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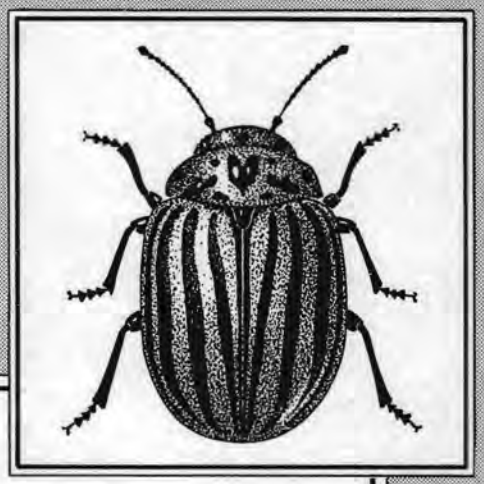

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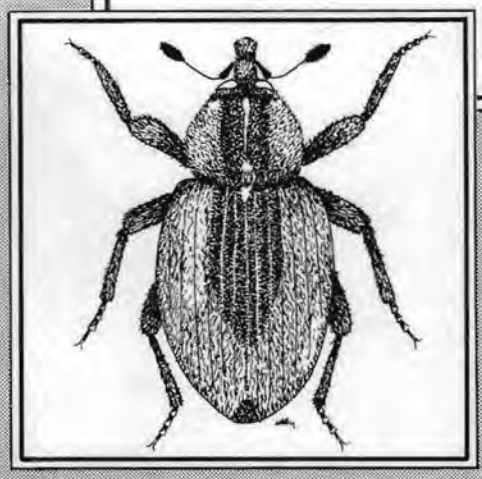
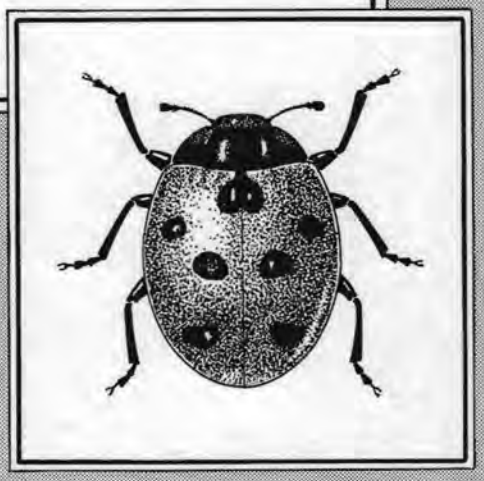
Otis Methods Development Center

Progress Report

Animal and Plant Health Inspection Service





October 1, 1985 - September 30, 1986

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

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Project Number: GM 6.1.5
Project Title: Regulatory Treatments
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: W.H. McLane and J.A. Finney

The main objective of this work is the development of new and improved treatments for regulated items moving from quarantined areas to non-infested locations. This project is primarily directed toward the development of treatments for recreational vehicles, mobile homes and outdoor household articles.

There is need for treatments to effectively control the hatch of gypsy moth eggs in regulatory situations. Compounds such as creosote have been used effectively in the past but may not be available in the future. Laboratory and field tests have identified a number of materials that are effective when applied to egg masses. However, most materials that are efficacious in laboratory studies have proven to be ineffective in the field. Furthermore, agents must be effective regardless of the time of year (stage of the egg) they are applied.

Two samples from Safer Agro-Chem Ltd., of Victoria, B.C., Canada were tested against viable gypsy moth egg masses in the laboratory.

Laboratory produced egg masses that were held in cold storage for 150 days were treated with various dosages of BTS and SAP. Application was made to the point of runoff with a hand held pipet. Each treatment was replicated 5 times with one untreated series acting as a check. Following treatment all egg masses were held in an incubation chamber for 14 days at 26°c with 50% relative humidity.

Table 1. Percent hatch from egg masses treated with BTS and SAP.

Material	Percent concentration	Percent hatch
BTS	100.0	0
BTS	50.0	0
BTS	25.0	1
BTS	5.0	10
SAP	100.0	0
SAP	50.0	0
SAP	25.0	0
SAP	2.5	0
SAP	1.0	0
Check		100

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period: October 1, 1985 - September 30, 1986
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.

In 1986 Bacillus thuringiensis was used to treat approximately 70 percent of all acreage sprayed for gypsy moth control in the United States. Nearly all other acreage was treated with the insect growth regulator, Dimilin. If the large Oregon spray program with multiple applications of Bt was excluded, the sprayed acreage would have been about 50/50, Bt and Dimilin. A fairly large area was also sprayed in Ontario, Canada with Bt.

Bacillus thuringiensis was sprayed at 12, 16 or 20 BIU in 96 or 128 ounces per acre with fixed-wing aircraft and helicopters. In experimental programs some low volume, new strains and encapsulant formulations were studied. All operational programs applied the HD-1 strain. In general Bt treatments were not as effective as most program managers had hoped. Of the acres treated with a single application nearly 50 percent were unacceptable. In some states, towns are withdrawing from the state spray program if only Bt can be used. Some states are starting to include more Dimilin in their programs. Established Bt manufacturers are starting to pull out of the market as new companies come forward with genetically engineered strains for laboratory and small field plot tests. Dimilin continues to give good, consistent control of gypsy moth larvae when applied at very low dosages (.25-1.0 ounces AI/acre). In 1986 experimental test, the ability of Dimilin to control gypsy moth with sprays prior to hatch and foilage development was demonstrated. However, Dimilin is not acceptable in a number of environmentally sensitive areas and some question its use in any area. Therefore, if control measures are taken, Bt will continue to be the material of choice for a number of managers.

Based on the inconsistent results obtained with Bt over the past few years we have devoted the majority of our time working with this material. This includes looking at more effective strains, new stickers, dosages and rates, encapsulant materials, UV screens and feeding stimulants. In laboratory tests some genetically engineered strains show great promise. Few new insecticide products were tested because of the lack of materials. All chemical companies have cut back drastically on the development of new insecticides.

However, during this reporting period a number of genetically engineered strains of Bt from Ecogen were tested.

Table 1. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation when treated with samples of Bt at 8.0 BIU/192 oz./acre.

Formulation	After 2 days		After 3 days		After 4 days	
	mor.	def.	mor.	def.	mor.	def.
3186-10	8	24	27	30	31	32
3186-11	17	4	34	13	59	21
3186-12	41	5	61	9	79	15
3186-13	21	12	45	16	79	16
3186-14	4	18	12	28	39	28
3186-15	9	8	25	16	63	18
3186-16	17	14	32	22	62	22
PO-291	14	16	27	28	53	30
PO-299	47	6	75	6	90	7
PO-302	68	5	77	7	97	7
PO-303	20	16	39	22	55	24
PO-304	44	3	58	10	86	10
PO-305	45	1	58	3	94	3
Check	0	48	1	64	2	80

Table 2. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation when treated with samples of Bt at 4.0 BIU/96 oz./acre.

Formulation	After 2 days		After 3 days		After 4 days	
	mor.	def.	mor.	def.	mor.	def.
3186-10	0	30	1	44	20	48
3186-11	6	19	16	26	76	28
3186-12	28	12	31	22	80	26
3186-13	18	16	29	20	72	23
3186-14	5	30	7	44	48	56
3186-15	8	14	18	18	72	30
3186-16	9	38	14	52	37	82
PO-291	5	20	25	22	66	22
PO-299	42	10	67	10	98	11
PO-302	33	11	54	13	94	19
PO-303	1	30	13	36	43	46
PO-304	28	6	56	9	94	10
PO-305	18	26	26	28	87	32
Check	0	74	0	84	1	94

Table 3. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation when treated with samples of Bt at 2.0 BIU/96 oz./acre.

Formulation	After 2 days		After 3 days		After 4 days	
	mor.	def.	mor.	def.	mor.	def.
3186-10	0	48	0	64	4	76
3186-11	0	26	1	38	14	46
3186-12	3	26	10	34	18	44
3186-13	3	34	4	48	14	60
3186-15	3	27	3	42	18	54
PO-299	3	26	4	36	20	48
PO-302	9	24	13	35	41	40
PO-304	0	14	0	24	3	28
PO-305	1	30	3	44	19	48
Check	0	84	0	96	0	100

Table 4. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation when treated with samples of Bt at 1.0 BIU/96 oz./acre.

Formulation	After 2 days		After 3 days		After 4 days	
	Mor.	Def.	Mor.	Def.	Mor.	Def.
3186-10	0	56	1	72	1	89
3186-11	0	38	0	58	10	68
3186-12	1	39	1	52	9	57
3186-13	0	48	2	64	8	73
3186-15	1	38	1	52	10	53
PO-299	3	34	4	48	17	60
PO-302	6	29	9	50	23	52
PO-304	4	31	5	46	15	48
PO-305	2	34	4	50	18	59
Check	0	54	0	80	1	98

Table 5. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation after a 4 day exposure to seedlings treated with new Bt strains at 8 BIU/gallon/area.

Material	Percent mortality	Percent defoliation
Dipel 8L	11	46
Thuricide 48LV	56	17
Ecogen 1886-12	19	44
Ecogen 1886-13	5	68
Ecogen 1886-14	9	54
Ecogen 1886-15	34	37
Ecogen 1886-16	3	68
Ecogen 1886-17	22	34
Check	0	90

A number of the new strains were found to be more efficacious than the 3186-10 standard HD-1 strain presently being used.

Testing continued with Javelin (SAN-415), Thuricide 48LV, Dipel 8L and a number of other SAN-415 formulations. SAN-415 is a new strain of Bt that has been more efficacious than the HD-1 strain when tested against gypsy moth larvae in the laboratory. However, field tests in 1984 and 1985 did not demonstrate its superiority. In 1986 field tests, SAN-415 did appear to be more effective than the HD-1 strains of Dipel 8L and Thuricide 48LV. The following are laboratory results with the various formulations. Mortality after a 2 and 3 day exposure to treated seedlings is included so that the reader can see the early activity with some of the formulations. Mortality with most formulations tends to equal out after 4 days. This early mortality is desirable to ensure as much kill as possible before rainfall and UV degradation.

Table 6. Percent mortality of 2nd instar gypsy moth larvae and percent defoliation of oak seedlings after larvae were exposed to seedlings treated with 4 Bt formulations.

Dosage BIU/96oz acre	SAN SC 353						SAN SC 355						Javelin						Dipel 8L (AQ)					
	mor.			def.			mor.			def.			mor.			def.			mor.			def.		
	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D
4	64	92	100	5	5	5	19	42	83	28	44	48	39	76	96	6	11	15	24	45	91	23	37	43
8	67	89	100	4	5	5	52	79	93	5	16	16	41	75	97	4	10	12	31	56	84	25	36	44
16	63	88	100	2	2	2	84	94	100	1	2	2	80	97	100	2	2	2	74	92	100	1	1	1
24	67	97	100	1	1	1	84	92	99	2	2	2	80	90	100	2	3	3	74	89	100	3	3	3
Check	0	0	0	100																				

mor. - Percent mortality of gypsy moth larvae
def. - Percent defoliation of oak seedlings.

2D - Readings made 2 days after larvae were first exposed to seedlings.
3D - Readings made 3 days after larvae were first exposed to seedlings.
4D - Readings made 4 days after larvae were first exposed to seedlings.

Table 7. Percent mortality of 3rd instar gypsy moth larvae and percent defoliation of oak seedlings after larvae were exposed to seedlings treated with 4 Bt formulations.

Dosage BIU/96oz acre	SAN SC 353						SAN SC 355						Javelin						Dipel 8L (AQ)					
	mor.			def.			mor.			def.			mor.			def.			mor.			def.		
	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D
4	25	25	82	21	34	34	11	18	48	32	52	52	17	32	75	22	34	40	3	10	28	48	72	72
8	42	68	94	9	21	21	30	47	82	22	26	27	18	40	79	24	36	47	1	7	19	46	72	100
16	47	75	92	16	18	18	53	74	91	6	7	7	45	70	97	11	13	13	20	38	75	29	43	44
24	59	78	97	6	9	9	37	50	97	22	24	24	58	83	98	3	4	5	44	67	92	8	10	10
Check	0	0	0	100																				

mor. - Percent mortality of gypsy moth larvae
def. - Percent defoliation of oak seedlings.

2D - Readings made 2 days after larvae were first exposed to seedlings.
3D - Readings made 3 days after larvae were first exposed to seedlings.
4D - Readings made 4 days after larvae were first exposed to seedlings.

Table 8. Percent mortality of 4th instar gypsy moth larvae and percent defoliation of oak seedlings after larvae were exposed to seedlings treated with 4 Bt formulations.

Dosage BIU/96oz acre	SAN SC 353						SAN SC 355						Javelin						Dipel 8L (AQ)					
	mor.			def.			mor.			def.			mor.			def.			mor.			def.		
	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D	2D	3D	4D
4	12		50	40		54	16		62	40		60	10		60	60		76	0		0	98		100
8	12		80	18		40	28		62	16		40	24		86	30		46	2		46	42		66
16		44	74		36	42		30	60		40	46		62	72		20	24		16	30		70	71
24		70	88		16	18		74	88		14	14		62	86		11	11		64	82		17	21
Check	0	0	0	100																				

mor. - Percent mortality of gypsy moth larvae
def. - Percent defoliation of oak seedlings.

2D - Readings made 2 days after larvae were first exposed to seedlings.
3D - Readings made 3 days after larvae were first exposed to seedlings.
4D - Readings made 4 days after larvae were first exposed to seedlings.

Table 9. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with various formulations of Bt.

Material	Dosage BIU/96 oz/acre	Percent mortality	Percent defoliation	Chi Square[1/] analysis
Javelin	12	83	18	d
Dipel 8L	12	33	39	b
SAN 415-SC 363	12	98	4	e
Javelin	8	57	19	c
Dipel 8L	8	23	50	ab
SAN 415-SC 363	8	89	9	d
Javelin	4	25	42	ab
Dipel 8L	4	14	62	a
SAN 415-SC 363	4	86	15	d
Check	—	0	99	-

1/ Percents followed by the same letter are not significantly different at the 5% level according to chi square analysis.

Table 10. Percent mortality of 3rd instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with various formulations of Bt.

Material	Dosage BIU/96 oz/acre	Percent mortality	Percent defoliation	Chi Square[1/] analysis
Javelin	12	42	41	cd
Dipel 8L	12	25	58	b
SAN 415-SC 363	12	88	5	e
Thuricide 48LV	12	46	16	d
Javelin	8	31	38	bc
Dipel 8L	8	13	89	a
SAN 415-SC 363	8	86	7	e
Javelin	4	38	39	bcd
Dipel 8L	4	6	92	a
SAN 415-SC 363	4	77	18	e
Check	--	1	100	-

1/ Percents followed by the same letter are not significantly different at the 5% level according to chi square analysis.

Table 11. Percent mortality of 4th instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with various formulations of Bt.

Material	Dosage BIU/96 oz/acre	Percent mortality	Percent defoliation	Chi Square 1/ analysis
Javelin	12	22	42	c
Dipel 8L	12	6	52	ab
SAN 415-SC 363	12	56	9	e
Javelin	8	12	42	bc
Dipel 8L	8	0	100	a
SAN 415-SC 363	8	54	16	c
Javelin	4	4	58	ab
Dipel 8L	4	0	100	a
SAN 415-SC 363	4	36	28	d
Check	--		100	-

1/ Percents followed by the same letter are not significantly different at the 5% level according to chi square analysis.

Table 12. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure to oak seedlings treated with 8 BIU/96 oz/acre of Bt and then exposed to rainfall.

Material	Inches rainfall	Percent mortality	Percent defoliation	Chi Square 1/ analysis
Javelin	-	19	16	b
	0.5	4	22	a
SAN 415-SC 363	-	72	5	c
	0.5	5	34	a
Thuricide 48LV	-	32	14	b
	0.5	3	42	a
Dipel 8L	-	6	49	a
	0.5	0	94	a
Check	-	0	96	-

1/ Percents followed by the same letter are not significantly different at the 5% level according to chi square analysis.

Table 13. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 3 day exposure to oak seedlings treated with 8 BIU/96 oz/acre of Bt and then exposed to rainfall.

Material	Inches rainfall	Percent mortality	Percent defoliation	Chi Square 1/ analysis
Javelin	-	15	11	cd
	1.0	3	18	ab
SAN 415-SC 363	-	19	5	a
	1.0	1	35	a
Thuricide 48LV	-	7	11	d
	1.0	0	29	ab
Dipel 8L	-	0	35	bc
	1.0	0	87	a
Check	-	1	88	-

1/ Percents followed by the same letter are not significantly different at the 5% level according to chi square analysis.

Table 14. Mortality of 2nd instar gypsy moth larvae and seedling defoliation following treatment with Larvo, Dipel and Thuricide.

