Hercon standard lure of the same age. However, these same dispensers at the other age intervals captured fewer moths than the standard. It is difficult to draw conclusions from these data. Dr. B. Leonhardt's analysis (Figure 1) shows a large discrepancy between the replicates, especially for the 4 Hercon treatments with nominally 2mg of lure. The release rate from Recon D R35-125-110-2 and Hercon L470-67-1 (2mil. PVC) were probably much too high for optimum insect catch. The new 0-1 day Hercon laminate formulation, L470-74-1 (7.0mg/in^2) captured more moths than L470-74-2 (5.0mg/in^2) treatment. However, both of these treatments captured fewer moths than the 0-1 day-old 1987 Hercon 500ug standard.

Project Number: GM 7.2.5
Project Title: Disparlure Dispenser Comparison - 1987
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leader: E. C. Paszek and V. C. Mastro

The 1987 Hercon dispenser, Lot D0117, was tested in multiple or fraction at 63ug, 500ug and 4000ug. Also compared were three new Hercon dispensers, L470-74-1, L470-74-2 and L470-66-4. Cotton wick USDA standards were tested at 1, 10, 100 and 1000ug/wick. They were replicated 4 times and 8 readings were made. The bioassay was conducted at Crane Wildlife Management Area.

Table 1. Disparlure dispenser comparison - 1987. Mean [1/] number of males captured per day per trip.

Dispenser type	n	Mean	S. E.
Cotton 1ug	32	0.75 c	0.191
Cotton 10ug	31	1.65 abc	0.395
Cotton 100ug	32	1.34 abc	0.358
Cotton 1000ug	32	1.09 c	0.334
1/8 Hercon D0117, 63ug	32	1.72 abc	0.438
1 Hercon D0117, 500ug	32	1.84 ab	0.447
8 Hercon D0117, 4000ug	32	0.94 c	0.246
Hercon L470-66-4	32	1.59 abc	0.483
Hercon L470-74-1 7.0mg/sq.in.	32	1.34 bc	0.507
Hercon L470-74-2 5.0mg/sq.in.	32	2.59 a	0.740

Means followed by the same letter are not significantly different according to Duncan's New Multiple Range Test at the 5% level. Analysis of variance performed on transformed data (log males +1) using replicate (4 reps) and reading (8 readings) as blocking variables. Actual means and standard errors are presented.

Results. The new Hercon formulation L470-74-2 (5.0mg/sq. in.) captured the most moths in this study. The following dispensers compared favorably to it. Hercon 1987 survey dispenser (500ug), New Hercon Laminate L470-66-4, 1987 Hercon at 63ug, and the cotton wicks at 100ug and 10ug. The cotton wicks at 1000 and 1ug captured fewer moths. The 8 Hercon wick dispensers equivalent to 4000ug captured few moths, probably due to repellant effects.

Project Number: GM 7.2.6
Project Title: Trap Design Comparison
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leader: E. C. Paszek, V. C. Mastro

In detection surveys it is desirable to use as efficient a trap as possible, for reasons which need not be restated here. The objective of this study was to compare the standard USDA gypsy moth detection trap (delta) with a modification of it (delta-open) and a widely used commercially available trap (Pherocon-IC[1/]). Similar comparisons in the past between the delta and the Pherocon-IC could not demonstrate significant differences between these traps in terms of number of males captured (Mastro, et al. Otis Laboratory Report 84-85). In the standard configuration, the delta trap has equilateral triangular entry ports which are 3.2cm on a side. In this study we included a modified delta trap (i.e. end not folded in) which provided larger entry ports (10cm - sides of equilateral triangle). Larger ports make it easier for males to enter the trap, thereby increasing efficiency. Drawbacks in increasing the entry port size include: increased capture of non-target insects, bird feeding on captured male moths and capture of small birds.

To test the efficiency of the three trap designs, traps were arrayed in 10 complete blocks and read and randomized 4 times. The area used for testing was Crane wildlife management area which had a light gypsy moth infestation in the year of testing.

Table 1. Trap design comparison - 1987. Mean number of males captured per day per trap.

Trap Type	n	Mean[1/]	S. E.
Pherocon - IC	40	1.50 b	0.2582
Delta - open	40	3.35 a	0.4060
Delta	40	2.64 b	0.5637

1/ Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test. Complete replicates (blocks), arrayed in a straight line (50m Inter Trap Spacing) were read and randomized 4 times = 40 observations/treatment. Analysis of variance was performed on log (n+1) transformed data using replicate and reading as blocking variables.

As in earlier studies, the number of males captured by the Pherocon-IC trap was not significantly different from the standard delta trap (Table 1). It also offers little in the way of cost or utility. Opening the ends of the delta trap to provide larger entry ports significantly increased its efficiency. This modification might merit consideration for survey programs where non-target capture or bird feeding are not of concern.

Project Number: GM 1.3.1
Project Title: Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leaders: John Allen Tanner, J. J. Baker, B. A. Fontes and Marc Matthews Jr.

The purpose of this project is to monitor the development of the laboratory strains and to determine if measurements fall 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). This year we began to evaluate several near wild strains for use as possible backup strains to the New Jersey strain.

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. In the wild or near wild strains data are collected on larval development, male and female pupal weights, sex ratio, pupal yields and female fecundity.

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 hatched from egg samples taken 21 days prior to the scheduled use of the masses (Tanner, et. al., 1986).

This method was first used in 1985 with New Jersey F28 egg masses and has subsequently been used with F29, F30 and F31 egg masses. The percentage of egg masses producing "straggling" larvae (MLS 1.00 - 1.25) dropped from 8% in the F28 to 1% in the F29 to 0.3% in the F30 generation but rose to 9% in the F31 generation (fig. 1.). This means that with this method we can not select for a "non-straggling" strain but we can select for egg masses that will produce normally developing larvae in the current generation.

The performance and reproductive data for the F28 through F31 generation colony insects are presented in Tables 1 and 2 respectively. At this time no attempt was made to statistically analyze the data. Selecting for "non-straggling" larvae still does not seem to have any overt effect on the growth and reproduction of the colony insects. With each passing generation there was a tendency for a slight acceleration in the growth rate of both male and female larvae (lower DT50's). Also with each passing generation there was an slight increase in the weight of the females and the number of eggs deposited per female, however, this probably is the result of the reduced competition in each cup caused by the reduction in number of neonates initially placed into each rearing cup.

Table 3 presents the developmental and reproductive data respectively for several near wild strains that may be used in the future as backup strains

for the New Jersey colony. The data are presented for informational purposes only and have not been statistically analyzed. At the present time we are trying to make these strains available year around for field and laboratory evaluations. Based on these evaluations it will be determined if any of these strains can be used as a backup strain for the New Jersey colony.

References Cited

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 Mean	28 SE(n=)	29 Mean	29 SE(n=)	30 Mean	30 SE(n=)	31 Mean	31 SE(n=)
Larvae per cup	9.8	0.05(640)	8.7	0.04(648)	8.0	0.03(613)	8.0	0.04(410)
Mean larval stage 1/	2.94	0.01(640)	2.79	0.01(648)	2.88	0.01(613)	2.76	0.02(410)
Female pupal weights (gms)	2.21	0.03(37)	2.22	0.04(36)	2.38	0.04(34)	2.40	0.03(23)
% Survival neonate to pupae	91.4	0.92(37)	91.4	1.11(36)	90.5	0.99(34)	89.4	1.96(23)
Developmental time to 50% pupation (DT50)								
female	29.0	0.05(635)	28.7	0.04(638)	28.0	0.05(606)	27.9	0.08(402)
male	27.0	0.05(640)	26.6	0.05(645)	25.6	0.05(603)	25.5	0.08(405)
Sex ratio M:F	1.0:1	0.02(37)	1.0:1	0.02(36)	1.0:1	0.02(34)	1.0:1	0.03(23)
% Adult female								
emergence	88.8	2.30(37)	95.5	0.82(36)	93.7	1.14(34)	92.4	1.84(23)
deformity	4.3	0.74(37)	4.2	0.59(34)	2.4	0.59(34)	3.5	0.93(23)

1/ Generation 28 - started March 25, 1985 through November 20, 1985.
Generation 29 - started November 27, 1986 through August 13, 1986.
Generation 30 - started August 18, 1986 through April 14, 1987.
Generation 31 - started April 21, 1987 through October 1, 1987.