Material	BIU 96 oz/acre	Percent mortality 3 days	Percent mortality 4 days	Percent defoliation 3 days	Percent defoliation 4 days
Dipel 8L	16	15	49	13	15
Thuricide 48LV	16	31	61	5	5
Larvo - A	16	0	0	74	82
Larvo - A	374.4	0	1	72	78
Larvo - B	16	0	0	70	82
Larvo - B	374.4	2	10	31	50
Larvo - C	16	0	0	74	88
Larvo - C	374.4	1	1	72	80
Larvo - D	16	1	1	70	76
Larvo - D	374.4	0	0	50	60
Check	--	0	0	66	82

Several laboratory tests were conducted with in-flight encapsulant materials and Thuricide 48LV. The materials were supplied by "Spray Control Systems". Two of the formulations, BTLS and Black LS were field tested in 1986. Results in the field were poor when applied against 3rd instar gypsy moth larvae.

Table 15. Larval mortality and seedling defoliation following a 2 and 4 day exposure to oak seedlings treated with encapsulated formulations of Bt with exposure to rainfall.

Formulation	Ratio	Oz/A	Inches rain	Mortality 2 day	Mortality 4 day	Defoliation 2 day	Defoliation 4 day
Thuricide 48LV	1x2	96	-	6	70	24	56
	1x2	96	0.5	0	26	56	98
	1x1	64	0.5	6	44	58	84
48LV + H_2O + BTLS	1x1x1	96	-	18	92	2	8
	1x1x1	96	0.5	10	78	10	23
48LV + BTLS	1x1	64	0.5	18	80	2	8
48LV + H_2O + BTA	1x1x1	96	-	8	50	23	56
	1x1x1	96	0.5	2	46	15	46
48LV + BTA	1x1	64	0.5	12	62	19	43
48LV + H_2O + Black LS	1x1x1	96	-	10	92	2	3
	1x1x1	96	0.5	16	84	7	24
48LV + Black LV	1x1	64	0.5	16	90	8	18
Check	-	--	-	0	0	100	100
"	-	--	0.5	0	0	100	100

A standard oak seedling test was conducted with Black LS, BTA and BTLS.

Table 16. Percent larval mortality and seedling defoliation following a 2 and 4 day exposure of 2nd instar larvae to oak seedlings treated with encapsulant materials and Thuricide 48LV.

Formulation	Ratio	Dosage rate	After 2 days mor.	After 2 days def.	After 4 days mor.	After 4 days def.
Thuricide 48LV	-	12 BIU/64 oz/A	28	1	85	7
	-	12 BIU/96 oz/A	15	2	85	6
48LV + BTA	1x1	12 BIU/64 oz/A	21	3	92	6
	1x1x1	12 BIU/96 oz/A	20	2	93	4
48LV + BTLS	1x1	12 BIU/64 oz/A	31	3	95	4
	1x1x1	12 BIU/96 oz/A	18	2	90	6
48LV + Black LS	1x1	12 BIU/64 oz/A	23	2	92	3
	1x1x1	12 BIU/96 oz/A	34	2	91	3
Check			0	92	0	100

During March of 1986, Mr. Alvaro Villaveces visited the Otis laboratory and worked with us for 4 days running oak seedling tests with BTLS, Black LS, Larvo, Bond and Thuricide 48LV.

Table 17. Percent larval mortality and seedling defoliation following a 2 and 4 day exposure of 2 instar gypsy moth larvae to oak seedlings treated with Bt and encapsulant and exposed to rainfall.

Formulation 1/	Ratio	Inches rain	After 2 days mor.	After 2 days def.	After 4 days mor.	After 4 days def.
Thuricide 48LV	1x2	-	25	3	74	8
	1x2	.25	21	3	49	16
	1x2	.50	2	13	40	26
48LV + BTA	1x1x1	-	27	5	88	9
	1x1x1	.25	33	2	82	6
	1x1x1	.50	37	2	88	5
48LV + BTLS	1x1x1	-	23	2	85	4
	1x1x1	.25	22	2	71	8
	1x1x1	.50	24	3	88	6
48LV + Black LS	1x1x1	-	31	2	86	5
	1x1x1	.25	44	2	84	4
	1x1x1	.50	46	1	89	4
Check	-	-	0	68	0	100

1/ Treatments made at 12 BIU/96 oz/acre.

Table 18. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following a 2 and 4 day exposure to seedlings treated with encapsulated Bt at 6 BIU/96 oz/acre then exposed to rainfall.

Material	Ratio	Inches rain	After 2 days mor.	After 2 days def.	After 4 days mor.	After 4 days def.
Thuricide 48LV + H_2O	1ml + 5ml	-	10	5	46	11
		.25	8	8	42	20
		.5	0	14	14	33
48LV + H_2O + BTA	1ml + 2.5ml + 2.5ml	-	6	6	45	19
		.25	7	4	33	18
		.5	6	3	50	12
48LV + H_2O + BTLS	1ml + 2.5ml + 2.5ml	-	50	3	97	4
		.25	19	8	69	15
		.5	12	7	53	15
48LV + H_2O + Black LS	1ml + 2.5ml + 2.5ml	-	21	3	72	6
		.25	4	4	46	12
		.5	5	6	59	7
48LV + AV1	1ml + 5ml	-	6	13	26	26
		.5	18	8	68	18
Check		-	0	82	0	100

Table 19. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following a 2 and 4 day exposure to seedlings treated with encapsulated Bt at 6 BIU/96 oz/acre then exposed to rainfall.

Material	Ratio	Inches rain	After 2 days mor.	After 2 days def.	After 4 days mor.	After 4 days def.
Thuricide 48LV + H_2O	1ml + 5ml	-	3	3	25	22
		.25	1	7	16	24
		.5	3	9	15	36
48LV + H_2O + BTA	1ml + 4ml + 1ml	-	10	3	56	14
		.25	4	2	31	20
		.5	4	2	40	12
48LV + H_2O + BTLS	1ml + 4ml + 1ml	-	7	3	47	13
		.25	3	3	38	13
		.5	4	3	37	8
48LV + H_2O + Black LS	1ml + 4ml + 1ml	-	2	5	30	17
		.25	4	4	46	12
		.5	10	3	53	14
Check		-	0	52	0	96

Table 20. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following a 2 and 4 day exposure to seedlings treated with encapsulated Bt then exposed to artificial sunlight and natural rainfall.

Formulation	Ratio	Dosage/Rate	Hours UV	Inches rain	Sticker	After 2 Days mor.	After 2 Days def.	After 4 Days mor.	After 4 Days def.
Thuricide 48LV	1x1	12 BIU/64 oz/A	--	-	--	42	2	89	5
			--	.25	--	24	3	74	14
			--	.5	--	13	5	68	15
48LV	1x2	12 BIU/96 oz/A	--	.25	1% Bond	16	2	83	4
			--	.5	"	23	3	90	10
			--	.25	3% Bond	22	2	97	3
			--	.5	"	59	2	100	3
48LV + BTA	1x1	12 BIU/64 oz/A	--	-	-	29	2	98	4
			--	.25	-	22	2	85	9
			--	.5	-	29	2	87	5
48LV + H_2O + BTA	1x.5x.5	12 BIU/64 oz/A	--	.25	-	7	3	56	5
			--	.5	-	15	3	56	5
			48	-	-	5	5	53	15
48LV + BTLS	1x1	12 BIU/64 oz/A	--	-	-	4	18	60	24
			--	.25	-	22	3	83	7
			--	.5	-	15	2	74	6
48LV + H_2O + BTLS	1x.5x.5	12 BIU/64 oz/A	--	.25	-	14	4	76	5
			--	.5	-	12	3	71	4
			48	-	-	1	4	35	16
48LV + Black LS	1x1	12 BIU/64 oz/A	--	-	-	15	1	68	6
			--	.25	-	17	1	67	3
			--	.5	-	8	2	62	2
48LV + H_2O + Black LS	1x.5x.5	12 BIU/64 oz/A	--	.25	-	16	3	66	4
			--	.5	-	12	3	59	4
			48	-	-	3	5	23	17
Check	-	-	--	-	-	0	70	1	97

Table 21. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation following a 3 and 5 day exposure to seedlings treated with encapsulant and Bt then exposed to artificial sunlight.

Formulation	Hours artificial sunlight	After 3 days		After 5 days	
		mor.	def.	mor.	def.
Thuricide 48LV 1/	24	23	6	72	11
	48	0	4	17	8
	72	0	2	1	5
48LV + BTA 2/	24	18	7	76	16
	48	1	3	25	15
	72	0	2	1	3
48LV + BTLS 2/	24	3	2	70	7
	48	0	4	19	7
	72	0	2	4	3
48LV + Black LS 2/	24	7	1	98	3
	48	0	1	71	3
	72	0	1	12	1
Check	--	0	80	0	100

1/ Material applied at 12 BIU/96 oz/acre, Ratio 1x2
2/ Material applied at 12 BIU/96 oz/acre, Ratio 1x1x1.

Table 22. Percent feeding of 3rd instar gypsy moth larvae with a 24 hour exposure time to oak leaves treated with various *Bt* encapsulant formulations.

Formulation comparison	Method of treatment	Percent feeding
Black LS	Brushed on	77
No Treatment	--	90
48LV + Black LS	Brushed on	5
No Treatment	--	42
48LV + Black LS	6 Droplets/leaf	42
No Treatment	--	50
48LV + BTLS	Brushed on	1
48LV	*	2
48LV + BTLS	6 Droplets/Leaf	32
48LV	*	32
BTLS	6 Droplets/Leaf	85
No Treatment	--	65
BTLS	Brushed on	70
No Treatment	--	98
48LV + BTLS	6 Droplets/Leaf	43
No Treatment	--	53
48LV + BTLS	Brushed on	4
No Treatment	--	33
48LV	Brushed on	8
No Treatment	--	27
No Treatment	--	70
No Treatment	--	68

Table 23. Percent feeding of 2nd instar gypsy moth larvae on oak foliage discs treated with various Bt encapsulated formulations.

Formulation	Percent feeding after 24 hours	Percent feeding after 48 hours
Thuricide 48LV + H_2O	2.0	2.0
48LV + BTA + H_2O	1.0	1.0
48LV + H_2O	0.8	0.8
48LV + BTLS + H_2O	3.0	4.0
48LV + H_2O	1.4	1.6
48LB + Black LS + H_2O	1.2	2.2

Table 24. Percent feeding of 2nd instar gypsy moth larvae on oak foliage discs treated with various Bt encapsulated formulations.

Formulation	Ratio	Dosage/Rate	Percent feeding after 24 hrs.	Percent feeding after 48 hrs.
Thuricide 48LV + H_2O	1x1	12 BIU/96 oz/A	0.8	0.8
48LV +BTA	1x1	12 BIU/64 oz/A	1.2	1.6
48LV + BTLS	1x1	"	1.4	2.8
48LV + Black LS	1x1	"	2.2	3.6
48LV + BTA + H_2O	1x1x1	12 BIU/96 oz/A	2.2	2.8
48LV + BTLS + H_2O	1x1x1	"	3.8	5.2
48LV + Black LS + H_2O	1x1x1	"	3.4	4.2
Thuricide 48LV		Neat	1.2	1.4
BTA		"	100.0	-
BTLS		"	100.0	-
Black LS		"	100.0	-
Check		-	100.0	-

Table 25. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 4 day exposure to seedlings treated with Thuricide 48LV and encapsulant at 12 BIU/96 oz./acre.

Formulation	Inches rain	Hours UV	Percent mortality	Percent defoliation
48LV			61	16
"	.01		30	50
"	.5		3	72
"		48	19	36
48LV + BTME			54	15
" "	.01		51	22
" "	.5		45	22
" "		48	60	7
48LV +BTBL			28	44
" "	.01		18	40
" "	.5		35	33
" "		48	68	14
Check			0	100

Thuricide 48LV and encapsulant materials were also sprayed by back-pack mist blower out of a fire tower at 56 feet onto oak seedlings. Seedlings were then returned to the laboratory for bioassay

Table 26. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 4 day exposure to seedlings that were treated with Bt and encapsulant sprayed from a fire tower.

Formulation	Inches rain	Hours UV	Percent mortality	Percent defoliation
Thuricide 48LV			44	9
" "	.1		7	64
48LV + BTBL			19	40
" "	.1		19	30
48LV + BTME			13	52
" "	.1		3	60
" "		48	49	20
Check			0	100

Dosage was 12 BIU/96 oz./acre

A study was started to identify the relationship between dosage and exposure time when laboratory reared 2nd instar gypsy moth larvae are exposed to Bt treated foilage. Tender oak foliage discs approximately 1.0 inches in diameter were treated in a laboratory spray chamber and then placed individually into plastic petri dishes. Twenty 2nd instar larvae were then exposed to oak discs for various periods of time and then removed to clean diet and reared for 14 days. Each test was replicated 5 times and defoliation and mortality readings were made and recorded. It would appear that gypsy moth larvae need only a short exposure time to Bt in order for it to be efficacious. Foliage was treated with 12 BIU/96 oz/acre. All test larvae were starved for 24 hours before being exposed to treated foliage. Untreated foliage was used as a check.

Table 27. Percent mortality of 2nd instar gypsy moth larvae after short exposure times to oak disc treated with Bt.

Material	Minutes exposure time	Percent defoliation of oak disc	Percent mortality days following exposure			
			2	4	7	14
Dipel	1	0	1	3	3	10
	15	2	2	14	34	49
	30	3	21	32	48	74
	60	5	0	5	12	33
	120	10	2	17	42	64
	180	21	1	2	2	4
	360	23	6	8	9	15
Thuricide 48LV	1	0	3	8	11	18
	15	2	46	62	72	82
	30	3	39	66	83	93
	60	2	45	65	69	80
	120	6	15	46	86	92
	180	4	39	72	84	90
	360	5	63	77	86	91
Check	1	0	0	0	1	5
	15	2	0	0	0	0
	30	2	1	1	1	2
	60	6	3	4	5	8
	120	20	0	0	0	0
	180	31	2	4	6	9
	360	63	0	0	0	0

Tests were conducted with Holdup, an encapsulant material from Greenshield of Washington, in anticipation of field studies during the spring of 1986. The material was tested on 50 acre plots and was applied by aircraft. Results were poor, however, the standard gave poor results also.

Table 28. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after exposure (4 days) to oak seedlings treated with Dipel 8L (oil) and Holdup.

Material	Percent Holdup	BIU/96 oz/ acre	Percent mortality	Percent defoliation
Dipel 8L		4	1	91
Dipel 8L + 40	33	4	8	47
Dipel 8L + 40 - 0	33	4	11	38
Dipel 8L + 40	42	4	3	54
Dipel 8L + 40 - 0	42	4	6	56
Dipel 8L + 40	46	4	8	50
Dipel 8L + 40 - 0	46	4	10	47
Dipel 8L		8	14	60
Dipel 8L + 40	17	8	12	54
Dipel 8L + 40 - 0	17	8	3	60
Dipel 8L + 40	33	8	19	50
Dipel 8L + 40 - 0	33	8	29	40
Dipel 8L + 40	42	8	21	36
Dipel 8L + 40 - 0	42	8	37	34
Check			0	100

Table 29. Percent mortality of 2nd instar gypsy moth larvae and seedling defolition after exposure to oak seedlings treated with Dipel 8L (oil) and Holdup.

Material	Percent Holdup	BIU/96 oz/ acre	Percent mortality days of exposure 2	4	Percent defoliation days of exposure 2	4
Dipel 8L		4	2	5	44	84
Dipel 8L + 40	29	4	12	23	21	54
Dipel 8L + 40 - 0	29	4	3	8	33	66
Dipel 8L + 40	38	4	13	22	28	52
Dipel 8L + 40 - 0	38	4	6	19	40	72
Dipel 8L + 40	45	4	21	52	16	34
Dipel 8L + 40 - 0	45	4	10	38	17	44
Dipel 8L		8	5	26	22	52
Dipel 8L + 40	8	8	17	32	18	44
Dipel 8L + 40 - 0	8	8	15	24	14	54
Dipel 8L + 40	25	8	7	17	30	58
Dipel 8L + 40 - 0	25	8	7	17	44	82
Dipel 8L + 40	42	8	33	73	10	24
Dipel 8L + 40 - 0	42	8	18	65	16	36
Dipel 8L		12	30	66	11	26
Dipel 8L + 40	6	12	24	56	13	31
Dipel 8L + 40 - 0	6	12	29	46	11	25
Dipel 8L + 40	13	12	36	64	20	37
Dipel 8L + 40 - 0	13	12	29	55	18	37
Dipel 8L + 40	38	12	49	92	8	13
Dipel 8L + 40 - 0	38	12	38	76	7	24
Check			0	0	92	99

Table 30. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after exposure to oak seedlings treated with Dipel 8L (aqueous) and Holdup.

Material	Percent Holdup	BIU/96/ oz/ acre	Percent mortality days of exposure 2	Percent mortality days of exposure 4	Percent defoliation days of exposure 2	Percent defoliation days of exposure 4
Dipel		4	2	42	28	70
Dipel 8L + 40	29	4	16	29	28	52
" " "	38	4	31	54	22	37
" " "	46	4	26	54	17	49
" " "	50	4	6	46	34	56
" " "	58	4	14	41	26	52
" " "	67	4	32	68	15	34
" " "	75	4	29	64	15	40
" " "	83	4	44	88	7	19
" " "	92	4	42	86	7	20
Dipel 8L		8	43	67	11	24
" "		8	35	72	9	35
Dipel 8L + 40	8	8	45	65	16	33
" " "	25	8	35	72	10	26
" " "	42	8	21	62	13	40
" " "	50	8	56	82	10	21
" " "	67	8	50	88	9	21
" " "	75	8	36	88	7	18
" " "	83	8	53	91	4	8
Dipel 8L		12	24	70	5	24
" "		12	49	83	8	18
Dipel 8L + 40	6	12	44	76	9	20
" " "	13	12	45	79	8	15
" " "	38	12	46	86	9	22
Check			0	0	100	100

Table 31. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after exposure to oak seedlings treated with Dipel 8L (aqueous) and Holdup.

Material	Percent Holdup	BIU/96 oz/ acre	Percent mortality days of exposure 2	4	Percent defoliation days of exposure 2	4
Dipel 8L		4	5	28	38	75
Dipel 8L + 40 - 0	29	4	15	45	30	56
" " " "	38	4	19	49	13	34
" " " "	46	4	12	36	30	52
Dipel 8L		8	31	61	15	33
Dipel 8L + 40 -0	8	8	35	78	8	22
" " " "	25	8	35	62	14	34
" " " "	42	8	10	45	26	39
Dipel 8L		12	24	63	16	30
Dipel 8L + 40 - 0	6	12	44	80	6	12
" " " "	13	12	44	75	11	28
" " " "	38	12	28	76	14	34
Check			0	9	88	100

Dipel 8L samples from the 1986 Oregon spray project were bioassayed with, laboratory reared, 2nd instar gypsy moth larvae acting as test insects. Sixteen of the samples were re-tested because of low activity in the original tests. Four gave increased activity when re-tested with the remaining giving results similar to those of the original test. It would appear that poor sampling techniques may have accounted for some samples giving poor results.

In the future if spraying material is to be bioassayed to determine activity, standard sampling techniques should be established and followed. We certainly encourage everyone to take samples and have them bioassayed to determine activity.

Table 32. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure of 2nd instar gypsy moth larvae to red oak seedlings treated with Bt at 16 BIU/96 oz/acre.

Batch number	Truck number	Date received	Percent mortality[1/]	Percent defoliation
88-909-BJ	6537	4/21/86	46 bc	27
88-910-BJ	6982	4/21/86	33 ab	29
89-966-BJ	6917	5/14/86	26 a	53
Dipel 8L	Std	--	56 c	21
Thuricide 48LV	Std	--	82 d	6
Check	--	--	0	100
88-902-BJ	6933	4/17/86	72 c	20
88-913-BJ	6548	4/21/86	41 a	32
88-927-BJ	455	4/26/86	40 a	40
Dipel 8L	Std	--	56 b	21
Thuricide 48LV	Std	--	74 c	17
Check	--	--	0	87
88-903-BJ	6581	4/17/86	42 de	22
88-905-BJ	6704	4/18/86	39 cde	30
88-906-BJ	6744	4/18/86	21 ab	50
88-907-BJ	6991	4/19/86	27 abc	48
88-904-BJ	6546	4/21/86	51 ef	19
88-912-BJ	6997	4/21/86	30 bcd	34
88-914-BJ	6918	4/21/86	17 a	38
88-925-BJ	6901	4/22/86	26 ab	30
89-941-BJ	6581	4/24/86	42 de	29
89-967-BJ	6706	5/14/86	25 ab	37
Dipel 8L	Std	--	61 f	13
Thuricide 48LV	Std	--	84 g	4
Check	--	--	0	80
---	6938	4/21/86	38 a	24
88-911-BJ	6990	4/21/86	38 a	37
88-926-BJ	6917	4/24/86	44 a	26
89-942-BJ	6933	4/25/86	46 a	22
89-943-BJ	6744	5/13/86	41 a	27
Dipel 8L	Std	--	78 b	14
Thuricide 48LV	Std	--	72 b	8
Check	--	--	0	98
88-908-BJ	6714	4/19/86	74 bc	15
88-916-BJ	6706	4/27/86	56 a	23
89-945-BJ	6731	5/12/86	55 a	25
89-944-BJ	6991	5/12/86	53 a	23
Dipel 8L	Std	--	63 ab	20
Thuricide 48LV	Std	--	81 c	4
Check	--	--	0	84

1/ Percents followed by the same letter are not significantly different at the 5% level according to chi square analysis.

Sixteen samples that gave low mortality in the first test were re-tested. Four samples gave higher mortality than in the first test. The remaining 12 samples gave similar or less activity than in the first bioassay.

Table 33. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation following a 4 day exposure of larvae to red oak seedlings treated with Bt at 16 BIU/96 oz/acre.

Batch number	Truck number	Date received	Percent mortality 1/	Percent defoliation
88-914-BJ	6918	4/21/86	8 a	36
88-925-BJ	6901	4/22/86	16 a	46
89-941-BJ	6581	4/24/86	30 b	24
88-912-BJ	6997	4/21/86	37 b	27
Dipel 8L	Std	--	39 b	19
Thuricide 48LV	Std	--	81 c	8
Check		--	0	100
--	6938	4/21/86	19 a	36
89-967-BJ	6706	5/14/86	22 a	47
89-943-BJ	6744	5/13/86	27 a	38
88-911-BJ	6990	4/21/86	29 a	47
Dipel 8L	Std	--	45 b	29
Thuricide 48LV	Std	--	89 c	3
Check		--	2	92
88-906-BJ	6744	4/18/86	21 a	35
88-905-BJ	6704	4/18/86	27 ab	33
89-966-BJ	6917	5/14/86	34 abc	31
88-927-BJ	455	4/25/86	35 bc	35
Dipel 8L	Std	--	42 cd	24
88-903-BJ	6581	4/17/86	42 cd	18
88-907-BJ	6991	4/19/86	47 cd	32
88-910-BJ	6982	4/21/86	51 d	21
88-913-BJ	6548	4/21/86	52 d	19
Thuricide 48LV	Std	--	89 e	3
Check			0	100

1/ Percents followed by the same letter are not significantly different at the 5% level according to chi square analysis.

Three Bt samples were bioassayed for the United Stated Department of Interior, National Park Service, National Capitol Region. The samples were as active as the laboratory standard when tested against 2nd instar gypsy moth larvae.

Table 34. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 2 and 4 day exposure to plants treated with Dipel 6L.

Formulation	BIU 96 oz./acre	Percent mortality 2 days	Percent mortality 4 days	Percent defoliation 2 days	Percent defoliation 4 days
Sample # 1	8	6	26	14	58
"	12	26	51	5	26
"	16	31	81	5	8
Sample #2	8	16	36	10	50
"	12	48	90	2	4
"	16	53	87	2	4
Sample #3	8	12	33	9	46
"	12	36	72	3	21
"	16	28	80	3	12
Lab Standard	8	9	18	14	52
"	12	33	77	4	12
"	16	38	82	2	10
Check	--	0	2	64	96

A sticker sample was tested with Dipel 8L and Thuricide 48LV.

Table 35. Percent mortality of 2nd instar gypsy moth and seedling defoliation after a 4 day exposure to oak seedling treated with Bt and R-27 sticker then exposed to rainfall.

Material[1/]	Percent sticker	Inches rain	Percent mortality	Percent defoliation
Thuricide 48LV			83	11
		.5	33	29
	.01	.5	47	19
	2.00	.5	36	29
	3.00	.5	17	44
	5.00	.5	13	40
	10.00	.5	11	36
Dipel 8L			60	20
		.5	1	99
	.01	.5	9	100
	2.00	.5	9	99
	3.00	.5	0	98
	5.00	.5	0	84
	10.00	.5	2	68
Check			0	100

1/ Material was applied at 12 BIU/96 oz/acre.

An ultraviolet degradation test was conducted with Thuricide 48LV.

Table 36. Percent mortality of 2nd instar gypsy moth larvae and oak seedling defoliation after exposure to oak seedlings treated with Thuricide 48LV and exposed to 24 hours of ultraviolet light.

Dosage BIU/96 oz/acre	Hours ultraviolet light	Percent mortality (days of exposure) 4	5	Percent defoliation (days of exposure) 4	5
8		88	97	.6	.6
8	24	38	58	38	46
4		56	78	18	22
4	24	10	38	50	64
Check		1	3	100	100

Table 37. Percent mortality of larvae and seedling defoliation following larvae exposure to oak seedlings treated with various amounts of SAP and BTS.

Material	Percent concentrate	After 1 day		After 2 days		After 3 days		After 4 days	
		mor.	def.	mor.	def.	mor.	def.	mor.	def.
SAP	100	34	1	50	1	85	1	93	2
"	75	33	1	52	1	85	1	95	1
"	50	20	1	33	1	41	1	85	3
"	25	14	1	17	1	20	2	43	15
BTS	100	0	6	1	32	1	40	29	52
"	75	0	7	0	29	2	40	23	56
"	50	0	16	0	36	1	48	28	56
"	25	0	25	0	52	0	74	14	84
Check		0	48	0	84	0	95	0	100

In test #3, SAP was tested on oak seedlings at 5 dosages. The material was diluted with water and applied in a laboratory spray chamber at a gallon per acre. Gypsy moth 2nd instar larvae were used as test insects.

SAP was diluted with water and applied at the rate of a gallon per acre to oak seedlings in the laboratory spray chamber. After two hours 2nd instar gypsy moth larvae were placed onto each plant.

Table 38. Percent mortality of larvae and seedling defoliation following larvae exposure to oak seedlings treated with SAP.

Mixture		After 1 day		After 2 days		After 3 days		After 4 days	
SAP	H_2O	mor.	def.	mor.	def.	mor.	def.	mor.	def.
1ml	79ml	0	30	0	60	0	82	0	96
5ml	75ml	0	8	0	26	2	30	1	64
10ml	70ml	0	3	1	8	2	21	5	28
20ml	60ml	6	1	17	3	25	4	26	7
40ml	40ml	20	1	28	1	34	1	42	1
Check		0	36	0	74	0	94	0	100

Viable gypsy moth egg masses were treated to the point of complete saturation with various amounts of BTS and SAP. Treated eggs were incubated at 26°C for a period of 9 days.

Samples from "Safer Agro-Chem Ltd.", Victoria, B.C., Canada were tested against 2nd instar gypsy moth larvae.

Tender oak seedlings were sprayed with BTSA-2S, BTSA-3S. BTSA-2F and SAP. The formulations were not diluted and were applied at a gallon per acre rate in a laboratory spray chamber. Another series of seedling were also treated with the same materials, using a brush to apply the formulation so that all foliage was completely covered with material.

Following a two hour drying period, twenty 2nd instar gypsy moth larvae were exposed to each seedling mortality and defoliation was recorded after days 1 and 4. Each test was replicated 5 times.

Table 39. Percent mortality of larvae and seedling defoliation following larvae exposure to oak seedlings treated with experimental samples from Safer Agro-Chem, Ltd.

Material	Type treatment	After 1 day		After 4 days	
		mor.	def.	mor.	def.
BTSA-2S	Spray	0	2	10	30
"	Brush	2	1	64	2
BTSA-3S	Spray	0	1	1	34
"	Brush	1	1	67	1
BTSA-2F	Spray	0	1	15	19
"	Brush	1	0	80	1
SAP	Spray	78	0	91	1
"	Brush	91	0	100	0
Check	---	0	8	0	75

Test #2 consisted of spraying tender oak seedlings in the laboratory spray chamber with various amounts of SAP and BTS concentrate. After 2 hours of drying time, 2nd instar gypsy moth larvae were exposed to treated seedlings. Material was applied at a gallon per acre.
Seedlings were treated with BTS and SAP in the laboratory spray chamber. Application was made at a gallon per acre.

Two formulations of Malathion used for grasshopper control were tested in the laboratory for washoff. Seedlings were tested in a laboratory spray chamber and dried for 2 hours or longer before being exposed to rainfall. Each test was replicated 5 times with 2nd instar gypsy moth larvae used as test insects.

Table 40. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 4 day exposure to oak seedlings treated with Malathion and then exposed to rainfall.

Malathion formulation	Dosage ounces/neat	Inches rain	Percent mortality	Percent defoliation
CR	8		90	24
ULV	8		79	31
CR	8	.1	19	78
ULV	8	.1	17	86
CR	8	.5	0	92
ULV	8	.5	0	100
CR	8	1.0	2	100
ULV	8	1.0	0	98
CR	16		100	6
CR	16	1.0	7	99
Check			0	94

Following treatment all seedlings were dried for 2 hours before being exposed to rainfall.

Table 41. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 4 day exposure to oak seedlings treated with Malathion and then exposed to rainfall.

Malathion formulation	Inches rain	Hours dried before rain	Percent mortality	Percent defoliation
CR		2	96	4
ULV		2	99	2
CR	.025	2	79	34
ULV	.025	2	89	14
CR	.050	2	55	36
ULV	.050	2	48	50
CR	.100	2	48	55
ULV	.100	2	43	60
CR	.100	24	89	22
ULV	.100	24	29	80
Check			0	98

Following treatment all seedlings were dried for 2 hours before being exposed to rainfall.

Project Number: GM 5.1.1
Project Title: 1986 Field Trials with Dimilin, Alsystin and *Bacillus thuringiensis*.
Report Period: October 1, 1985 - September 30, 1986
Report Type: Final
Project Leaders: W.H. McLane, J.A. Finney and T. Roland

Field trials were conducted in West Virginia, Pennsylvania and Rhode Island. Dimilin 25W and 2F(in 72 crop oil) were applied to gypsy moth infestations in West Virginia, at 4 time intervals from pre hatch to 2nd instar larval stage.

Alsystin 4F, Dimilin 25W and 2F(in 72 crop oil) were applied to gypsy moth infestations in Pennsylvania, at 4 time intervals from pre hatch to 2nd instar larval stage.

In Rhode Island, Dipel 8L, Thuricide 48LV and SAN 415 were sprayed against 3rd instar larvae. The *Bt* was used with the in-flight encapsulated materials BTLS, Black LS and Holdup.

All applications were made with the APHIS Ag-truck aircraft with a conventional spray system equipped with flat fan nozzles. The aircraft was piloted by Mr. Tim Roland, Aircraft Operations, Moore Field, Mission, Texas.

West Virginia

Insect growth regulators have proven to be extremely effective insecticides for use against gypsy moth larvae. Their mode of action is by contact as well as ingestion. Normally, excellent population reduction can be achieved with application of 0.25 to 1.0 ounces active ingredient per acre. After application onto foliage, peak mortality of 2nd instar gypsy moth larvae will occur in 5-7 days.

Because of their exceptional contact activity, it may be possible to achieve good control of early instar larvae by applying pre-foliage and pre-hatch treatments. Such treatments might also eliminate the threat of re-establishment by wind-blown early instar larvae. During 1976, Dimilin was applied to 3 fifty acre plots in Pennsylvania on April 30th. Foliage had not developed except on witch hazel that had 5% expansion. Approximately 50% of the gypsy moth eggs had hatched, but larvae had not left the egg masses. Average defoliation of treated areas was 10% with 65% in adjacent, untreated woodlands. Based on egg mass counts, populations were reduced by 87% in treated areas with a 72% increase in untreated check areas. Work continued with early application of Alsystin in 1984 and 1985. However, results were inconclusive due to poor timing of applications and the generally poor state of the insect population.

In 1986, early applications of Dimilin were applied in West Virginia at 4 time intervals to 100 acre woodland plots near Sleepy Creek State Public Hunting, Fishing and Camping Area. Treatments were replicated 3 times with all plots located along the top of a ridge at approximately 1800 feet elevation.

Table 1. Early application treatment schedule with Dimilin.

Material	Dosage/Rate	Treatment date
Dimilin 25W	.03 lbs AI/gal/acre	4/2/86 no budbreak/no hatch
Dimilin 2F(oil)	.03 lbs AI/gal/acre	4/14/86 no foliage/ 10% hatch
Dimilin 2F(oil)	.03 lbs AI/gal/acre	4/24/86 budbreak/general hatch
Dimilin 2F(oil)	.03 lbs AI/gal/acre	5/12/86 regular timing/2nd instar

Main objectives of the study were:

1. Determine if early treatments of Dimilin and Alsystin will prevent the establishment of wind-spread early instar gypsy moth larvae in areas outside of an isolated treatment plot.