2/ 9th Post-neonate infest date.

Table 2. Reproductive data of New Jersey colony insects.

	Generation 1/							
	28		29		30		31	
	Mean	SE(n=)	Mean	SE(n=)	Mean	SE(n=)	Mean	SE(n=)
% of females depositing egg masses	81.9	2.96(37)	84.4	2.9(36)	74.8	3.7(34)	80.5	2.9(23)
# eggs deposited per female	958.1	21.8(37)	997.2	19.6(36)	1021.4	27.6(34)	1030.3	26.1(23)
# colony eggs per colony mating	764.5	23.7(37)	873.3	19.6(36)	833.1	23.4(34)	789.5	31.3(23)

1/ Generation 28 - started March 25, 1985 through November 20, 1985.
Generation 29 - started November 27, 1986 through August 13, 1986.
Generation 30 - started August 18, 1986 through April 14, 1987.
Generation 31 - started April 21, 1987 through October 1, 1987.

Table 3. Performance and reproductive data of near wild strains.

	Michigan F^2		North Mountain F^2		Maine F^3		Sleepy Creek F^2		Ashumet F^3	
	Mean	SE(N)	Mean	SE(N)	Mean	SE(N)	Mean	SE(N)	Mean	SE(N)
Larvae per cup	9.6	0.25(28)	9.6	0.16(23)	9.9	0.18(42)	9.8	0.21(28)	9.7	0.38(41
Mean larval stage 1/	2.41	0.12(28)	2.54	0.11(23)	3.19	0.08(42)	2.40	0.13(28)	2.57	0.10(39
Pupal weights (gms)										
Male	0.54	0.02(25)	0.62	0.03(23)	0.56	0.01(40)	0.60	0.02(27)	0.62	0.10(41
Female	2.23	0.09(24)	2.23	0.11(22)	1.88	0.05(40)	2.07	0.09(23)	2.24	0.11(38
% survival neonate to pupate	29.2	3.49(28)	25.8	4.82(23)	47.5	3.64(42)	23.0	4.18(28)	32.5	4.19(39
Sex ratio M:F	1.6:1	0.26(24)	2.3:1	0.29(22)	1.9:1	0.21(40)	2.0:1	0.40(23)	2.1:1	0.19(38
% of females depositing egg masses	92.1	1.73(23)	77.9	3.81(8)	77.4	2.33(53)	80.9	5.61(14)	86.0	3.14(31
# eggs - deposited per female	895.7	49.2(23)	628.9	117.2(8)	732.7	33.6(53)	842.8	70.2(14)	971.6	148.5(31)
# eggs per mating	828.4	52.9(23)	509.7	105.0(8)	578.2	30.4(53)	702.9	86.5(14)	734.4	36.2(31)

1/ Mean larval stage was determined 14 days after the neonates were placed into 6 oz. fluted rearing cups.

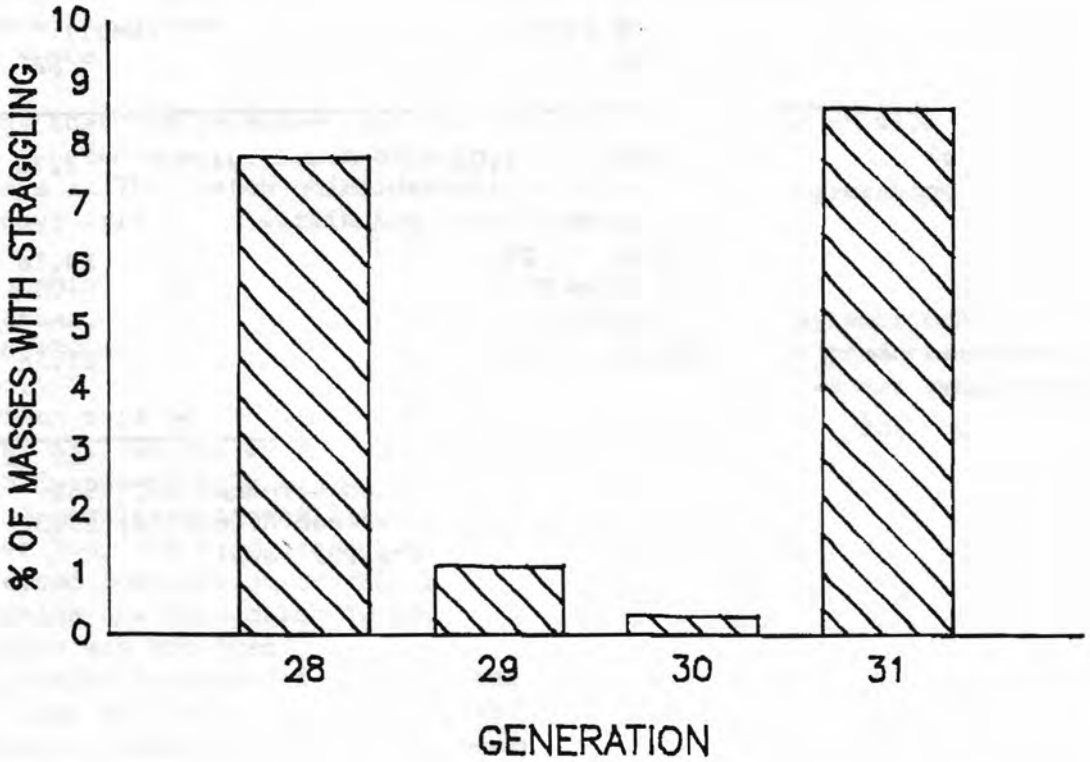


Figure 1. The percentage of New Jersey laboratory strain gypsy moth egg masses that produced "stragling" larvae.

CORRECTION

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: Table 2. pg 124 should read.

Table 2. Reproductive data of New Jersey colony insects.

	Generation[1/2/]			
	28 (N=36)		29 (N=37)	
Parameter	mean	SE	mean	SE
% of females depositing egg masses	80.9	3.0a	84.7	2.7a
# eggs deposited per female	958.5	22.4a	995.7	19.1a
# colony eggs per colony mating	761.6	24.2a	873.2	23.5a

1/ Generation 28 - started March 25, 1985 through November 20, 1985.
Generation 29 - started November 27, 1985 through August 13, 1985.
2/ Means in each row were nonsignificant at the .05 level.

Project Number: GM 3.3.1
Project Title: Development and Evaluation of Improved Rearing Techniques
Report Period: October 1, 1986 - september 30, 1987
Report Type: Interim
Project Leaders: John Allen Tanner, J.G.R. Tardif, J.J. Baker, B.A. Fontes and Marc Matthews Jr.

This project concerns the development and evaluation of new rearing techniques and the improvement of presently use techniques. Unreliable or inefficient techniques will be modified or discarded.

The F1 sterile male program is now the major user of reared insects. Currently, we can provide up to approximately 35,000 pupae/day. However, these numbers will have to be increased considerably in the future. To do this, the rearing facility will not only have to be enlarged, but new rearing techniques will have to be developed that will efficiently use all the available space.

The F_1 eggs that are released into test plots near the time of wild egg hatch must have a hatch profile which coincides with that of the wild. Eggs chilled 140-180 days at $7^{o}c$, hatch coincidentally with wild eggs; F_1 eggs held longer than 180 days will hatch within the refrigerator.

Wild eggs begin to hatch around March 15 in southern areas and around May 5 in northern areas. This means that with the standard 28-35 day embryonation period and 180 day chilling period, all the F_1 eggs must be produced over a 91 day period. In anticipation of higher production demands, we are looking for ways to widen this 91 day production "window". In the 1986 laboratory report (Tanner, et al.) we presented hatch data that indicated we could increase the production "window" by 26 to 30 days by using selected combinations of embryonation-acclimation-chilling periods and still have a minimum percent hatch above 20%. We also presented hatch data which indicated that by using other selected combination of the embryonation-acclimation-chilling periods we could increase the production "window" by 45 to 58 days if a 15% minimum percent hatch was accepted. Table 1 lists the seven best combinations of embryonation-acclimation-chilling periods tested and also gives the percent hatch and time to 50% hatch (HT50) observered when F_1 eggs were exposed to these periods. Table 2 presents the percent survival of the F_1 neonates to the adult stage.

The hatch profile is presented in figure 1 and the male and female pupation curves are presented in figures 2 and 3 respectively.