2. Determine if pre-hatch and pre-foliage treatments can be effective in preventing defoliation and/or effecting population reduction.

3. Establish the earliest date that Dimilin and Alsystin can be effectively applied for controlling gypsy moth larvae.

4. Compare an oil formulation of Dimilin at two rates with Dimilin 25W applied at one rate.

Methods and Techniques

Plots were selected based on location and condition of the resident gypsy moth population. The Sleepy Creek area was selected because of the large egg masses present, little defoliation in 1985 and plots being accessible by a woods road.

Using a compass, topographical maps, rope and surveyor tape, plots were established and allocated to the various treatments. All plots were marked at each end with tape along the access road that ran through the center of each plot lengthways. Each plot was a square of 2,090 feet per side. The aircraft applied the spray material in a swath width of 75 feet, therefore, 14 swaths were treated on each side of the markers and/or wood road going parallel to the ridge. At time of treatment a yellow 30-inch balloon was flown at the end of each plot for aircraft guidance. An observation aircraft equipped with radio was flown overhead to assist in the guidance of the spray aircraft.

The aircraft was equipped with a 50-mesh in-line screen and quick drain valves. All 8006 flat fan nozzles utilized 50-mesh screens as the material was applied at 120 mph, 50 feet above the tree tops at approximately 40 PSI. Aircraft calibration was checked over the actual spray plots with each formulation.

Dimilin 25W and water was mixed in a nurse tank before being pumped into the aircraft. Dimilin 2F was mixed with 72nd dormant crop oil in the aircraft hopper. All aircraft operations were conducted out of the Martinsburg, West Virginia airport.

Treatment evaluation consisted of pre and post-spray egg mass counts, hatchability tests, defoliation estimates and larval collection and rearing. In early March nine 1/40 acre plots in each 100 acre spray plot were surveyed for egg masses. After fall foliage drop, post-spray counts were made using the same technique as described for pre-spray counts. At the time of pre-spray counts, egg masses were collected from each plot and incubated in the laboratory to establish percent hatch. Hatch was recorded at 80% or better.

In early July defoliation estimates were made in each sprayed and untreated check plot to determine percent defoliation. Defoliation was also observed from the air.

On May 1st, following general hatch and distribution of 1st instar larvae, 50 newly hatched larvae were collected at both the 1-6 foot and 20-40 foot levels in all plots treated with Dimilin by the first 3 applications. Larvae were also collected in all check plots. Collected insects were placed on artificial diet and reared over a period of time to record mortality. This technique was to determine if wind-blown larvae would survive if blown outside the treatment area.

Results

Table 2. Results of early Dimilin treatments in West Virginia.

Treatment date	Formulation	Pre EM/A	Post EM/A	Percent defoliation	Percent change (EM)
April 2	Oil 2F	6,566	527	13	-92
	25W	3,305	7,443	23	+56
April 14	Oil 2F	298	43	5	-86
	25W	1,858	444	7	-76
April 27	Oil 2F	1,449	47	5	-97
	25W	1,874	17	8	-99
May 12	Oil 2F	2,650	33	5	-99
	25W	2,073	80	5	-96
Check		4,470	521	13	-88

TABLE 1. Percent mortality of collected larvae from 3 early Dimilin treatments in West Virginia.

Date	Formulation	Percent mortality
April 2	Oil 2F	2
	25 W	4
April 14	Oil 2F	2
	25 W	6
April 27	Oil 2F	8
	25 W	16
Check		0

Although pre-spray egg masses averaged only 2,990 per acre, it appears that a population collapse may have occurred in the experimental area. Excellent control occurred in nearly all treatment plots, however, an 88% reduction took place in our check plots.

It would appear, based on the extensive defoliation just to the east of our plots, that strong southwesterly winds caused a movement of small larvae off the ridge top and out of the majority of our spray area. Therefore, the ridge tops are not suitable experimental areas to locate small plots. This would account for the population reduction recorded in all check plots. Based on the amount of defoliation just to the east of the plots where wind-blown larvae would have landed, one would say that larvae can survive if moved by wind out of an area that has received an early Dimilin application. This is further evident by the small amount of mortality that occured in larvae that were collected and reared on artificial diet. A problem with this analysis is the fact that we were not able to collect larvae high up in the trees and therefore may not have had samples representive of the population that might be succeptable to blow-out. Additional studies should be conducted making use of better sampling techniques.

Although little can be said, based on egg mass counts, defoliation estimates and our general observations within each plot indicate that we achieved good control with treatments 2, 3 and 4. In plots treated before foliage development we observed shot-holes in leaves after development of 50-75% expansion. At this time few if any larvae could be found in treated plots. This indicates that larvae moved from treated bark to foliage and fed, then went back to the bark and repeated this procedure until a lethal dosage had accumulated. During this period larvae would normally be too large to be spread by the wind velocity that was experienced. Therefore, population reduction was not due completely to blow-out that appeared to occur prior to foliage development.

Except for our first treatment we were able to record little difference between the oil and water base formulations. The oil appeared to be more effective than water in the first treatment. The main advantage of oil would

be to minimize evaporation. The main disadvantages would be the extra cost, handling and hang-up of spray. The durability of Dimilin 25W is excellent so we see no advantage of an oil base formulation to give longer residual. We feel it would be much more advantageous to develop a good 4 flowable formulation.

It appears that successful control of gypsy moth larvae can be achieved by applying Dimilin 25W in water or Dimilin 2F in oil at 0.03 lbs AI/gal/acre by aircraft as soon as any hatch appears on the south side of trees. This would allow an additional 2-3 weeks of treatment time. However, this study should be finalized in 1987 with early operational treatments being made to 5,000 acre plots at time of first hatch and at general hatch.

The West Virginia project was a cooperative effort by the West Virginia Department of Agriculture; United States Department of Agriculture, Forest Service, Morgantown, West Virginia; United States Department of Agriculture, Animal and Plant Health Inspection Service, Otis Methods Development Center, Otis ANGB, Massachusetts; and APHIS Aircraft Operations, Moore Field, Mission, Texas.

We extend special thanks to the West Virginia, Department of Agriculture personnel for their exceptional help and cooperation in the project.

Pennsylvania

Studies in Pennsylvania were very similar to those conducted in West Virginia with the objectives being the same. In addition to Dimilin being sprayed over 120 acre plots, Alsystin 4F was also tested on 500 acre sites.

Table 4. Early application treatment schedule with Dimilin and Alsystin in Pennsylvania.

Material	Dosage/Rate	Treatment dates
Alsystin 4F	.06 lbs AI/gal/acre	4/1/86 no budbreak/ no hatch
Dimilin 25W	.03 lbs AI/gal/acre	4/18/86 no foliage/ 2% hatch
Dimilin 2F(oil)	.03 lbs AI/.5 gal/acre	4/29/86 5% foliage/ general hatch
		5/14/86 regular timing/ 2nd instar1/

1/ Dimilin was not applied on the last treatment date.

Alsystin was applied at .06 lbs AI/gal/acre and all Dimilin work in West Virginia, and Pennsylvania was with .03 lbs AI/gal and .5 gal/acre. Treatments in Pennsylvania were not replicated.

Plots were located on the southeast side of Blue Mountain in Tuscarora State Forest near Mifflintown, Pennsylvania. Mixing and loading operations were located at Mid-State Airport, Lewistown, Pennsylvania.

Methods and Techniques

All methods and techniques were very similar to those in West Virginia, with the following exceptions:

1. Pre and post-spray egg mass counts were made at 6 sites in each treatment and control plot using a 1/40 acre evaluation area.

2. Most corners were located and marked with balloons at time of treatment. The Cessna Ag-truck aircraft navigated off marker balloons and was not aided by an observation aircraft.

3. Larvae were collected for rearing in only the Alsystin plots of the first 3 treatments. As was the case in West Virginia, we had difficulties in trying to collect from high up in the trees.

Results

Table 5. Results of early Alsystin and Dimilin treatments in Pennsylvania.

Date	Formulation	Pre EM/A	Post EM/A	Percent change
April 1	Alsystin	6,760	120	- 98
	Dimilin 2F oil	4,500	0	-100
	Dimilin 25W	3,500	180	- 95
April 18	Alsystin	9,393	0	-100
	Dimilin 2F oil	4,000	27	- 99
	Dimilin 25W	4,000	100	- 98
April 29	Alsystin	5,603	43	- 99
	Dimilin	1,500	0	-100
	Dimilin 25W	1,800	0	-100
May 14	Alsystin	6,187	0	-100
Check		2,656	1,200	- 55

Defoliation was less than 10 percent in all treatment plots and 80 percent in check plots.

The 500 acre plot sprayed with Alsystin on April 1, 1986 was evaluated by state personel in mid-May and they found a number of larvae still actively feeding. They retreated 450 acres of the plot with Dimilin, and left 50 acres for our post-spray evaluation. For that treatment all egg counts and defoliation estimates were made within the 50 acres that were not retreated.

Based on egg mass reduction and defoliation estimates Dimilin and Alsystin were very effective at all time intervals studied. There appeared to be

little difference between dosages and only a slight difference between oil base and water base Dimilin formulations.

Two weeks after Alsystin was applied on April 29, thousands of dead larvae were observed in a 45 minute observation period. Within the Alsystin plot treated on April 18th only small numbers of live larvae could be observed in small isolated spots near the southern border. No post-spray egg masses were found within this plot.

As was the case in West Virginia, after foliage had developed to 50-70%, shot-holes were observed in most leaves indicating feeding by gypsy moth larvae. However, in most cases no larvae could be found, indicating accumulative effects of the spray.

Heavy virus infection occurred in larvae that were collected and reared in the laboratory. As a result we were unable to determine if larvae that may blow-out of a plot after early treatment might survive. As in West Virginia, we had great difficulty in collecting young larvae high up in the trees.

In conclusion, we can say that successful control of gypsy moth larvae can be achieved by applying Alsystin or Dimilin at .03 lbs AI/acre by aircraft as soon as hatch appears on the south side of trees. It also appears that successful control can be achieved with applications of Alsystin or Dimilin up to 2 weeks prior to any hatch.

This was a cooperative program with the Pennsylvania Division of Forest Pest Management, Mobay Chemical Corporation; Uniroyal Chemical Company and the United States Department of Agriculture, Animal and Plant Health Inspection Service. We thank the personnel involved in the program for their excellent help and cooperation.

Rhode Island

In 1985 *Bacillus thuringiensis* (*Bt*) was the material of choice for treating more than half of the total acres sprayed by air for control and/or eradication of gypsy moth infestations. Thuricide and Dipel were applied in a number of states as a single application at dosages of 12-20 BIU (Billion International Units) per acre. Rate of application ranged from 64-128 ounces per acre. In some states, isolated infestations were treated with multiple applications at 16 BIU in 96-128 ounces per acre. Large and small plots were treated along with narrow strips along roadways. Various types of helicopters, single and multi-engine fixed wing aircraft were used. Formulations were mixed in nurse tanks and dispersed by aircraft through flat fan and beecomist nozzles.

Where multiple applications of *Bt* were made, results were good based on post-spray adult trapping. Results with single applications varied greatly. One reason for this is the fact that where single applications are made, gypsy moth populations are generally high (5,000-10,000+ egg masses per acre). Pressure of migrating larvae from adjacent, unsprayed land is too great, especially with strip spraying. In some states, the use of *Bt* was a disaster, and in others, foliage protection was achieved.

Two new formulations of Bt were tested on 50 acre plots in Rhode Island during 1986. Holdup, a liquid polymer system and sticker agent was tested with oil base Dipel 8L. This material is a product of Greenshield of Washington. In laboratory tests, a combination of Holdup and Dipel 8L have resulted in faster kill, 20-30 percent more mortality and less defoliation than tests with Dipel 8L alone (Table 6). An encapsulant material developed at the University of Georgia was tested with Thuricide 48LV. Field studies on individual apple trees demonstrated longer residual with the addition of the encapsulant material (Table 7).

Table 6. Percent mortality of 2nd instar gypsy moth larvae and defoliation after a 3 and 4 day exposure to oak seedlings treated with Dipel 8L (oil) and Dipel 8L (oil) with Holdup at 12 BIU/96 oz/acre

	Percent Mortality - Defoliation			
	3 days		4 days	
Formulation	mor.	def.	mor.	def.
Dipel 8L	41	25	59	40
2ml 8L + 6ml 1128540	56	9	86	10
2ml 8L + 3ml H_2O + 3ml 1128540	83	3	95	3
2ml 8L + 6ml 1128540-0	81	6	94	6
2ml 8L + 3ml H_2O + 3ml 1128540-0	74	6	97	6
Check	2	88	3	91

Table 7. Percent mortality of 3rd instar gypsy moth larvae after a 4 day exposure to treated apple foliage collected from the field.

Material	Days after treatment											
	2	3	7	10	14	18	22	25	29	36	39	42
Thuricide 48LV	92	87	90	73	23	27	40	23	33	47	23	0
Thuricide 48LV + H1(1x1)	92	90	90	67	93	53	97	90	100	87	73	7
Thuricide 48LV + H4(1x1)	87	80	80	83	83	37	93	83	100	90	47	7
Thuricide 48LV + H5(1x1)	43	--	70	47	67	73	27	--	80	17	--	--
Thuricide 48LV + H5(2x1)	93	--	100	30	73	77	23	--	67	10	--	--
Thuricide 48LV + H6(2x1)	83	--	93	33	73	73	53	--	53	3	--	--
Thuricide 48LV + 3% Bond	90	87	93	97	67	30	60	37	57	30	100	7
Dipel 8L	83	97	80	87	50	63	83	60	83	37	50	3
Dipel 8L + 3% Bond	95	97	100	97	100	63	93	100	100	77	47	20
Bactospeine	95	80	87	80	50	43	93	77	23	23	43	7
Sevin XLR	100	100	100	100	100	100	100	100	100	100	73	100
Dimilin	100	100	100	100	100	100	100	100	100	100	100	100
Check 1	18	0	10	13	3	0	10	3	0	27	0	0
Check 2	0	6	3	10	7	0	0	0	0	0	20	0
Check 3	0	--	7	0	3	13	0	--	0	0	--	--

The following table is a summary of work conducted in 1986 Rhode Island experimental spray plots.

Table 8. 1986 Rhode Island field work with Dipel 8L, Thuricide 48LV, Holdup, encapsulant materials and SAN-415.

Formulation	Dosage BIU/96 oz/acre	Acres treated
Dipel 8L 1/	4	150
Dipel 8L + Holdup	4	150
Dipel 8L 1/	8	150
Dipel 8L + Holdup	8	150
Dipel 8L 1/	12	150
Dipel 8L + Holdup	12	100
Thuricide 48LV	8	150
48LV + 26% BTLS 2/	8	150
48LV + 26% BLACK LS 2/	8	150
48LV + 13% BTLS 3/	8	50
SAN 415 (NRD-12)	8	100
SAN 415 (NRD-12)	12	50
SAN 415 (NRD-12)	12 (Strip 2000x500')	23
SAN 415 (NRD-12)	16 (Strip 2000x500')	23
Check (untreated)		400

1/ Same amount of Holdup used as water.
2/ Encapsulant was 26% of total formulation.
3/ Encapsulant was 13% of total formulation.

The main objectives were:

1. Compare aerial applications of Dipel 8L(oil) with formulations of Dipel 8L(oil) mixed with Holdup.

2. Compare aerial applications of Thuricide 48LV to formulations of Thuricide 48LV mixed with the encapsulants BTLS and BLACK LS.

3. Compare SAN-415 (NRD-12 strain) to Dipel 8L and Thuricide 48LV (HD-1 strain).

4. Determine if Bt can be effectively used to protect foliage in strips 500 feet wide surounded by heavily defoliating gypsy moth populations (2,00-6,00 egg masses per acre).

Methods

A total of 38 experimental spray plots were established in Rhode Island at Arcadia Management Area, Wichaboxit Management Area and Alton Jones, University of Rhode Island Campus. Egg masses were large and averaged from 82-5,252 per acre with a mean of 1,072. Areas selected were mainly on state lands and had experienced little defoliation in 1985.

Using a compass, topographical maps, rope and surveyor tape, plots were established and allocated to the treatments listed in Table 8. Boundary lines were surveyed and marked in flourescent orange tape and each corner tree was marked with double flourescent orange tape and a tag identifying corner and plot number. Minimum distance between plots was 400 feet. Plots were located so that there would be a maximum number of corners on or near roadways.

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

Within the center 10 acres of each plot, 20 prism points were established, 5 points on 4 parallel lines. During March and early April, pre-spray egg mass counts were made at each prism point. New egg masses were counted and 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 6 oak trees at random in the center 10 acres of some 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.

Six, 8 inch funnels were utilized in the center of some plots to collect frass following treatment. Funnels were located on stakes approximately 3 feet above the ground. Each funnel had a small plastic tube with screen end attached for collecting the frass. Frass collection continued until pupation had started.

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.

Flow rates of each formulation were established at the laboratory with a miniature sprayer and new stainless steel flat fan spray tips. Final aircraft calibration was made over the first spray plot treated in each series. Spray distribution was checked with "Kromekote" cards placed in open areas on the ground. Encapsulant formulations appeared to be sticking to the spray cards when treatments were made early in the morning. It was reported that when mid-morning treatments were made some droplets rolled off from collection cards.

All applications were made with a Cessna Ag-truck aircraft equipped with a conventional spray boom and 8006 flat fan nozzles. The aircraft sprayed a 75 foot swath at 120 mph, approximately 50 feet over the target foliage. All nozzles were pointing straight down.

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 Westerly Airport.

Bond, an agricultural spray sticker was used with all standard Bt application at a rate of 2.0 percent by volume. When Bt was mixed with Holdup, BTLS or Black LS, no sticker was added to the mix.

All treatments were made in the early morning hours before temperatures became too high. No applications were made in the late afternoon or early evening. The majority of larvae were in the early 3rd instar when spraying was conducted.

Results

Table 9. Results with Dipel 8L and Holdup, 1986.

Formulation	BIU/ 96 oz./Acre	Pre EM/A	Post EM/A	Average defoliation	Percent change (EM)
Dipel 8L	4	305	2,685	29	+780
Dipel 8L + Holdup	4	250	4,257	59	+1603
Dipel 8L	8	661	2,146	55	+225
Dipel 8L + Holdup	8	539	2,350	71	+336
Dipel 8L	12	1,971	2,948	56	+50
Dipel 8L + Holdup	12	2,068	2,309	52	+12
Check		396	3,381	91	+754

Table 10. Results with Thuricide 48LV and encapsulant materials, BTLS and Black LS.

Formulation	Pre EM/A	Post EM/A	Average defoliation	Percent change (EM)
Thuricide 48LV	401	3,579	48	+793
Thuricide 48LV + BTLS 26%	514	2,099	46	+308
Thuricide 48LV + BTLS 13%	1,165	5,678	100	+387
Thuricide 48LV + Black LS 26%	411	2,765	67	+573
Check	396	3,381	91	+754

Table 11. Results with the SAN-415(NRD-12).

BIU/ 96 oz./acre	Pre EM/A	Post EM/A	Average defoliation	Percent change(EM)
8	82	1,468	14	+1690
12	5,252	615	46	-88
12 (strip 500x2000')	1,032	7,621	17	+638
16 (strip 500x2000')	1,032	7,664	50	+643
Check	396	3,381	91	+754

Table 12. Total numbers of gypsy moth life stages recorded under burlaps.

Formulation	BIU/ acre	Life stages		
		Live larvae	Dead larvae	pupae
Thuricide 48LV	8	176	89	95
Thuricide 48LV + 26% BTLS	8	126	33	71
Thuricide 48LV + 13% BTLS	8	632	128	815
Thuricide 48LV + 26% Black LS	8	174	144	348
Dipel 8L	4	234	87	21
Dipel 8L + Holdup	4	326	79	39
Dipel 8L	8	677	236	512
Dipel 8L + Holdup	8	1,012	128	618
Dipel 8L	12	408	169	97
Dipel 8L + Holdup	12	420	69	65
Check		589	166	186

Table 13. Average grams of total frass collected for each treatment following spraying.

Treatment	Dosage BIU/acre	Average grams of frass
Dipel 8L	4	.41
Dipel 8L + Holdup	4	.42
Dipel 8L	8	.45
Dipel 8L + Holdup	8	.74
Dipel 8L	12	.56
Dipel 8L + Holdup	12	.47
Thuricide 48LV	8	.25
Thuricide 48LV + 26% BTLS	8	.29
Thuricide 48LV + 13% BTLS	8	.25
Thuricide 48LV + 26% Black LS	8	.40
Check		.37

At defoliation time numbers of larvae on tree trunks were observed in all plots as the defoliation estimates were made. A rating of 0-10 was given each treatment based on the numbers of larvae observed. The higher the number the less effective the treatment.

Table 14. Rating assigned each treatment based on numbers of larvae observed at peak defoliation time.

Treatment	Dosage BIU/acre	Rating
Dipel 8L	4	5.3
Dipel 8L + Holdup	4	4.3
Dipel 8L	8	5.3
Dipel 8L + Holdup	8	6.5
Dipel 8L	12	5.0
Dipel 8L + Holdup	12	4.5
Thuricide 48LV	8	5.6
Thuricide 48LV + 26% BTLS	8	4.8
Thuricide 48LV + 13% BTLS	8	9.0
Thuricide 48LV + 26% Black LS	8	7.3
SAN 415	8	2.0
SAN 415	12	5.0
SAN 415 (Strip)	12	4.0
SAN 415 (Strip)	16	4.0
Check		9.6

Based on all data collected, nearly all Bt treatments gave very poor results. Dipel with Holdup was no better than Dipel alone. Formulations of BTLS and Black LS mixed with Thuricide 48LV gave no more kill than Thuricide 48LV alone. This may have been due to the encapsulant drying out and not sticking to the foliage. However, material was observed on some foliage and spray cards indicated that some material did stick.

Results with SAN-415 were much more encouraging than in past years. Good foliage protection was achieved in the 50 acre plots and strip areas. It shows that strips of 500 feet can be successfully treated for foliage protection with SAN-415. The 2 strips had 100 percent defoliation all around them and they remained green except for 75 feet on each side where migration occured. However, the population was not reduced based on egg masses. We should continue to work with SAN-415 in 1987 as results this year were surely more impressive than in past years.

Project Number: GM 5.1.2
Project Title: Spray Deposit Assessment
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: W. H. McLane, J. A. Finney, T. Roland, W. Yendol, R. Reardon and M. McMannus

At this time all data from a drift study conducted at Mission, Texas in September, 1985, has not been submitted to analysis. Results of this work will be reported at a later date.

In June, 1986, a foliage canopy penetration test was conducted at Black Moshannon Airport near Philipsburg, Pa. The project was a cooperative program between APHIS, FS and Pennsylvania State University.

Bacillus thuringiensis was applied over 25 acre blocks (1043 x 1043) of primarily oak species. Applications were made with flat fan and micronair (AU-5000) nozzle systems using a Cessna Ag-truck aircraft. The aircraft was calibrated for a 75 foot swath and applied material 50 feet above the canopy. Thuricide 48LV and Dipel 8L were selected to conduct this work. Both neat and diluted formulations were made.

Meteorology. Meteorological recordings were taken one hour prior to, during, and one hour after the spray period. The balloon-mounted Tethersonde, consisting of an airborne monitoring station employing FM radio telemetry, was used to transmit continuous metereological data to a ground receiving station. The recorded data included elapsed time, wet and dry bulb temperatures, relative humidity, pressure, wind speed and wind direction. Readings were taken at 50 feet above the canopy (spray height) and 10 feet above the canopy, allowing calculation of lapse rates and turbulence intensities in the spray block area.

Sampling Procedure. Some 30 oak trees were selected from each of the treated blocks and with the aid of tree climbers, groups of leaves were removed from the canopy. Samples were taken at two levels -- in the upper and lower third of the canopy. Each of the levels consisted of four stations corresponding to the four cardinal directions or along and across the flight line, plus one central sample for each level. Thus, ten canopy sample areas per tree.

Groups of leaves were removed from each of the canopy sampling stations for droplet analysis. Five to ten leaves from each sample station were subjected to photographic analysis. With the use of a 35 mm camera and color positive film, each leaf was photographed. These photographs were used to determine droplet density and size with the aid of the Optomax Image Analyzer.

A portion of the remaining leaves from each station served to estimate deposit density (ng/cm sq.). The area of these leaves was measured with a Li-Cor Meter and then subjected to alcohol washing. The total residue was determined by flourometric analysis.

Other collection methods included aerial lines with collecting devices attached at 10 foot intervals and ground positioned collectors. These included acetate sheets, Kromekote cards and other collecting devices. Aerial

lines were constructed 3 feet above the canopy, so as to determine the droplet characteristics just prior to entering the upper zone area of the canopy.

At this time all data from the 1986 Black Moshannon test have not been completely evaluated. Therefore, complete results will be reported in the next annual report.

The Northeast Application, Formulation Working Group conducted an aerial application technology workshop at Pennsylvania State University on September 23-25, 1986. The workshop consisted of 2 days of classroom instruction and one day of hands-on field instruction with 3 types of aircraft. Approximately 60 people attended the session, including 33 trainees from the northeastern states. Additional training sessions are planned so that all individuals wishing to attend such training will have that opportunity.

On October 2-3, 1985, a workshop and review of 1985 state spray projects was held at the Otis Methods Development Center, Otis Air National Guard Base, Mass. Attendance was by invitation and the purpose of the meeting was to review the results of the previous spring's operational use of Bacillus thuringiensis against gypsy moth, and to discuss the possible establishment of operational guidelines for future use of Bt against gypsy moth in cooperative federal suppression programs.

Forty-five, state, federal university and industry people were present. This was the first attempt to get a group such as this together to jointly review their programs.

The main interest was; how can we get Bt to give suitable control of gypsy moth larvae? A number of states reported very poor results with Bt in their 1985 spray program. Most states felt that the Forest Service should establish operational guidlines for the use of Bt. As a result of this meeting, guidlines were established by the Forest Service.

Mr. McLane suggested that a Bt work group be formed and meet annually or semi-annually to; a) review the results of operational use of Bt against gypsy moth, b) review the state of the art of Bt use, c) analyze and evaluate recent results and recommend appropriate revisions to the existing guidelines on operational use of Bt as new developments and improvements become available, d) provide input and guidance for research and development as well as operational use of Bt, and e) collect and make available published and unpublished reports on the use of Bt against gypsy moths. We suggested the committee be composed of representatives from federal and state agencies, academia, commercial manufacturers of Bt, and applicators.

A group was formed and held its first meeting at the Gifford Pinchot Institute (U.S. Forest Service) in Milford, PA on January 14-15, 1986. The group voted to call themselves the Cooperative Gypsy Moth Bt Committee. The committee consists of the following personnel:

Name	Organization
Normand R. Dubois*	U.S. Forest Service
Richard Reardon	U.S. Forest Service
Timothy Rowland	USDA, APHIS
Winfred McLane**	USDA, APHIS
Ralph Webb	USDA, ARS

William Slippey	Pennsylvania
John Kegg	New Jersey
William Yendol (Jon Bryant)	Pennsylvania State University
A. Temple Bowen, Jr.	Zoecon Corporation
Robert Fusco (Steve Sears)	Abbott Laboratories
Chris Stafford	Microbial Resources Ltd.
Carol Voss	Ag. Rotors

** - Chairman
* - Recording Secretary

The committee will make recommendations to the appropriate state and federal agencies. They in turn will accept or reject the recommendations and develop their own policy relative to the control of the gypsy moth.

Committee Membership

Officers

a. Chairperson: Will be appointed to the position by voice vote by the full committee. The Chairperson will organize and set the dates of the meetings and present to the full committee any matter brought up by individual committee members.
b. Recording Secretary: Will handle all correspondence and prepare meeting reports.

Both the Chairperson and Secretary will be voted in by the committee and serve at the committee's discretion.

Committee full membership

a. Academia, federal and state agencies will be permanently represented on the committee. Individuals will serve for an indefinite period of time at the discretion of the academic institutions or agencies.

b. Bt industry membership: One position on the committee will be available for one member of each Bt manufacturing company operating in the U.S. Changes on the committee such as addition of a new company, deletion of a former producer of Bt will be by vote of the committee except industry representatives already on the committee.

c. State representatives: Two states will be represented by one individual each. Membership for the individual state representative will be on a rotating staggered term of 2 years (initially one state's representative will be for a 3 year term so that both states don't rotate the same year). A state cannot represent itself for 2 successive terms. The full committee will select the next representative northeastern states, however, one individual from outside the northeastern area will be invited to participate on the committee for a 2 year term.

d. Applicator's representative: one representative from the commercial applicators will be invited to serve on the committee for a 2 year term.

Selection of a new representative will be made at the discretion of the committee.

e. Periodically Specialists will be invited to participate at specific meetings at the discretion of the committee.

The main item on the committee's agenda was to review Bt use guidelines that were established by the Forest Service as a result of the Otis meeting.

The following are provided as minimum standards for all gypsy moth suppression projects. Individual cooperators may have additional standards that are more stringent.

Aircraft and Spray Systems

- All spray systems must be clean prior to calibration. Responsible project personnel will inspect all systems to ensure compliance.

- All spray aircraft systems must be calibrated and verified by project personnel before operational use. Ideally, the actual tank mix to be applied should be used to check the flow rate.

- All aircraft must be equipped with boom timers.

- All nozzle systems must have new tips at the begining of the project. Stainless steel tips are preferred.

- All tips on an aircraft spray system must be the same size unless specified otherwise.

- All spray systems and mixing tanks must be equipped to maintain spray materials in suspension.

- All aircraft spray systems must be charaterized and verified by project personnel before operational use in a program. Characterization for swath width and droplet size is critical to the success of aerial application programs. If characterization trials are not or cannot be conducted, swath widths can be assigned from APHIS tables or from recent data from spray trials.

Metereological Conditions During Application

- Average winds must be above 2 mph and less than 10 mph at ground level at the highest point of elevation in the target area or in an open area close to the spray block.

- Temperatures should be less than 80 degrees Farenheit in the target area.

- Relative humidity should be greater than 50 percent.

- Foliage must not be wet to the point of run off.
If .5 inches or more of rain occurs within 24 hours following application, retreatment is recommended.

- If retreatment is necessary because unexpected heavy rains have washed off the initial application, the USDA Forest Service will cost share a second application of the same insecticide.

Spray Deposit Assessment

- The insecticide application must be monitored to insure the spray is reaching the target area, that sensitive areas are avoided and that proper

response is made in case an accident or dump occurs. This can be accomplished through aerial observation (chase planes) or ground monitoring during the time of application.

Dosage/Rates of Bt Application

- At least 12 BIU's/acre must be used.

- At least 96 oz/acre must be applied.

- The Forest Service will fund up to 2 applications of Bt.

- Bt treatment of strips less than 500 feet wide will generally not be approved for funding.

- The Forest Service encourages the use of stickers with Bt because they can save up to 50 percent of the applied material if rain occurs following treatment. However, some commonly used stickers can be harmful to car finishes and this should be a consideration regarding their use in residential areas.

Biological Parameters for Bt

- Fifty percent of the larvae should be in the second instar with most of the remainder in the first instar.

- White oak or other target foliage should be 50 percent expanded at the time of application.

- If a second application is planned, it should be applied 7 (± 3) days after the first treatment. If retreatment is needed because of heavy rain, it should be applied as soon as the foliage is dry.

- If larval development and foliage expansion are not in concert, larval development usually will dictate when the spray should be applied.

General

- The Forest Service will not fund ground applications of any insecticides.

- Radio contact between the airport, spray aircraft, chase planes and ground crews has proven to be essential to well managed suppression projects. Radio contact provides the opportunity to correct operational problems, suspend application during adverse weather conditions and improve the rescue opportunities during emergencies.

These standards are intended to supplement insecticide label directions. They are not intended to replace label requirements or imply endorsement of any particular product.

FPM personnel are available for technical assistance on all phases of the gypsy moth suppression program. If you have any questions concerning the standards, please contact the FPM office serving your State.

The "inconsistent results" that occured with Bt in 1985 also occured in some cases with other insecticides. Because of the complex nature of suppression projects and a lack of a good data base on operational factors such as application methods used, weather conditions and population levels at the time of treatment, the reasons for the inconsistent results are difficult to identify. We will be asking cooperators to collect specific information on their 1986 projects in selected areas. Cooperating States will be contacted by FPM personnel regarding the specifics of this data collection.

The above guidelines were received by the Bt committee at their January meeting. Some changes were recommended and presented to Forest Service for consideration. Some of the original guidelines were not changed.

The following are provided as minimum guidelines for all gypsy moth suppression projects. Individual cooperators may have additional standards that are more stringent.

Aircraft and Spray System

- All spray systems must be cleaned, inspected by officer-in-charge, and be ready for operational use before calibration.

- All spray aircraft systems must be calibrated before operational use. Ideally, the actual tank mix to be applied should be used to check the flow rate.

- All aircraft must have boom timers to maintain a constant check on the flow rate.

- All nozzle systems must have new tips at the begining of the project. Stainless steel tips are preferred.

- All tips on an aircraft spray system must be the same size unless specified otherwise.