Table 1. Hatch data for F_1 eggs subjected to selected combinations of embryonation-acclimation-chilling periods to increase the F_1 sterile male production "window".[1/]

	Length (days)						
Embryonation period (% RH)[2/]	28(55)[3/]	84(80)	56(80)	28(55)	28(80)	84(80)	56(80)
Acclimation period (7°C)	0	0	0	30	30	0	30
Chill period (°C)[4/]	180(7)	150(7)	180(7)	180(5)	195(5)	180(7)	180(5)
Number of egg masses sample	15	15	15	15	15	14	15
Total pre-egg incubation period[5/]	208	234	236	238	253	264	266
Increase in the F_1 production "window" (days)[6/]		26	28	30	45	56	58
Percent hatch[7/]	25.1±4.4	30.1±4.4	27.1±3.2	25.1±2.5	17.0±3.0	15.7±3.9	16.7±3.0
Time to 50% hatch HT50(days)[7/8/]	1.5±0.1	3.7±0.4a	2.1±0.1a	4.1±0.2a	4.5±0.2a	2.0±0.4	4.9±0.4a

1/ Based on a 15% minimum percent hatch.
2/ ( )Percent relative humidity during embryonation.
3/ Control
4/ ( )Temperature during egg chilling.
5/ Total pre-egg incubation period = embryonation period + acclimation period + chill period.
6/ Increase in the F_1 "window" = pre-egg incubation period of treatment - pre-egg incubation period of control.
7/ ± S.E.
8/ Means within the row followed by the letter a were significant different at $P<.05$ when compared to the control, t-test unpaired comparison.

Table 2. Survival to the adult stage of neonates hatch from F_1 eggs subjected to selected combinations of embryonation-acclimation-chilling periods to increase the F_1 sterile male production "window".

	Length (days)						
Embryonation period (% RH)[1/]	28(55)[2/]	84(80)	56(80)	28(55)	28(80)	84(80)	56(80)
Acclimation period (7°C)	0	0	0	30	30	0	30
Chill period (°C)[3/]	180(7)	150(7)	180(7)	180(5)	195(5)	180(7)	180(5)
Number of neonates sample	286	278	299	291	247	209	270
Percent survival[4/5/]	63.8±3.6	67.3±3.6	70.9±2.6	68.1±3.0	66.7±3.2	56.6±3.9	60.0±3.5

1/ ( )Percent relative humidity during embryonation.
2/ Control
3/ ( )Temperature during egg chilling.
4/ ± S.E.
5/ Means within the row were nonsignificant at $P<.05$ when compared to the control, t-test unpaired comparison.

Accepting the 15% minimum hatch level will allow us to select embryonation-acclimation-chilling periods that will extent the F_1 production "window" up to 58 days without reducing the ability of the F_1 larvae to survive to the adult stage (table 2) nor radically change their growth rates (fig. 2 and 3) when compared to the control F_1 larvae. The higher HT50's (table 1) and the shift to the right in the accumulative percent hatch curve indicates that the hatch of the noncontrol F_1 eggs will be delayed slightly compared to the control eggs. However, once hatch begins it is completed in 3 to 5 days in all the treatments. If the delay in hatch is determined to be a problem, the noncontrol F_1 eggs could be held at ambient temperature a few days before they are released into the field.

The percent hatch of the partially sterilized eggs produced during the 1986 program was equal to or slightly lower than the eggs produced during the 1984 program (fig.4), but was considerably reduced when compared to the 1985 program. Larval "straggling" was less of a problem in the 1986 program than in the 1985 program and probably was not, as originally thought, the reason for the lower hatch observed in the 1984 program (Tanner, et al. 1986).

References Cited

Tanner, J.A., J.G.R.Tardif, J.J. Baker, H. Hamilton and B.A. Fontes. 1986. Development and Evaluation of Improved Rearing Techniques. October 1, 1985 -September 30, 1986:126-137.

Correction

Tanner, J.A., J.G.R. Tardif, J.J. Baker, H.Hamilton and B.A.Fontes. 1986. Development and Evaluation of Improved Rearing Techniques. APHIS Laboratory Report, October 1, 1985-September 30, 1986:Table 3 pg 130 should read.

Table 3. Percent of hatch of F_1 eggs given different combinations of embryonation and acclimation-chilling periods.

Acclimation-chilling period[2/]		Embryonation period (% RH)[1/]					
7°C	5°C	28(55%)	28(80%)	56(55%)	56(80%)	84(80%)	112(80%)
120	30			15.2	25.7	14.7	7.3
150	30			14.4	26.2	11.7	3.7
180	30	25.1	22.7	5.3	16.7	5.7	2.0
195	30	15.0	17.0	6.0	10.0	3.3	2.0
0	150			22.5	30.2	30.1	13.3
0	180			18.9	27.1	15.7	4.0
0	210	25.1[3/]	24.7	4.7	10.0	2.7	1.3
0	225	15.6	13.8	3.0	7.3	1.3	0.3

1/ ( ) percent relative humidity used during the embryonation period.
2/ Acclimated eggs were initially exposed to 7°C for 30 days then chilled at 5°c. Nonacclimated eggs were chilled continuously at 7°C.
3/ Control

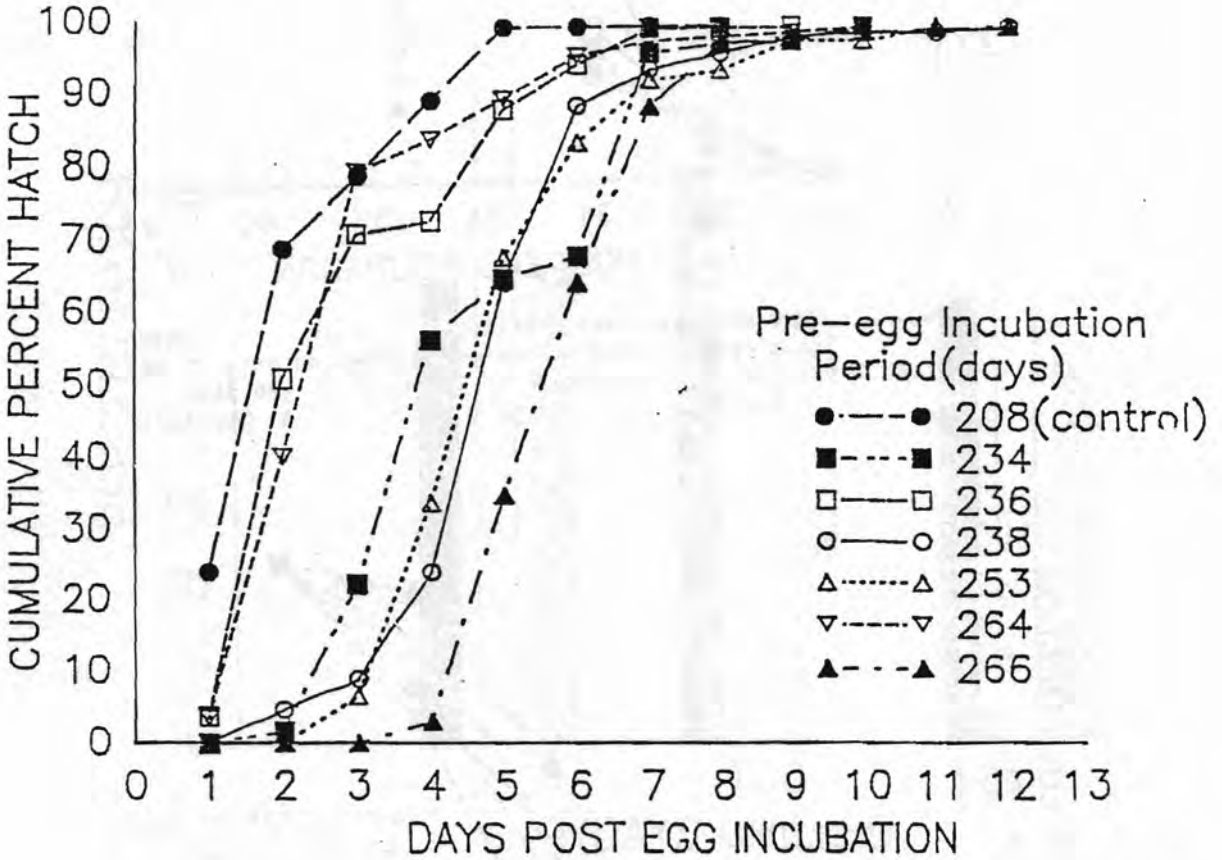


Fig. 1. Cumulative percent hatch of F_1 eggs exposed to pre-egg incubation periods of: A) 208 days (control) B) 234 days C) 236 days D) 238 days E) 253 days F) 264 days G) 266 days.