- All spray systems and mixing tanks must be equipped to create and maintain suspendibility of the spray material.

- All aircraft spray systems must be characterized before operational use in a program. Characterization for swath width and droplet size is critical to the success of aerial application programs. Swath widths can be assigned using the tables in the APHIS guidelines or from recent data from spray trials.

Metereological Conditions During Application

- Average winds must not exceed 10 mph at ground level in an open area in or near the block.

- Spray application should not proceed if emitted spray does not penetrate the canopy.

- Relative humidity should be greater than 50%.

- Foliage should be dry or at least not be wet to the point of run off.

- If . 5 inches or more of rain occurs within 24 hours, retreatment is recommended.

-If retreatment is necessary because unexpected heavy rains have washed off the initial application, the Forest Service will cost share a second application of the same insecticide.

Spray Deposit Assessment

-The insecticide application must be monitored to ensure the spray is reaching the target area. This can be accomplished through arerial observation (chase planes) or ground monitoring during the time of application.

Dosage/Rates of Bt Application

- At least 12 BIU's/acre must be used.

- At least 96 oz/acre must be applied.

- We recommend up to 2 applications of Bt for suppression.

- We encourage the use of stickers with Bt to reduce rain wash off. However, some commonly used stickers can be harmful to car finishes and this should be a consideration regarding their use in residential areas.

- Formulations should not be made up until just prior to application and, if not used, refer to label guidelines for storage.

Biological Parameters for Bt

- Fifty percent of the larvae should be in the second instar with most of the remainder in the first instar.

- Target foliage should be greater than 30 percent expanded at the time of application.

- If a second application is planned, it should be applied 7 ($\pm$3) days after the first treatment depending on population density. If retreatment is needed because of heavy rain, it should be applied as soon as conditions are acceptable.

If larval development and foliage expansion are not in concert, larval development usually will dictate when the spray should be applied.

General

- The Forest Service will not fund ground applications of any insecticides.

- Radio contact between the airport, spray aircraft, chase planes and ground crews has proven to be essential to well managed suppression projects. Radio contact provides the opportunity to correct operational problems, suspend application during adverse weather conditions and improve the rescue opportunities during emergencies.

In addition to the guidelines, the committee made the following recommendations.

1. That training be provided for state personnel involved in organizing, managing and operating a Bt gypsy moth spray program.

2. That a standardized spray contract be developed as a guide for the different state agencies when negotiating for a spray contract (the spray contract developed for the state of Pennsylvania could be used as a model). The contract should also include clearly defined severe penalty clauses to permit its effective enforcement.

3. That the committee prepare and make available current swath width data tables for use when actual calibration trials cannot be conducted.

4. That the committee (or its appointee) collect all pertinent literature (publications, reports, references, etc...) concerning the use of Bt against gypsy moth.

Gypsy moth - Bt lab and field research activities presently in progress and being considered for the immediate future.

Priorities were established for Bt gypsy moth studies that are either presently in progress or are being contemplated for the immediate future. These studies are listed below in order of their decreasing priority as established by the committee.

Priority	Description
1st	Effect of droplet size on field efficacy of Bt
2nd	Studies on canopy penetration
3rd	Efficacy of different types of rotary atomizers
4th	Bt strain NRD-12 field efficacy
5th	Equipment development: a) dual boom system for inflight drop size changes b) develop a calibration system for nozzles c) develop collection devices for deposit efficacy.
6th	Laboratory studies on new formulations, including adjuvants such as stickers, feeding stimulants, etc.
7th	Determine larval distribution in the canopy at spray time
8th	Effectiveness of strip spraying
9th	Bt + "Holdup" combinations
10th	Bt + Dimilin combinations
11th	Effect of encapsulation on field effectiveness

In addition to these studies, another series of suggested studies considered important for improvement of use of Bt against gypsy moth, were proposed for future consideration. It should also be noted here that the committee recognized that some of these studies may well be in various stages of progress. These studies are also listed below in their order of priority.

Priority	Description
1st	Effect of formulations, water based vs oil based, neat vs diluted, on Bt effectiveness
2nd	Relationships between population quality and response to spray tactics
3rd	Pre-spray egg mass density vs Bt dose response (measured by larval reduction)
4th	Bt residual activity over time

5th	Effect of increased volume rate/acre
6th	Effect of flight height over canopy on Bt deposit
6th	Effect of foliage wetness on Bt field effectiveness
8th	Effects of foliage size and age on Bt effectiveness
8th	Development of a simple, quick and inexpensive technique for estimating Bt field deposit
10th	Follow up effect (on virus load) of Bt treatment in succeeding years
11th	Comparison between fixed wing aircrafts and helicopters on spray deposit efficiency
12th	Development of a simple, quick inexpensive technique for estimating wind speed above the canopy

Project Number: JB 4.1.1
Project Title: Evaluation of Traps and Other Techniques for Controlling Japanese Beetles in and Around Airports
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: W.H. McLane and J.A. Finney

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. For example, during 1986, aircraft inspections in California resulted in a total of 24 live beetles being found. A total of 3,627 live and dead beetles were discovered on aircraft originating from airports in the eastern United States. This was an increase of 3,599 over the number found in 1985.

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. Recently, at Dulles International Airport, Ron Addington developed a trap design that eliminates the handling of captured beetles. A tube sock, with the toe end cut off and treated with 5% Sevin dust (Sevin 80S), was 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 dust and mortality results. Beetles pass through the trap and fall to the ground, eliminating the need to empty traps. Socks are dusted with Sevin on a weekly basis or more frequently if rain occurrs. Laboratory and field studies have demonstrated the residual of some insecticides and/or formulations to be greater than that of Sevin 80S.

Trap tests during the 1985 season demonstrated a longer residual with Sevin XLR 5% and Malathion EC 5% than with the standard Sevin 80S dust at 5%.

In 1986 Sevin and Malathion continued to be tested with the sock type trap at the Otis Methods Development Center.

Table 1. Treatments applied to sock type Japanese beetle traps during the 1986 trapping season.

Material	Formulation applied
Sevin 80S	5% Dust
Sevin XLR PLUS	7% EC
Sevin XLR PLUS	5% EC
Sevin XLR PLUS	3% EC
Sevin XLR PLUS	1% EC
Malathion	5% EC
Malathion	3% EC
Standard Ellisco Trap	

An open grassy field, about 50 acres in size, was selected as the test site. Japanese beetle grub population in the area was approximately 15 grubs per square foot. Six parallel lines of traps were established so that traps were 50 feet apart in all directions. The bottoms of the collection cans of Ellisco traps were removed to make an opening for beetles to fall through. The toe end of tube socks (K-Mart) were removed and the ankle end pulled up completely over the perforated collection can. The open top of a cardboard container was attached to the end of each sock and fitted with a plastic funnel so that captured beetles were not able to climb back up into the treated sock. A container was placed into the ring top to collect all trapped beetles to estimate trap efficiency. In an operational program, all trapped beetles fall out the open end of the sock onto the ground. Traps were suspended approximately 3 feet off the ground by a steel rod. Each treatment was replicated 6 times with a treatment trap on each line. After each collection, traps were relocated on the lines.

On July 18, 1986, traps were put out and treated with insecticides. Dust was applied to the inside of the main trap funnel and outswide of sock by a small hand-held duster. Emulsifiable concentrates were applied from a hand-held pump-up garden sprayer to the sock and inside of the trap funnel to the point of complete saturation. All traps were baited with standard lure and pheromone purchased from Tracy Company. Six standard Ellisco traps (unaltered) were included as checks. Each trap was given a code number to identify the various treatments.

As time and weather permitted, collection containers were placed onto each trap and beetles were collected and returned to the laboratory. Beetles were held in the original collection containers for 24 hours at 22°C and then mortality readings were made and recorded.

Beetle populations were so high that quart containers would fill up in 1-2 hours. Therefore, collection containers were placed on traps and removed after a period of only 20-30 minutes.

Table 2. Percent mortality of Japanese beetles 24 hours after capture in insecticide treated sock type traps over a period of time.

Material	Percent mortality days after trap treatment				
	4 days	6 days	10 days	14 days	18 days
Sevin 80S 5%	19	0	0	2	1
Sevin XLR(PLUS) 7%	47	33	75	98	46
Sevin XLR(PLUS) 5%	46	38	69	77	13
Sevin XLR(PLUS) 3%	43	10	58	9	1
Sevin XLR(PLUS) 1%	23	0	5	9	0
Malathion 5%	37	54	23	18	7
Malathion 3%	26	6	5	5	5
Ellisco Standard	13	0	4	2	0

Table 3. Percent mortality of Japanese beetles based on total of all beetles collected throughout the testing period.

Material	Totals for test period			Percent mortality
	Alive	Dead	Total	
Sevin 80S 5%	4047	165	4212	4
Sevin XLR 7%	1457	2059	3516	59
Sevin XLR 5%	1928	1615	3543	46
Sevin XLR 3%	2609	651	3260	20
Sevin XLR 1%	3063	340	3403	10
Malathion 5%	2448	777	3225	24
Malathion 3%	3316	433	3749	12
Ellisco Standard	3849	149	3998	4

Although the overall kill with Sevin and Malathion was not as dramatic as in 1985 tests, it was clear that the materials were much more effective than Sevin 5% dust.

At Dulles International Airport the beetle population appeared to be on the decline in 1986. However, a trapping program was again implemented to remove the threat of the beetle movement around aircraft. The trapping technique was again effective as Dulles was able to stay unregulated throughout the summer.

A total of 336 sock type traps were put into operation at Dulles in early July. Thirty traps were treated with 5% Sevin XLR PLUS and the remainder with standard Sevin 5% dust. A small hand duster was used to apply the dust and a pump-up (Solo) back-pack for Sevin XLR PLUS treatments. Dust was applied directly into the funnel part of the trap and XLR was applied into the funnel and on the outside of the sock. One quart of Sevin XLR PLUS, 5% liquid, serviced 25 traps.

By August 1st, it was obvious that Sevin XLR PLUS was far superior to Sevin 5% dust. At that time all traps were treated with Sevin XLR PLUS. In 1987 all traps will receive the Sevin XLR PLUS.

Field personnel at Dulles felt that considerable time was saved with Sevin XLR PLUS. They reported that beetles did not hang on the outside of traps as they do with Sevin dust treated traps. Large piles of dead beetles were observed under all Sevin XLR PLUS treated traps. They had no problems in the mixing, handling, or application of materials.

Project Number: GM 5.2.3
Project Title: Sterile F_1 Demonstration Project - Bellingham, WA
Report Period: October 1, 1985 - September 30, 1986
Project Leaders: V. C. Mastro, C. P. Schwalbe, and A. Pellegrini-Toole
Project Cooperators: Eric Lagasa, Washington State Department of Agriculture

This project was initiated in 1985 when 34,000 F_1 egg masses were released in an isolated sparse gypsy moth population. A description of the site, and details of the release and some preliminary evaluations were presented in the last annual report (GM 5.2.3, pp 98-107). In 1986 an additional release of F_1 egg masses was made at the site. Following is a description of the complete 1985 results and the preliminary results of the 1986 evaluation.

Last year's laboratory report presented a partial tabulation of the data generated by placing monitor F_1 females in 1985. In all, 830 egg masses were recovered from monitor females, 5 of which had characteristics of a wild type male parent while 88 had the characteristics of a mating involving a F_1 male parent. In addition, 734 egg masses contained all unembryonated eggs (e.g. either the result of no mating or a mating involving an F_1 male parent) and 3 egg masses had characteristics that, when tested statistically against control values, could not be separated from either mating type. Computed overflooding ratios were 17.6 F_1:1 wild and 164.4 F_1:1 wild, respectively, excluding and including egg masses with no embryonated eggs (Figures 1 and 2).

Figure 1 presents the seasonal occurrence of F_1 and wild type matings produced by monitor females and the numbers of males captured in pheromone baited traps. Matings of monitor females by wild type males occurred throughout the flight period (e.g. Julian dates 208, 220 and 224) and apparently there was relatively good synchrony of activity of the F_1 and wild adults. Generally, the largest proportions of monitor females that produced egg masses containing unembryonated eggs occurred on days when the smallest numbers of males were captured (Figure 2). Conversely, on days when the largest numbers of males were captured (Julian date 224) fewer monitor females produced an unembryonated egg mass. Undoubtedly, some monitor females producing unembryonated egg masses had mated with F_1 males, but, in the 1985 data, it was impossible to separate these from females which did not mate. In 1986, in an effort to distinguish which monitor females producing unembryonated egg masses had mated with F_1 males, reports were kept on when oviposition was initiated (i.e. before or after the female was recovered). Unmated females, unless stressed, normally do not begin to oviposit until two or three days after eclosion. Monitor females are placed when one day old (i.e. the day after eclosion) and recovered the same day or the following morning. Thus, if females were found ovipositing at the time of recovery, this was taken as presumptive evidence she was mated.

Results of sampling female larvae and pupae in 1985 are in agreement with data generated from evaluations of collections of immature males. Of the 372 field collected females reared to the adult stage and mated with normal males in the laboratory, 309 produced egg masses which could be evaluated for sterility. Females producing egg masses characteristic of a fertile male x F_1 female mating type totaled 241 while 9 produced egg masses which had characteristics of a fertile male x fertile (i.e. wild) female mating type. Of the remainder,

49 females produced egg masses containing all unembryonated eggs and 12 females produced egg masses which statistically could not be separated from either of the above mating types. Theoretically, because F_1 egg masses produce a skewed sex ratio of 2 males:1 female, the female ratio in the field should be approximately one half of the male ratio. Computing a female ratio from these data results in a 26.8:1 F_1:wild ratio, n=250. This is approximately one half of the ratios reported earlier for male evaluation (i.e. chromosome evaluation, 57.3S:1F and mating-egg mass evaluation 62S:1F). A tabulation of females determined to be fertile is presented in Table 1.

Table 1. Date of collection and location of females that were determined to be fertile "wild" (based on laboratory mating and egg mass evaluation), Bellingham, WA. 1985.

Collection date	Location
120	Medical Center
130	1400 Maplewood
164	1402 Oregon
165	1421 Victor
175	3021 Kulshan
176	3021 Walnut
183	3011 Elm
193	3019 Walnut
200	2926 Elizabeth

The ratio of males to females in larval and pupal collections throughout the season remained skewed in favor of males (Figure 3). One would expect just such a skewed ratio from past studies of F_1 gypsy moths.

Within the 9 square mile grid of traps (36 traps/sq mi.) which was centered over the F_1 release area, 869 males were trapped in 1985. Of these, 745 were captured in 12 traps which covered the area of the release. The pattern of remaining captures generally circumscribed a pattern of catch within two inter-trap lines around the release site, however, there were a few single catches radiating from the release site to the northeast and one single male was caught on the outer line in the northeastern part of the grid (e.g. approximately 1.5 miles from the center of the release area). Also one male was captured along the extreme western edge of the grid. Males were also captured in trap grids surrounding the 9 square mile area; 2 single males were captured to the west of the release site and a single and one trap with 2 males to the southwest and 3 singles to the south.

After the 1985 flight season the entire release area was searched for egg masses. A total of 63 egg masses were found and shipped to the Otis Methods Development Center for evaluation. These eggs can potentially be the progeny of four mating types:

1. F_1 male x F_1 female
2. F_1 male x wild female
3. wild male x F_1 female
4. wild male x wild female

Mating types 1 and 2 (i.e. those types involving an F_1 male parent) cannot be separated either by comparing the proportion of the eggs which are embryonated or hatched. Types 3 and 4, however, can be separated from each other and from mating types 1 and 2.

One of the 63 egg masses collected was determined to be from a type 4 (fertile male x fertile female) mating. Characteristics of 7 egg masses were of type 3 matings, and 36 egg masses were of pairs involving a F_1 male parent (i.e. type 1 or type 2). In 19 cases the male parent could not be determined but the female parent was an F_1. Computing mating ratios from the above data provide the following: sterile to fertile male ratio 5.1:1 (n=43) and sterile to fertile female ratio 26:1 (n=27), the female ratio being almost exactly the same as computed from larval collections. The male ratio, however, is much lower than predicted from both mating-egg mass evaluation and chromosome analysis. It should be noted that although in 19 cases the male parent could not be determined, in all cases embryonation was very low (below 30 percent) and the male parents were probably F_1 steriles. This low male ratio projected from the post-season egg masses is unexplained.

Overall, the ratio of sterile egg masses (where at least one parent was an F_1) to fertile egg masses (both parents fertile wilds) was 62:1.

1986 Program

The purpose and expected impact of the 1985 release in the Bellingham site was suppression and not eradication (reasons are given in the last annual report). Based on preliminary evaluation data from 1985, 12,769 F_1 egg masses were released on April 7, 1986. The objective in 1986 was eradication. Egg masses were distributed so the heaviest concentrations were at sites where 1985 male larval and pupal samples had found wild insects and where in the spring of 1985 wild egg masses were found. Samples from all production lots of eggs were held in a small cage at the site and hatch from these was regularly checked. Because no wild egg masses were available for sampling in 1986, egg hatch synchrony cannot be compared.

During 1986 it was only possible to collect small numbers of immature insects (total=93). Five males from the larval collection were dissected for chromosome evaluation and all were F_1's. Twenty-seven males were reared to the adult stage for mating-egg mass evaluation of which 24 pairs produced egg masses characteristic of F_1 males and 1 pair produced an egg mass characteristic of a wild male parent. One of the remaining pairs produced an egg mass which could not be separated from either mating type and the other failed to produce an egg mass. Data from field collected immature females reared and held for mating egg mass evaluation are not complete and will be presented in the next annual report.

As in 1985, monitor F_1 females were placed throughout the flight period. Sixty-seven of these females produced egg masses which were characteristic of an F_1 male x F_1 female mating and an additional 49 produced egg masses with no embryonated eggs but the female had begun to oviposit by the time she was recovered. No monitor females produced an egg mass characteristic of a fertile male mating although one produced an egg mass which could not be separated statistically from either mating type. An additional 117 females produced totally unembryonated egg masses after the female was recovered from the field and 146 failed to produce any egg mass.

Traps were again placed in 1986 to monitor male flight. In the 12 core traps which covered the F_1 release area 209 males were captured in 1986. This is ca. 28% of the number of males captured in the same traps in 1985 (i.e. n=745). This roughly corresponds to a reduced F_1 release in 1986, ca. 35% of the number released in 1985 (i.e. 1985, 34,000 F_1 egg masses, 1986, 12,769 egg masses). Within the nine square mile area a total of 244 males were captured. Projections from 1985 and 1986 indicate that the overflooding ratios achieved should have reduced the native population in Bellingham to the point of eradication. In 1987 the site will be monitored with the same array of traps used in previous years to determine if the objective was achieved.

Figure 1. MONITOR FEMALE AND MALE TRAPPING DATA FROM A PILOT STUDY IN BELLINGHAM,WA.1985

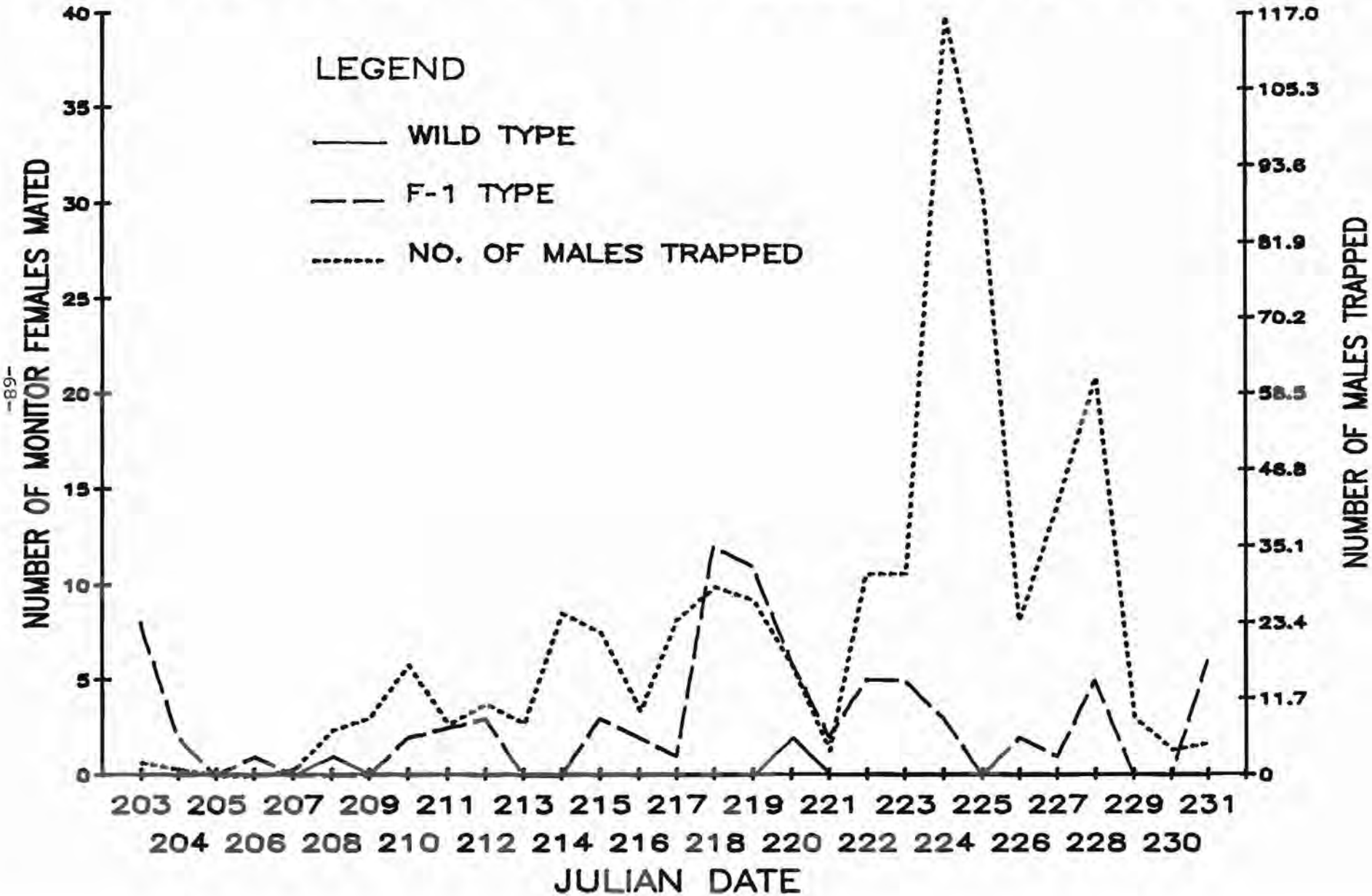


Figure 2. MONITOR FEMALE AND MALE TRAPPING DATA FROM A PILOT STUDY IN BELLINGHAM, WA 1985

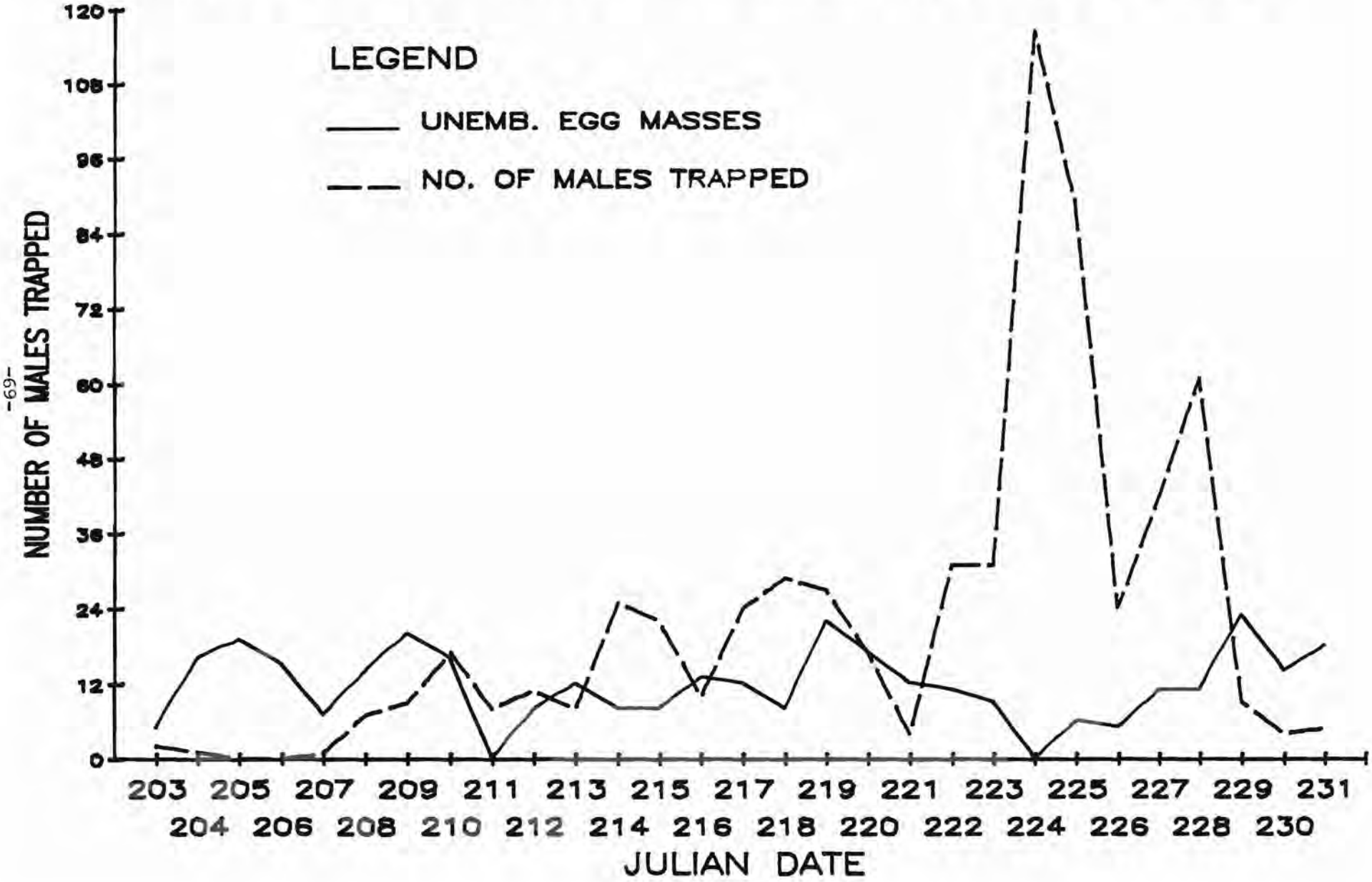
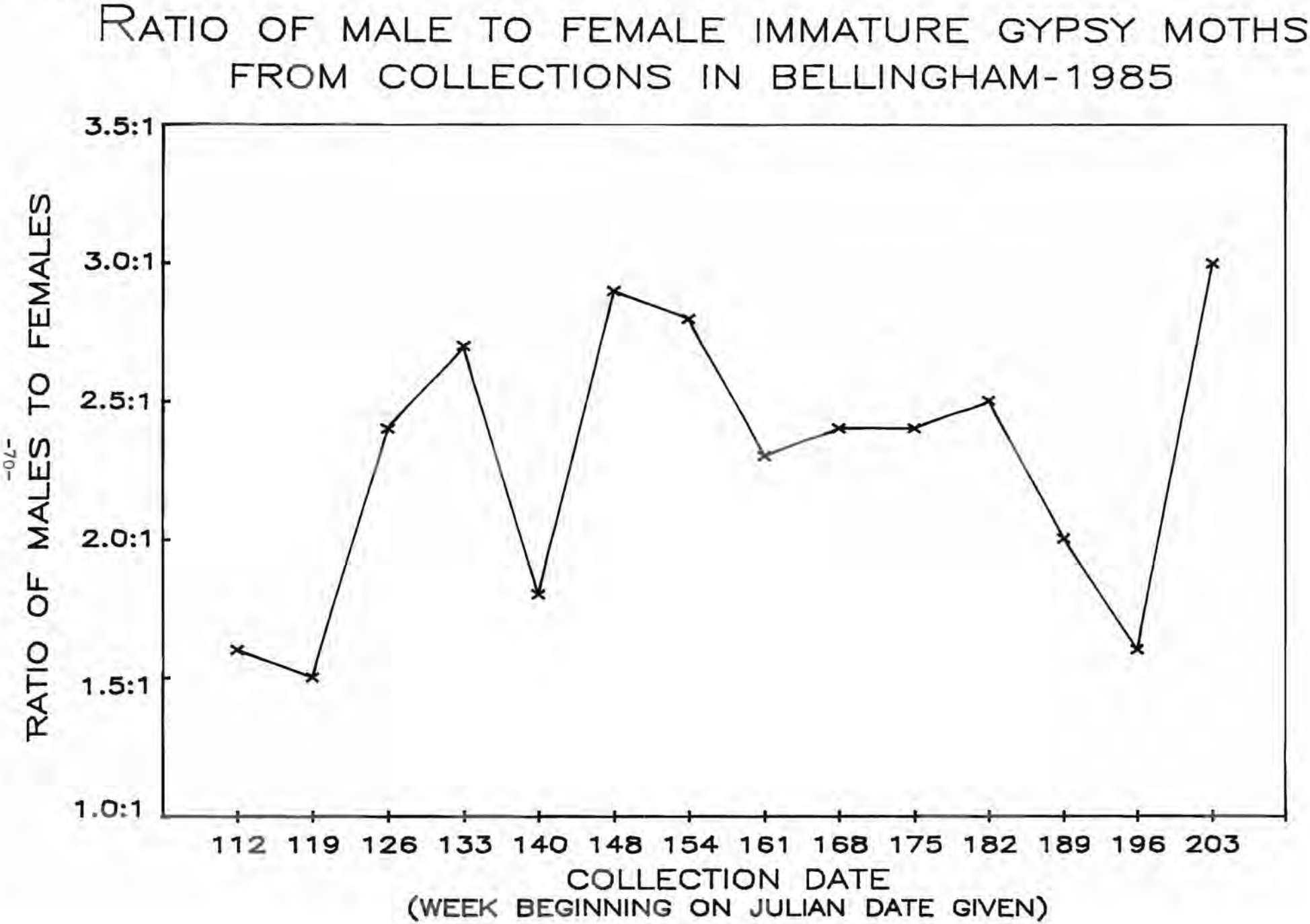


Figure 3.


RATIO OF MALE TO FEMALE IMMATURE GYPSY MOTHS
FROM COLLECTIONS IN BELLINGHAM-1985
RATIO OF MALES TO FEMALES
3.5:1
3.0:1
2.5:1
2.0:1
1.5:1
1.0:1
112 119 126 133 140 148 154 161 168 175 182 189 196 203
COLLECTION DATE
(WEEK BEGINNING ON JULIAN DATE GIVEN)

Project Number: GM: 5.2.4
Project Title: Sterile F_1 Demonstration Project - Darke County, Ohio
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: V. C. Mastro, C. P. Schwalbe and A. Pellegrini-Toole
Cooperators: R. Barth, K. Roach, and D. Gerold

A release of F_1 sterile insects was conducted at the Darke County site in 1985. Preliminary results in 1985 indicated that no further release was necessary in 1986. Details of this release and the preliminary evaluations were presented in the 1985 Annual Report (GM 5.2.4). The following report summarizes the remainder of the 1985 data and the results of monitoring the site in 1986.

Detailed in the last report are the overflooding ratios calculated from males collected as immatures; both from those dissected for chromosome evaluation (187S:1F, n=188) and for males reared to the adult stage and mated for subsequent egg mass evaluation (395.5S:1F, n=792). Females, were also collected as immatures and reared to the adult stage, when they were mated to normal males for egg mass evaluation. Because those crosses needed to be held for hatch to determine degree of sterility, the results are reported here, instead of in the 1985 report. In all, 369 pairs were mated using field collected immature females and normal laboratory reared males. Sixty of these pairs did not produce an egg mass. Of the remainder, evaluation of the egg masses found that 11 pairs had a fertile female parent while 282 pairs produced egg masses which indicated the female parent was sterile. Of the 282 pairs which were designated sterile only 97 produced egg masses which contained embryonated eggs. The remainder produced egg masses containing all unembryonated eggs which can be the result of no mating or a mating involving an F_1 female parent. In addition, the characteristics of egg masses from 16 pairs could not be distinguished from either of the two mating types (i.e. normal male x wild female or normal male x F_1 female). A calculated overflooding ratio based on all pairs which produced egg masses is 282S:11F (i.e. 25.6:1) and a ratio based only on egg masses containing at least one embryonated egg is 97S:11F or 8.8:1. The overflooding ratios established from male evaluations (e.g. chromosome analysis or mating evaluation) indicate that this female F_1 to wild ratio is low. Theoretically, the ratio of F_1 females to wild females should be approximately one half of the male ratio because of a skewed sex ratio (e.g. 2 males:1 female) resulting from F_1 egg masses. Using data from male chromosome analysis one would expect a 93:1 ratio of F_1:wild female immatures. Data from an F_1 release made in Sandwich, Massachusetts in 1985, however, indicated a slow decay of the proportion of F_1 females over the course of the larval and pupal development period while male overflooding rates held nearly constant. Seasonal analysis of the female data from Darke County in 1985 is difficult because of the small sample size and the small number of fertile insects, however a higher proportion of fertile females were found in the collections from later in the season (Figure 1).