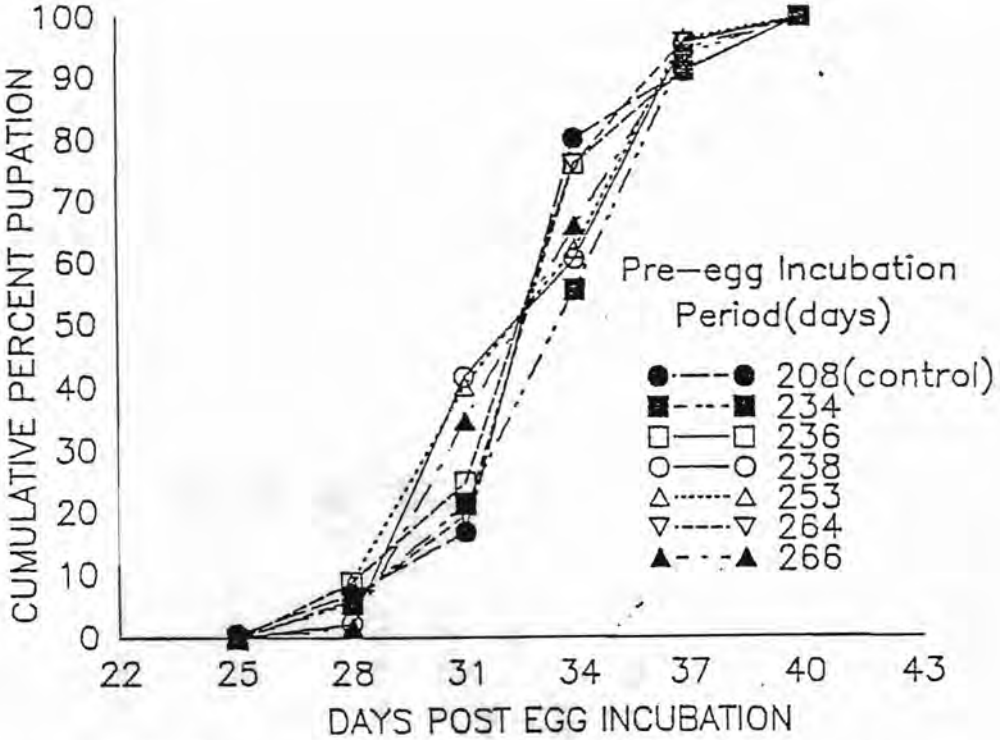


Fig. 2. Cumulative percent pupation of F_1 male larvae hatch from eggs exposed to pre-egg incubation periods of: A) 208 days (control) B) 234 days C) 236 days D) 238 days E) 253 days F) 264 days G) 266 days.

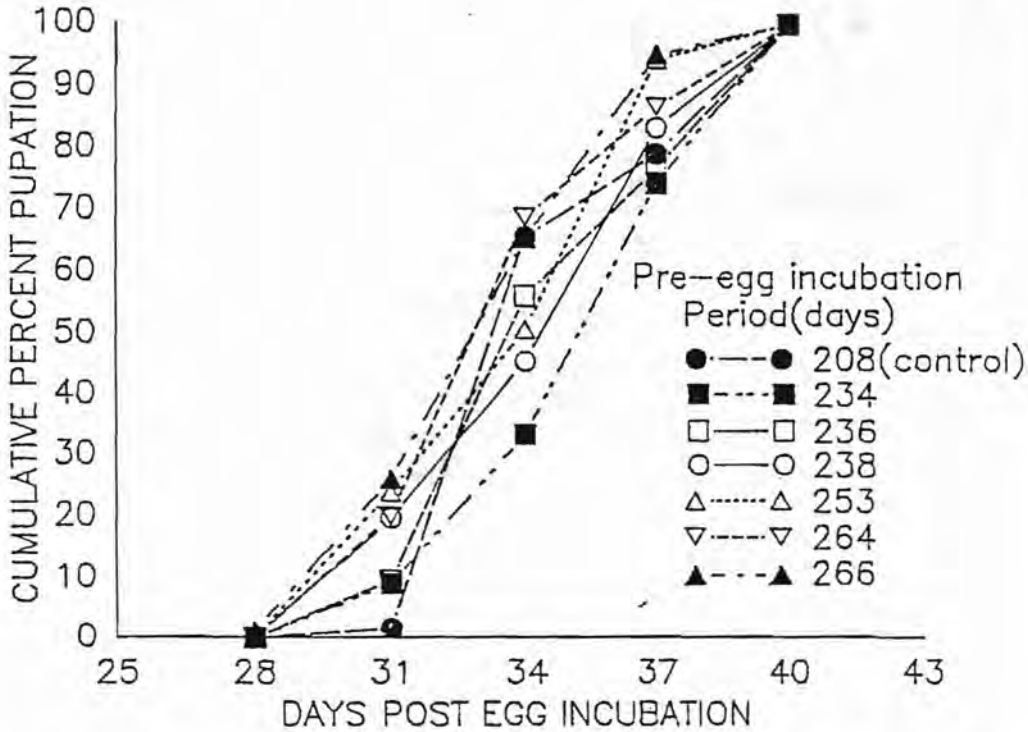


Fig. 3. Cumulative percent pupation of F_1 female larvae hatch from eggs exposed to pre-egg incubation periods of: A) 208 days (control) B) 234 days C) 236 days D) 238 days E) 153 days F) 264 days G) 266 days.

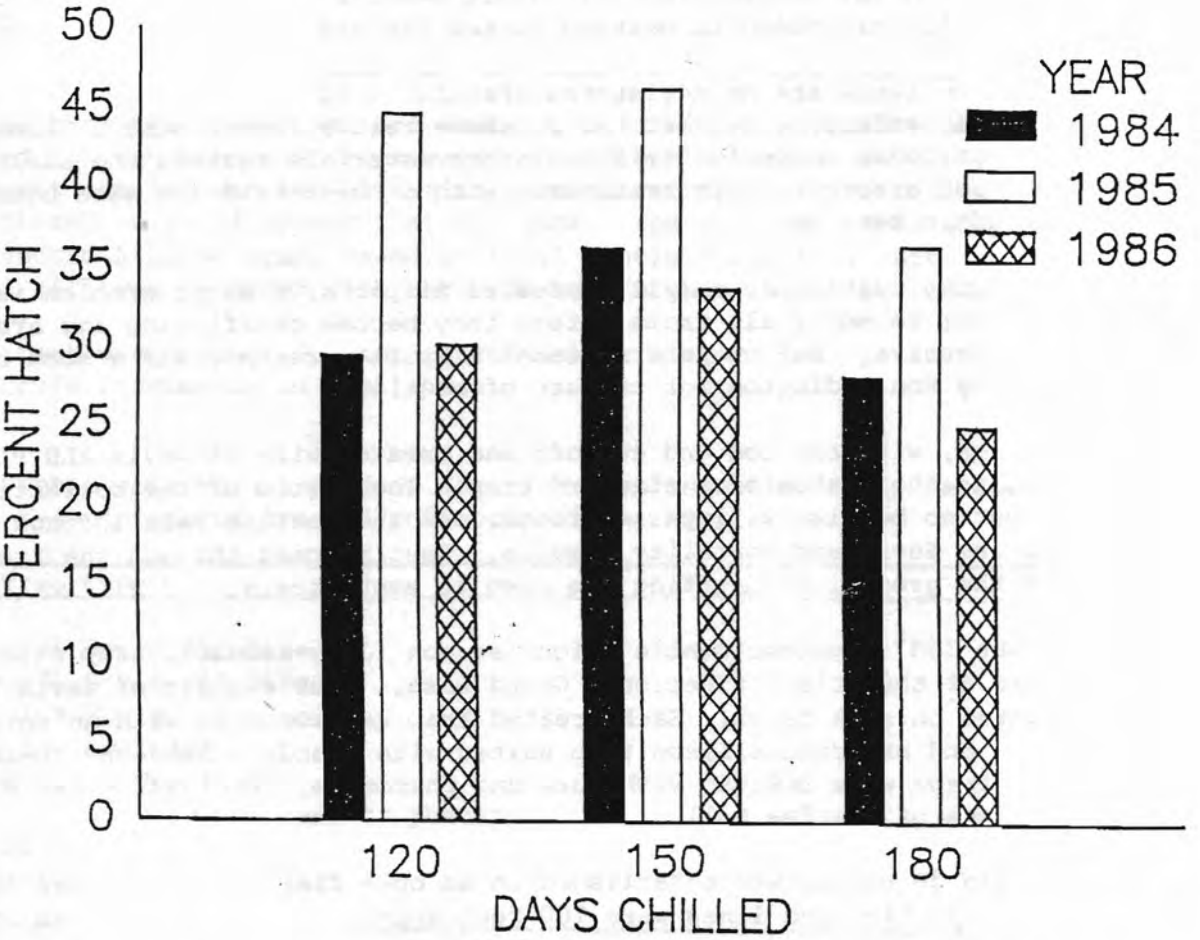


Fig. 4. The percent hatch of embryonated F_1 partially sterilized eggs produced in the fall of 1984, 1985 and 1986.

Project Number: JB 4.1.1
Project Title: Evaluation of Traps and Other Techniques for Controlling Japanese Beetles in and Around Airports and Nurseries
Report Period: October 1, 1986 - September 30, 1987
Report Type: Interim
Project Leaders: W. H. McLane, J. A. Finney and T. Ladd

Japanese beetle infestations are known to occur at nearly all major airports in the eastern United States and, at times, adult populations are high enough to warrant regulation of airports. Adult beetles are attracted to aircraft and can easily hitchhike to western states and establish new infestations.

At this time there are no registered granular soil insecticides that are consistently effective against the Japanese beetle larval stage. Limited spraying is conducted with Sevin and other materials against the adult stage. Trapping and aircraft cabin treatments with d-Phenothrin are also used to control adult beetles.

When trapping beetles at heavily infested airports, a major problem is having the manpower to empty all traps before they become overflowing and are no longer effective. Dulles International Airport personnel use a sock trap designed by Ron Addington for capture of beetles.

A tube sock, with the toe end cut off and treated with 5% Sevin XLR Plus is attached to the bottom of a standard trap. The bottom of the collection can is cut out so beetles will pass through. As the beetles pass through, they contact the Sevin and mortality results. Beetles pass through the trap and fall to the ground, eliminating the need to empty traps.

During the 1987 Japanese beetle flight season (July-August), trap studies were conducted at the Otis Air National Guard Base. Four dosages of Sevin XLR Plus were tested on sock traps. Each treated trap was compared with an untreated sock trap and standard Ellisco trap baited with standard lure and pheromone. All sock traps were treated with lure and pheromone. Evaluation was based on total volume of beetles collected.

A grid with 36 points was established in an open field. Points were 50 feet apart on each line and lines were 100 feet apart. A trap was placed at each point and trap placement was rotated after each collection. Fifty mesh screen bottom wooden boxes (18 x 18 x 6 inches) were used under each sock trap to collect dead beetles. At time of beetle collection, the contents of each trap or box were measured volumetrically. Sevin was applied to the sock part of sock traps with a small hand sprayer to the point of run-off. Only one treatment was made throughout the test period.

Table 1. Total volume (ml) of dead Japanese beetles collected in sock traps, standard Ellisco traps and untreated sock traps in 11 collections.

Treatment	Volume (ml)
Sevin XLR Plus 25% (sock)	45,835
Sevin XLR Plus 15% (sock)	36,050
Sevin XLR Plus 10% (sock)	39,625
Sevin XLR Plus 5% (sock)	37,875
Untreated (sock)	4,999
Standard Ellisco trap	54,575

The results from the sock traps are probably underestimates since some beetles that passed through the sock traps were not collected because on windy days the sock exit opening was not always centered directly over the collection box. It therefore would appear that the sock traps were nearly as effective as the standard Ellisco trap, based on total beetle mortality. Little additional kill was recorded with Sevin dosages higher than 5 percent.

A similar test was conducted later in the season comparing traps and lower rates of Sevin treatments on sock traps.

Table 2. Total volume (ml) of dead Japanese beetles collected in various types of traps over 15 collection intervals.

Trap type	Volume (ml)
Sock trap (5% Sevin XLR Plus)	29,485
Sock trap (3% Sevin XLR Plus)	22,025
Sock trap (1% Sevin XLR Plus)	13,905
Sock trap (no treatment)	932
Standard Ellisco Trap	98,830
Standard Ellisco Trap (no lure or pheromone)	889
Lure and Kill Trap	89,995
Blitz-A-Beetle Trap	10,902

In this test the sock trap was less effective than the standard Ellisco and Lure and Kill traps. Perhaps the loss of cadavers due to high winds was a greater problem. However, this experiment showed that lowering pesticide application reduces effectiveness. The Blitz-A-Beetle trap gave poor results.

Japanese beetle adults continued to be a problem at some of our major airports in the eastern United States. Standiford airport at Louisville, Kentucky, was regulated on June 26, 1987. Live beetles continued to be found on aircraft in California that originated in Kentucky despite cabin and storage area treatments with d-Phenothrin (Sumithrin). At first, the d-Phenothrin was suspect as some of the material being used was 10 years old. Old and new samples were sent to 3 laboratories for analysis. Each laboratory found that the old material had lost no more than 16 percent of its active material, and most old cans sampled had lost no more than 5 percent. They did find that

most old cans had lost a great deal of their propellant.

When cargo planes, such as the 747, are treated after all cargo has been loaded on board, the pesticide is not getting to beetles that may be trapped in the ball-mat area. In this case, the ball-mat area should be treated before all cargo is loaded. Treatments should be applied around the door area as it is closed. In some cases, it appears the material is not killing the beetles regardless of how much contact they have with it.

A number of exposure tests were conducted at the Otis Methods Development Center with native beetles. Most were designed to determine kill after various exposure times to surfaces treated with chemical. Two old and 2 new samples of d-Phenothrin were bioassayed to determine activity. Five plastic petri dishes were sprayed with each material. Five native Japanese beetle adults were then placed onto each dish for various intervals, and transfered to clean dishes and held for observation of mortality.

Table 3. Percent mortality of Japanese beetle adults following exposure to petri dish surfaces treated with a number of d-Phenothrin samples.

Sample	Exposure time	Percent mortality after:1/			
		1 hour	24 hours	48 hours	72 hours
KY 1 (new sample)	1 hour	0	12	24	68
	2 hours	0	8	20	76
Bulk 1 (new sample)2/	1 hour	0	24	40	76
	2 hours	0	24	48	64
KY 3 (old sample)	1 hour	0	8	16	64
	2 hours	0	24	36	76
KY 4 (old sample)	1 hour	0	16	52	76
	2 hours	0	16	48	84
Control	1 hour	0	0	0	28
	2 hours	0	4	12	40

1/ Hours in untreated petri dishes after initial exposure
2/ New sample just received from Bulk Chemical Company

Mortality for both old and new samples was similar, but less than desired for regulatory treatment.

Metal plates were treated with 4 dosages of Sevin XLR and one dosage of d-Phenothrin. The materials were applied to the point of run-off and each treatment was replicated 5 times. Five live native Japanese beetle adults were then exposed to each replication for various periods of time. Beetles were exposed to treated surfaces immediately after treatment, 1 and 3 days

later. After exposure, beetles were held in plastic petri dishes and mortality readings made over periods of time.

Table 4. Percent mortality of Japanese beetle adults following exposure to metal plates with insecticide 1 hour prior to exposure.

Material	Exposure time	Percent mortality after: 1 day	2 days	3 days
Sevin XLR 25%	1 min.	92	96	100
Sevin XLR 25%	5 min.	88	92	100
Sevin XLR 25%	15 min.	88	92	100
Sevin XLR 15%	1 min.	80	100	100
Sevin XLR 15%	5 min.	80	96	100
Sevin XLR 15%	15 min.	92	92	100
Sevin XLR 10%	1 min.	72	92	100
Sevin XLR 10%	5 min.	76	92	100
Sevin XLR 10%	15 min.	88	88	100
Sevin XLR 5%	1 min.	76	92	100
Sevin XLR 5%	5 min.	84	92	100
Sevin XLR 5%	15 min.	88	100	100
d-Phenothrin 10%	1 min.	36	92	100
d-Phenothrin 10%	5 min.	24	64	100
d-Phenothrin 10%	15 min.	24	68	100
Control	15 min.	0	0	0

Table 5. Percent mortality of Japanese beetle adults following exposure to metal plates treated with insecticides 24 hours prior to exposure.

Material	Exposure time	Percent mortality after: 1 day	2 days
Sevin XLR 25%	15 sec.	52	88
Sevin XLR 25%	30 sec.	68	96
Sevin XLR 25%	1 min.	71	96
Sevin XLR 25%	5 min.	80	96
Sevin XLR 25%	15 min.	92	100
Sevin XLR 15%	15 sec.	48	76
Sevin XLR 15%	30 sec.	40	80
Sevin XLR 15%	1 min.	74	100
Sevin XLR 15%	5 min.	68	96
Sevin XLR 15%	15 min.	83	88
Sevin XLR 10%	15 sec.	32	72
Sevin XLR 10%	30 sec.	56	96
Sevin XLR 10%	1 min.	78	96
Sevin XLR 10%	5 min.	85	100
Sevin XLR 10%	15 min.	92	100
Sevin XLR 5%	15 sec.	20	56
Sevin XLR 5%	30 sec.	44	76
Sevin XLR 5%	1 min.	68	84
Sevin XLR 5%	5 min.	68	100
Sevin XLR 5%	15 min.	82	100
d-Phenothrin 10%	15 sec.	20	77
d-Phenothrin 10%	30 sec.	16	92
d-Phenothrin 10%	1 min.	4	48
d-Phenothrin 10%	5 min.	12	84
d-Phenothrin 10%	15 min.	15	77
Control	15 min.	0	0

Table 6. Percent mortality of Japanese beetle adults following exposure to metal plates treated with insecticide 72 hours prior to exposure.

Material	Exposure time	Percent mortality after 1 day
Sevin XLR 25%	15 sec.	53
Sevin XLR 25%	30 sec.	52
Sevin XLR 25%	1 min.	78
Sevin XLR 25%	5 min.	90
Sevin XLR 25%	15 min.	100
Sevin XLR 15%	15 sec.	50
Sevin XLR 15%	30 sec.	23
Sevin XLR 15%	1 min.	38
Sevin XLR 15%	5 min.	83
Sevin XLR 15%	15 min.	100
Sevin XLR 10%	15 sec.	46
Sevin XLR 10%	30 sec.	39
Sevin XLR 10%	1 min.	48
Sevin XLR 10%	5 min.	88
Sevin XLR 10%	15 min.	100
Sevin XLR 5%	15 sec.	32
Sevin XLR 5%	30 sec.	82
Sevin XLR 5%	1 min.	70
Sevin XLR 5%	5 min.	92
Sevin XLR 5%	15 min.	100
d-Phenothrin	15 sec.	10
d-Phenothrin	30 sec.	37
d-Phenothrin	1 min.	41
d-Phenothrin	5 min.	87
d-Phenothrin	15 min.	100
Control	15 min.	0

Four samples of d-Phenothrin were tested at various exposure times.

Sample 1	New sample from Louisville, Kentucky
Sample 2	New sample from Bulk Chemical Company
Sample 3	Old sample from Louisville, Kentucky
Sample 4	Old sample from Louisville, Kentucky

For each treatment, the bottoms of 5 plastic petri dishes were sprayed over for various periods of time using the aerosol spray cans containing 10% active material. Spray was released approximately 20 inches over the dishes. Five native adult Japanese beetles were then placed in each dish for various exposure times. Following exposure, beetles were held in clean petri dishes and mortality readings were made over time.

Table 7. Percent mortality of Japanese beetle adults following exposure to plastic petri dishes treated with d-Phenothrin for 20 seconds.

	Exposure time																			
	4 hours				5 hours				6 hours				24 hours				48 hours			
	Percent mortality after: (hours)																			
	4	24	48	72	4	24	48	72	4	24	48	72	4	24	48	72	4	24	48	72
I	4	16	32	88	0	8	48	76	0	12	48	88	0	36	44	68	12	48	68	68
II	0	16	36	60	0	4	16	44	0	12	44	64	0	24	48	60	24	56	76	84
III	4	12	40	64	0	12	20	64	0	4	20	72	0	48	68	84	36	80	80	92
IV	4	4	12	56	0	4	52	88	0	8	36	68	8	36	56	76	24	48	76	88
Control	0	8	12	28	0	4	8	32	0	8	20	40	0	4	16	40	8	36	52	80

Table 8. Percent mortality of Japanese beetle adults following exposure to plastic petri dishes treated with d-Phenothrin for 25 seconds.

	Exposure time																			
	4 hours				5 hours				6 hours				24 hours				48 hours			
	Percent mortality after: (hours)																			
	4	24	48	72	4	24	48	72	4	24	48	72	4	24	48	72	4	24	48	72
I	0	20	28	56	0	10	20	30	0	4	20	56	4	16	32	64	21	30	65	82
II	0	12	44	64	0	0	24	36	0	4	20	40	0	0	44	48	28	64	68	100
III	0	8	20	56	0	40	80	88	0	48	100	100	0	8	24	44	32	60	72	80
IV	0	4	12	36	0	8	28	36	0	20	36	68	0	28	40	84	48	60	68	88
Control	0	0	20	40	0	0	12	32	0	16	28	44	0	0	16	28	4	16	28	40

Table 9. Percent mortality of Japanese beetle adults following exposure to plastic petri dishes treated with d-Phenothrin for 30 seconds.

	Exposure time																			
	4 hours				5 hours				6 hours				24 hours				48 hours			
	Percent mortality after: (hours)																			
Sample	4	24	48	72	4	24	48	72	4	24	48	72	4	24	48	72	4	24	48	72
I	0	4	8	28	0	0	16	32	0	8	24	36	16	36	40	60	28	52	72	84
II	0	12	28	48	0	8	16	36	0	12	28	52	0	8	32	44	32	64	72	80
III	4	44	64	76	0	48	76	100	0	36	96	100	0	16	28	52	28	60	68	76
IV	0	0	8	32	0	8	24	52	0	20	44	76	0	4	12	40	28	60	92	100
Control	0	0	20	40	0	0	12	32	0	16	28	44	0	8	16	28	4	16	28	40

These data demonstrate little difference between activity of old and new samples. A fair amount of mortality did occur in all control dishes. The data do clearly show that prolonged exposure to heavy dosages of d-Phenothrin in does not result in complete mortality of all Japanese beetle adults. In fact, mortality that takes place can occur over a long period of time. If live beetles are observed on an aircraft 4-8 hours after treatment, mortality may still occur, but there is a good chance that some will survive. Live beetles should be held on foliage to see if mortality occurs at a later date.

Work was conducted with 2 old (8-10 years) samples from Louisville, Kentucky. Petri dish bottoms were sprayed over for various periods of time and beetles were exposed and mortality readings made. Each test was replicated 5 times with 5 beetles per dish.

Table 10. Percent mortality of Japanese beetle adults following exposure to plastic petri dishes treated with d-Phenothrin sample #1.

Dosage in seconds of spray times	Exposure time in hours	Percent mortality after: 1 hour	24 hours	48 hours
5	1	0	0	20
5	2	0	12	28
5	3	0	0	20
5	4	0	8	48
5	5	0	0	24
5	6	0	4	12
10	1	0	0	16
10	2	0	8	28
10	3	0	4	32
10	4	0	4	24
10	5	0	20	20
10	6	0	12	28
15	1	0	0	28
15	2	0	12	32
15	3	0	0	16
15	4	0	12	44
15	5	0	4	16
15	6	0	16	36
20	1	0	0	20
20	3	0	8	32
20	6	0	20	56
25	1	0	8	24
25	3	0	8	44
25	6	0	8	44
Control	6	0	0	0

Table 11. Percent mortality of Japanese beetle adults following exposure to plastic petri dishes treated with d-Phenothrin sample #2.

Dosage in seconds of spray times	Exposure time in hours	Percent mortality after: 1 hour	24 hours	48 hours
5	1	0	0	16
5	2	0	4	24
5	3	0	4	20
5	4	0	12	32
5	5	0	4	16
5	6	0	20	48
10	1	0	4	12
10	2	0	0	12
10	3	0	0	20
10	4	0	16	40
10	5	0	4	24
10	6	0	16	32
15	1	0	4	24
15	2	0	12	76
15	3	0	8	44
15	4	0	0	4
15	5	0	4	32
15	6	0	20	40
20	1	0	0	16
20	3	0	4	24
20	6	0	8	24
25	1	0	4	32
25	3	0	8	52
25	6	0	12	24
Control	6	0	0	1

Additional testing was conducted with the following samples:

Sample 1	New sample from Louisville, Kentucky
Sample 2	New sample from Bulk Chemical Company
Sample 3	Old sample from Louisville, Kentucky
Sample 4	Old sample from Louisville, Kentucky

Table 12. Percent mortality of Japanese beetle adults following a 4 hour exposure to plastic petri dishes treated with various amounts of insecticide.

Material	Material sprayed over dishes in sec.	Percent mortality after: 1 hour	24 hours	48 hours	72 hours
I	20	0	48	68	72
I	30	0	12	60	68
I	60	0	4	24	48
II	20	0	4	52	80
II	30	0	52	76	80
II	60	0	44	84	96
III	20	4	20	44	56
III	30	8	24	44	64
III	60	0	0	16	60
IV	20	0	4	36	48
IV	30	8	8	28	48
IV	60	4	20	52	68
Diazinon	Rinse	32	92	96	96
Sevin	Rinse	32	100		
Control	-	0	4	16	32

Table 13. Percent mortality of Japanese beetle adults following a 6 hour exposure to plastic petri dishes treated with various amounts of insecticide.

Material	Material sprayed over dishes in sec.	Percent mortality after: 1 hour	24 hours	48 hours	72 hours
I	20	8	52	72	76
I	30	0	24	44	80
I	60	4	36	76	92
II	20	20	52	68	84
II	30	0	28	56	84
II	60	4	80	100	
III	20	0	16	56	84
III	30	8	80	96	100
III	60	4	44	80	88
IV	20	8	12	40	64
IV	30	20	44	68	80
IV	60	0	44	76	84
Diazinon	Rinse	88	100		
Sevin	Rinse	60	100		
Control	-	0	12	16	44

Table 14. Percent mortality of Japanese beetle adults following a 24 hour exposure to plastic petri dishes treated with various amounts of insecticide.

Material	Material sprayed over dishes in sec.	Percent mortality after: 1 hour	24 hours	48 hours	72 hours
I	20	40	92	100	
I	30	28	40	48	
I	60	32	52	52	64
II	20	0	64	88	96
II	30	20	84	88	100
II	60	64	100		
III	20	12	24	60	96
III	30	32	64	84	100
III	60	8	48	64	96
IV	20	16	48	60	92
IV	30	28	68	80	92
IV	60	40	76	88	100
Diazinon	Rinse	92	100		
Sevin	Rinse	100			
Control	-	0	4	12	36

These results indicate that the use of d-Phenothrin for treating cargo holds for Japanese beetles is not a fully effective regulatory treatment. Prolonged exposure to high residues rarely resulted in total mortality. However, before completely discounting the efficacy of the treatment, testing under operational conditions is needed. The fumigant (aerosol) effects of d-Phenothrin was not studied here, and this may be an important exposure method. None-the-less, live beetles have been found on treated aircraft, illustrating the need for improvement of treatment materials and procedures.

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 fall. 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 workplan 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. The work plan that was used (developed jointly with T. Ladd, ARS) is presented below:

Japanese Beetles in Nurseries: Procedures for the Determination of Infestations and Establishment of Larval-Free Zones

I. Introduction: This program proposes procedures i.e. the use of survey traps and the establishment of larval-free zones, that, respectively, may either demonstrate the absence of Japanese beetles from plant nurseries or eliminate larvae from the soil of roots surrounding nursery plants.

II. Survey Procedure: Negative captures of Japanese beetles in survey traps may be accepted as evidence that an infestation of Japanese beetles does not exist. In areas not know to be infested, negative captures in survey traps (5-9 traps per sq. mile) will be accepted as evidence of continued non-infestation. Where beetles are captured in survey traps and in areas where infestations are know to exist, negative captures in traps, arranged as indicated below, will be accepted as evidence of non-infestation. For nurseries, the protocol requires that Japanese beetle traps baited with phenethyl propionate-eugenol-geraniol (3:7:3) and sex pheromone (Japonilure) be placed at 200-ft intervals around the perimeter of a nursery and that additional traps also be placed in holding and receiving areas and within established beds of attractive host plants. Traps should be in place before emergence of adults commences (as early as 15 June), be rebaited in mid-season (August 1), and remain in position until October 1. All traps should be examined every two weeks for captures of adult beetles.

III. Establishment of larval-free zones: Where beetles are captured in traps or where infestations are know to occur, larval-free zones, established in the root zones of plants should be established and plants dug and shipped in order to take advantage of periods when larvae are susceptible and adults are not present to reinfest nursery stock. Such a period occurs during September (late summer) when adult flights are over.

A. Action elements: The integration of two action elements, or procedures, will contribute to the establishment of larval-free zones. These procedures are the use of clean cultivation while growing plants and the incorporated use of soil insecticides.

1. Clean cultivation and fallow: Food for larvae in nurseries can be supplied either by the roots of host plants or by roots of grasses and weeds growing in association with either host and non-host plants. When herbicides and cultivation procedures are used to prevent the growth of weeds and grasses, infestations of larvae can be greatly reduced. Two factors contribute to such reductions. First, female beetles prefer to oviposit in plant-covered soil; they tend to avoid bare soil. Secondly, the absence of weed and grass roots, i.e. the build-up of organic matter for use as food, decreases chances for larval survival.

   a. General Procedures: Nursery plants for shipment into uninfested areas should be grown under clean cultivation during June 1 - October 1. To obtain such conditions by June 1, the use of herbicides and cultivation may have to begin as early as May 1.

2. Soil insecticides: Soil insecticides currently registered for use against Japanese beetle larvae in turf (diazinon and insofenphos) will be utilized to establish larval-free zones in cultivation, such insecticides could eliminate larvae from plants since a lack of living plant matter in soil surrounding nursery plants would both remove larval food sources and reduce binding and absorption of insecticides. Binding of insecticides by organic matter, i.e. by thatch and decomposing plant tissues, is one of the greatest impediments to expression of insecticidal activity in soil.

   a. General procedures: Soil insecticides would be incorporated into the upper layer of clean-cultivated soil adjacent to nursery plants and watered in. After appropriate waiting periods, depending on insecticide, plants could be dug and prepared for shipment.

B. Plan of Work and Protocols For Establishing Larval-Free Zones

1. Insecticides: Two insecticides presently labelled for control of Japanese beetle larvae in turf (diazinon and isofenphos) will be tested in nurseries under conditions of clean cultivation. Both insecticides have been reported to give high degrees of control of larvae (up to 100%) in turf plots.

   a.

Insecticide	Formulation	Lbs a.i./acre
Diazinon	4E	5.5
Diazinon	4E	11.0
Diazinon	4E	22.0
Isofenphos	2I	2
Isofenphos	2I	4
Isofenphos	2I	8

2. Application sites: Nurseries in N. Y., Ohio, and New Jersey. Insecticides to be applied to plots containing selected nursery

plants (ash, linden, Amelanchier, and witch hazel) grown in clean cultivation in soils free from chlordane residues.

3. Methods of Application: Insecticides will be applied by hand using a compressed-gas sprayer. After application, incorporate insecticides into top 2-3 inches of soil. Incorporation of insecticide in soil immediately adjacent to plants may have to be done manually. Treatments should receive 1/2 inch of water by irrigation immediately following application.

4. Replication: Four replications of three plants per treatment in a randomized complete block design. Blocks to include larvae in controls as well as in grass-covered pots. The latter may aid in measurement of larval survival. Plots to be separated by 1-3 buffer plants. Plots will be artificially infested during July-August with eggs obtained from field collected female beetles. Five eggs per plant will be placed individually one inch deep in soil surrounding each test plant.

5. Time of application: Late summer, August 21 - September 15.

6. Data and evaluation: Sample whole plants 30-40 days after application of insecticides and before the emergence of adults for the presence of surviving life forms (i.e. larvae or pupae). Soil to be examined should approximate that included in a normal root ball.

During June 3-11 ARS and APHIS employees, state representatives and personnel from cooperating nurseries met to visit test sites at the three participating nurseries in New Jersey, New York and Ohio. The purpose of the visits was to verify that sites are ready and appropriate for test purposes, prepare plans for establishing plots for testing, alert all concerned of the procedures to be followed, and to ascertain that equipment for incorporating insecticides and irrigating plots after insecticidal treatment would be available. Sites visited and persons meeting there are indicated below:

June 3	June 10	June 11
Princeton Nurseries Allentown, N. J.	Warner Nurseries Willoughby Hills, OH	Schichtel's Nursery Springville, N. Y.
T. Ladd, ARS	T. Ladd, ARS	T. Ladd, ARS
T. Flanagan, APHIS	J. Moyseenko, ARS	J. Moyseenko, ARS
W. McLane, APHIS	W. McLane, APHIS	W. McLane, APHIS
H. Mumma, APHIS	H. Smith, APHIS	L. Kershaw, APHIS
B. Edmonds, APHIS	G. Ruckel, Warner Nurseries	B. Bohne, N.Y. Dept. Agri
T. Scudders, Princeton Nurseries		R. Jablonski, N.Y. Dept. Agri.
D. Voorhees, N.J. Dept. Agri.		C. Cranston, Shichtel's Nur.
		F. Hoerner, Shichtel's Nur.

Experimental plots at cooperating nurseries were infested with Japanese beetle eggs on the dates indicated below. Eggs were placed 1-2 inches deep in the ground about 4 inches from the bases of trees and covered with soil. Eight containers with established turf also were infested with 5 eggs each and placed in each nursery. Weather conditions prevailing at the various test sites are indicated below:

	Princeton Nurseries Allentown, N.J.	Warner Nurseries Willoughby Hills, OH	Schichtel's Nursery Springville, N.Y. *
Date	July 11	July 10	July 14
Soil	Slightly moist to dry	Slightly moist to dry	Wet
Air Temp.	94°	91°	65°
Organic Matter In Soil	None	Considerable - roots and plant parts	Considerable - roots, stems and other parts

Experimental plots at cooperating nurseries were treated with different rates of diazinon and isofenphos insecticides on the dates indicated below. Insecticides were incorporated into the soil by hand cultivation to a depth of 2 inches and watered in. Because soil at nurseries in New York and New Jersey were quite wet at the time insecticides were applied, only one-quarter of an inch of water was applied to plots at these locations. Conditions at the specific sites are indicated below:

	Princeton Nurseries Allentown, N.J.*	Warner Nurseries Willoughby Hills, OH	Schichtel's Nursery Springville, N.Y. **
Date	August 11	August 6	August 18
Air Temp.	67° F	82° F	75°-80° F
Soil Temp. (3")	66° F	70° F	75° F
Water (inches)	.25-.5	.5-1.5	.25

Experimental insecticide plots at cooperating nurseries were examined by ARS and APHIS personnel for the presence of Japanese beetle larvae on the dates indicated in Table 1. Soil (16-in. diam. x 12-in. deep) surrounding individual trees was removed by hand, placed on plastic sheeting, and carefully examined for living scarab larvae (white grubs). Those found were placed in alcohol and returned to the laboratory for identification. Results obtained at the three nurseries are presented in Table 1.

Few Japanese beetle grubs were found at nurseries in New York or Ohio; none were found in treated plots in Ohio. In New Jersey, however, living grubs were recovered from most of the individual insecticide plots. Reductions in numbers of grubs in New Jersey attributable to insecticidal activity ranged from 30% (diazinon - 5.5 lbs A.I./acre) to 57% (diazinon - 8.8 lbs A.I./acre; isofenphos - 4 lbs A./I./acre). Average numbers of grubs per individual plant in the New Jersey nursery ranged from 2.8 in the best insecticide plots (diazinon and isofenphos) to 6.8 in the controls.

In New Jersey it was noticed that larvae had burrowed deeply into soil surrounding plant roots. Therefore, separate diggings were made on several plants that had not been treated with insecticides to determine the vertical distribution of larvae in the soil. Soil was excavated by layers to a depth of 12 inches. Results are indicated in Table 2. Forty-eight percent of all larvae found were in the top 6 inches of soil; 29% in the 6-9 inch zone; and 23% below 9 inches. Thus, more than half of all larvae were 6-12 inches below the soil surface. (By way of contrast, Fleming, in his 1972 bulletin "Biology of the Japanese Beetle", notes that in turf in New Jersey during September, 97% of larvae are found in the upper 2 inches of soil with 99+% in the first 4 inches.) With such a large proportion of larvae occurring at these depths, it seems very unlikely that surface-applied insecticides will ever be able to be used to clean up infested field-grown nursery stock. Other procedures, such as systemic soil insecticides, should be tested for possible applications to prevent the infestation of field-grown nursery stock during periods of adult activity.

Table 15. Japanese beetle larvae found in insecticide-treated plots (3 trees/plot) and turf-covered pots at nurseries cooperating in tests to develop improved insecticidal treatments for Japanese beetles.

Insecticide	New Jersey a/	Ohio b/	New York c/
lbs. A.I. per acre	9/15/87	9/9/87	9/22/87
	Average number larvae per plot		
Isofenphos - 8	9.0a	0.0a	0.0a
Isofenphos - 4	8.8a	0.0a	0.5a
Isofenphos - 2	11.0ab	0.0a	0.0a
Diazinon - 22	9.0a	0.0a	0.5a
Diazinon - 11	8.8a	0.0a	0.0a
Diazinon - 5.5	14.3ab	0.0a	0.3a
Control	20.5 b	0.5 b	0.5a
	Percent survival in 6-inch grass-covered pots e/		
	10	13	30

a/ Princeton Nursery, Allentown, New Jersey, Maple and Ash.
b/ Warner Nurseries, Willoughby Hills, Ohio, Sunburst Locust.
c/ Schichtel's Nursery, Springville, N.Y., Maple and Ash.
d/ Averages followed by the same letter are not significantly different at the 5% level.
e/ Survival of larvae in 6-inch grass-covered pots at Wooster, Ohio. Set 1 = 38%, Set 2 = 65%.

Table 16. Japanese beetle larvae found at different depths in soil surrounding the roots of nursery stock at Princeton Nurseries, Allentown, N.J.a/

Soil depth (inches)	No. larvae in Maple	No. larvae in Ash	Total number	Percent at indicated depth
0-6	9	6	15	48
6-9	1	8	9	29
9-12	5	2	7	23

a/ 3 ash and 3 maple plants examined.

Soil sample cores were taken during early October at the New Jersey test site and sent to the National Monitoring and Residue Analysis Laboratory at Gulfport, Mississippi, for analysis. Samples were taken at depths of 0-3, 3-6 and 6-9 inches.

Of the twenty-one soil samples tested, Isofenphos residue values ranged from none detected (ND) to 0.15 ppm. Diazinon residues ranged from none detected to 0.04 ppm.

Table 17. Diazinon and Isofenphos ressidue values found in soil samples from Princeton nursery experimental plot.

Material	Soil depth (inches)	Dosage (percent active)	Results (ppm)
Isofenphos	0-3	2	0.06
Isofenphos	3-6	2	0.02
Isofenphos	6-9	2	0.02
Isofenphos	0-3	4	0.12
Isofenphos	3-6	4	0.04
Isofenphos	6-9	4	0.02
Isofenphos	0-3	8	0.15
Isofenphos	3-6	8	0.06
Isofenphos	6-9	8	0.07
Diazinon	0-3	5.5	0.02
Diazinon	3-6	5.5	< 0.01
Diazinon	6-9	5.5	< 0.01
Diazinon	0-3	11	0.04
Diazinon	3-6	11	0.01
Diazinon	6-9	11	< 0.01
Diazinon	0-3	22	0.04
Diazinon	3-6	22	0.03
Diazinon	6-9	22	< 0.01
Control	0-3	-	< 0.01
Control	3-6	-	< 0.01
Control	6-9	-	< 0.01

Limit of detection (LOD) = 0.01
< 0.01 ppm = below detection limit (BDL)

Methodology

Method PR0016

Analysis of Malathion, Guthion, Diazinon, or Isofenphos from Soil or Sediment

Instrument Parameters

Reference NMRAL Notebook, #003, page 10.

Nearly all material was found in the upper 0-3 inches of soil. Based on the depth that some of the grubs were found, it seems the material did not get deep enough to be effective.

This test work suggests that the technique as used will not be a suitable way to certify nursery stock moving into Canada.