Three hundred and thirty one egg masses were recovered from monitor females which were placed during the 1985 flight period. Of these, 7 were determined to be wild while 300 were determined to be sterile. Those classified as sterile include both egg masses which contained some embryonated eggs (n = 54) and those containing no embryonated eggs. In addition the degree of

embryonation in 24 egg masses was such that they could not be statistically separated from either mating type (e.g. F_1 male x F_1 female or wild males x F_1 female). An overflooding ratio calculated using all egg masses determined to be sterile is 300S:7F or 42.9S:1F and is, again, lower than earlier observed male overflooding rates. In addition 472 monitor females failed to produce any egg mass.

In August of 1985, after adult male flight, 16 "wild" post-season egg masses were recovered. These egg masses were held for hatch and evaluation. Potentially, these egg masses can be from four different mating types.

1. F_1 male x F_1 female
2. F_1 male x wild female
3. wild male x F_1 female
4. wild male x wild female

Separation of egg masses resulting from the first two types of matings is not possible, however, the third and fourth types can be separated from each other and from types 1 and 2.

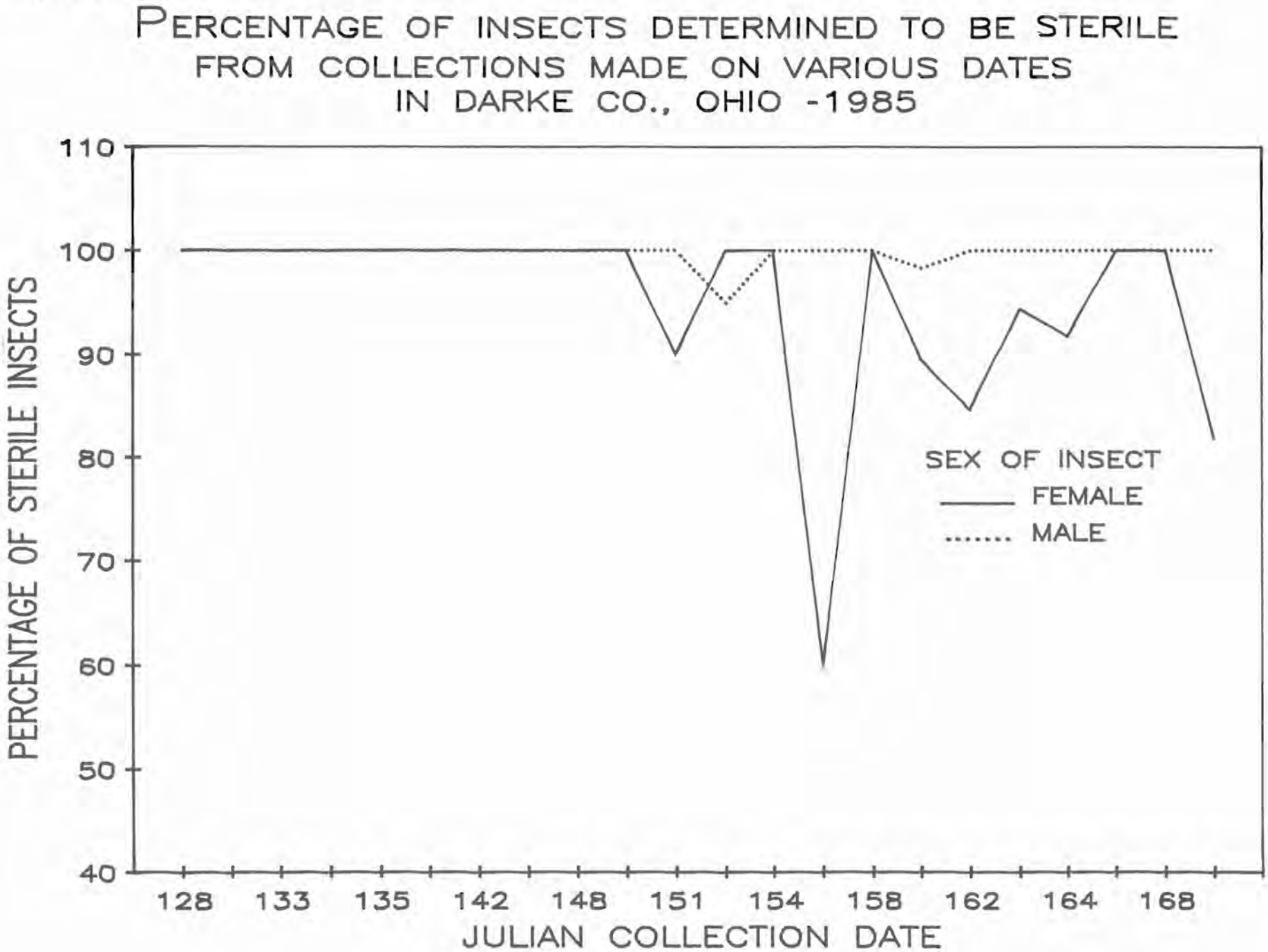
Based on the proportion of embryonated eggs in individual masses, of the 16 egg masses collected, 5 could be classified as belonging to either groups 1 or 2 (i.e. the two groups with an F_1 male parent) and 9 classified as having a wild male parent. Two egg masses could not be classified as either having an F_1 or a wild male parent. Using the proportion of embryonated eggs which hatched the 9 egg masses which were classified as having a wild male parent and the 2 egg masses with unknown male parentage were separated into mating types 3 and 4. Nine of these egg masses were classified as having an F_1 female parent (i.e. mating type 3 - wild male x F_1 female), one was classified as having a wild male parent (wild male x wild female) and one could not be clearly separated from either mating type.

The outcome of the evaluation of the post season egg masses is in conflict with results of all other monitoring techniques. If only post season egg mass results are considered, the male overflooding ratio would be calculated as 5 F_1 males:9 wild males and the remale overflooding ratio would be calculated as 9 F_1 females:1 wild female. In other studies conducted within the endemic gypsy moth area results are similar, e.g. the results of post-season egg mass evaluation do not agree with results of other monitoring techniques. In the case of Darke County data this may simply be due to sample size. However, some as yet unexplained mechanism may favor the longevity in the field of egg masses with a wild male parent. This disparity in results of monitoring, using different techniques, is not apparent from the other isolated sites.

In 1986, as in 1985, the site was again monitored with pheromone baited milk carton traps placed on a grid (36 traps/sq mi., 880 ft. inter-trap distance). Two males were captured in a single trap which was located in the center of the 1985 release area and near the site of the wild egg mass finds in 1984. One male was found on Julian date 191 and the second on 194. It is unknown if these were insects native to the site or if they were associated with the truck stop and roadside park within the release site. Plans for 1987 are to again monitor the site using the same trapping density.

Although all the estimates of the overflooding ratio do not entirely agree, the trapping of only two males in 1986 with an intensive trapping grid (36 traps/sq. mi.) is a marked reduction in insect density from the 97 males captured in 1984 when a lower trap density was used for delimitation and when 27 wild egg masses were located. Monitoring in 1987 may establish whether the infestation was eradicated or if additional treatment is necessary.

Figure 1.


PERCENTAGE OF INSECTS DETERMINED TO BE STERILE
FROM COLLECTIONS MADE ON VARIOUS DATES
IN DARKE CO., OHIO -1985
PERCENTAGE OF STERILE INSECTS
110
100
90
80
70
60
50
40
128
133
135
142
148
151
154
158
162
164
168
JULIAN COLLECTION DATE
SEX OF INSECT
FEMALE
MALE

Project Number:	GM 6.2.1
Project Title:	Sterile F_1 Demonstration Project - Southern Shores, Dare County, North Carolina.
Report Period:	October 1, 1985 - September 30, 1986
Report Type:	Interim
Project Leaders:	V. C. Mastro, A. Pellegrini-Toole and C. P. Schwalbe
Cooperators:	H. Singletary, K. Wilson, W. Westcott, K. Hicks and T. ODell

The objectives of this demonstration project were to:

1. Demonstrate the utility and efficiency of the sterile F_1 technique when applied to an isolated population.

2. Gather and compare information about the establishment, growth, and survival of F_1 sterile and wild insects.

Methodology and protocols followed in this demonstration project were similar to other F_1 demonstration projects (GM 5.2.3, GM 5.2.4, GM 6.2.2, GM 6.2.3 and GM 6.2.4). The site of this particular project offered a unique opportunity for gathering information about the competitiveness of F_1 sterile insects and the gypsy moth in general. The release site is as far south as we have ever released sterile F_1 insects, providing an opportunity for observation of gypsy moth establishment and feeding on various southern species of shrubs and trees. Also, in all of the isolated sites where F_1s have been released to date, the average temperatures during the early development period were higher than at this site producing input on the competitiveness of F_1 insects under these elevated temperature conditions.

Over several years, trapping in and around the Southern Shores, Dare County site had indicated that there was a reproducing population in the area, however, these earlier surveys failed to delimit the population. In 1985, trapping succeeded in capturing 167 males in an area where, subsequently, egg masses were found. Past experience with gypsy moth trapping in this area and, indeed, all along the Outer Banks of North Carolina has shown that at times large numbers of males are captured which are not considered to be native to the area. Separation of native males from those presumed migrants has been based on the timing of capture. In theory, seasonal phenology would be accelerated with the high early season temperatures experienced in this area compared to further north. This should result in an earlier adult flight period. Frequent checking of traps is employed so that the influx of migrants can be identified. Males captured after the influx occurs are considered suspect migrants; those before are considered natives. Based on this information: of the 167 males captured in 1985, 70 were captured before July 4, and were considered natives and the remaining 97 were considered suspect migrants. Egg mass surveys during the fall and winter succeeded in locating 7 egg masses and one additional egg mass was found in the spring of 1986.

To estimate overflooding ratios that would be achieved by releasing various numbers of F_1 egg masses an estimate of the native population is necessary. For this estimate, we assumed that the 70 males captured in 1985 before July 4th represented 40% of the total male population. There are several reasons why this estimate may be in error: 1) traps were not placed on a grid, 2) core traps (those where egg masses were later found later) were not in place when

flight began, 3) all males captured after July 4th may not be migrants, and 4) a capture rate of 40% is too high for the actual trap density. However, based on a 40% capture rate and 70 males captured, an estimate of the native population was 175 males and, assuming a 1:1 sex ratio, 175 females. Assuming that all 175 females produced viable egg masses, the spring egg mass population in Southern Shores was estimated at 175. To achieve a sterile:fertile ratio of 100:1 would require approximately 43,750 F_1 sterile egg masses. On March 19, 1986 we released approximately 46,000 F_1 egg masses in Southern Shores. These egg masses were distributed in a manner which concentrated the greatest densities of F_1s in the area where male trap catches indicated the wild population was concentrated, "core area" (Figure 1). As distance from the core area increased, density of distribution of F_1s was decreased.

To monitor hatch synchrony of wild and F_1s, egg mass samples of all production lots of F_1s and three available wild egg masses were placed in a small wire shelter at the time of F_1 release. These samples were checked daily and any hatching neonates were removed and recorded.

To facilitate larval sampling, trees on a grid system were banded with burlap throughout the release area. Larval development and overflooding ratios were monitored by collecting samples from under burlap bands, recording the stage at collection and then shipping the insects to the Otis Methods Development Center (OMDC), where samples were processed and typed (i.e. sterile F_1 or fertile wild determined). Normally 100, and after May 19th, 200 immature insects were collected per week.

Males were evaluated for sterility using two techniques. Male larvae up to the stage in their development where the 4/5 instar ecdysis was reached could be dissected for chromosome analysis, or reared to the adult stage and mated with normal females after which sterility was evaluated based on proportion of egg mass embryonation. The strain of all males beyond the 4/5 ecdysis was determined using the mating/egg mass evaluation technique. The strains of field collected females was determined based on rearing to the adult stage, mating to normal males and subsequent egg mass evaluation.

Additional monitoring of overflooding ratio was provided by placing F_1 virgin, 1-day old females in small mating shelters throughout the area. In all, thirty monitor females sites were established; twenty-five were placed in the core area and five additional sites were established in an area where chromosome analysis of early collections of male larvae indicated there was a wild population. Females were placed as early in the morning as possible and recovered the following morning. If a female had begun to oviposit by the time she was recovered, this was noted and any portion of an egg mass was also recovered. Females, after recovery, were placed in containers for oviposition and held an additional 48 hours. All egg masses were shipped to OMDC for evaluation of mating type.

A grid (36/sq. mi.) of disparlure (Hercon wicks containing 500 ug) baited milk carton traps were placed over approximately a nine square mile area centered over the release site. Traps in the F_1 release area were checked as often as possible, usually daily, and as distance from the release area increased, checking frequency decreased. By monitoring adult captures, the seasonal pattern of adult flight could be defined and if infestation did occur outside of the treated area (if there was more than one infestation or the boundaries drawn were too conservative) the pattern of captures would indicate that there was additional infestation.

Results and Discussion

Monitoring of egg mass samples revealed that hatch from F_1 egg masses initiated and peaked well before hatch from wild egg masses (Figure 2 & 3). This asynchrony of hatch was probably due to high temperatures on the days following release. F_1 eggs which had completed diapause began to hatch almost immediately in response to these temperatures. Percentage hatch computed from data collected from the egg mass samples averaged 37.02%. This amounted to 93% of the expected hatch rate (i.e. 40%). The observed hatch rate should have provided nearly the numbers of neonates required to obtain the desired overflooding ratio in the adult stage. However, at the time when hatch of F_1 egg masses was peaking, very little new foliage was available for establishment with the exception of willow (Fraxinus) and possibly muscle wood, Carpinus caroliniana. How this scarcity of new foliage affected establishment of F_1 neonates and, eventually, adult overflooding ratios is unknown.

Larval collections began on April 18th. Initially 100 samples of early instar larvae were collected weekly from foliage. As the season progressed and large larvae began to use the burlap bands, samples were collected from under bands and the sample size increased. In all, 1423 larvae or pupae were collected. We attempted to dissect as many of the males collected as early instar as possible. In all, 161 were dissected for chromosome analysis, 151 of which were determined to be sterile and 5 of which were determined to be fertile (wild). The calculated sterile:fertile overflooding ratio is 30.2:1 or approximately 97% of the dissected larvae were F_1s.

Following is a list of sites where insects determined by chromosome analysis to be wild were collected.

Collection date (Julian)	Insect number code	Grid point
120	157	L03-1
132	340	M16
139	403	L03
139	531	N6
148	593	L03-1

It should be noted that after the initial find of a wild male at grid point L03 (which was well outside of what was considered the core infested area) larval samples were collected from this area regularly instead of the planned random sampling as was conducted in other non-core areas. Undoubtedly, higher numbers of fertile insects were ultimately collected from this area is because of this intensive sampling.

An additional 474 males were reared to the adult stage and back mated with normal females for strain determination by egg mass evaluation. Evaluation of

these resulting eggs identified the male parents of 30 of these matings as wild, 419 as sterile and 9 being of unknown parentage. In addition, 16 pairs failed to produce any egg masses. Evaluations of egg masses produced by pairs with unknown male parents and normal laboratory-reared females are based on the proportion of the total eggs which are embryonated. Due to the large variance around the mean proportion of embryonation for both mating types (i.e. those involving an F_1 or fertile male) there is a range of values where, statistically, an egg mass cannot be separated from either mating type. Thus 9 egg masses of unknown parentage were lost from the evaluation as falling in this range. A summary of the males determined by egg mass evaluation to be wild is presented in Table 1.

Table 1. Males collected as immatures and determined to be fertile by using mating egg mass evaluation techniques.

Collection date (Julian)	Insect number code	Grid point	Stage at collection 1/
154	997	D-23	5
148,160	653,1177	G-9	4,P
153	950	I-22	5
153	947	I-17	P
148	619	JO-3	4
152	979	JO-5	5
153,153,158	818,835,1290	J-8	P,P,P
168	1333	J-14-2	P
160	1068	K-6	P
139,168	488,1341	K-9,K-9-2	5,P
139,148,148	526,750,758	K-10,K-10-3,K-10-3	4,5,5
139	411	LO-3	4
153	859	LO-3-9	5
153	856	LO-3-15	P
160	1004	LO-3-19	P
154	981	LO-5	5
160	1139,1140	M-24	P,P
177	1395	M-9	P

1/ Numbers refer to the larval instar, P refers to the pupal stage.

Some pairs produced egg masses which contain no embryonated eggs and these could be the result of no mating or a mating with a F_1 male parent. Following are two estimates of overflooding rates computed by either including or excluding these unembryonated egg masses.

F_1:wild = 14:1 (n=449) includes egg masses containing no embryonated eggs
= 6.7% fertile

F_1:wild = 11.3:1 (n=369) includes only egg masses containing greater than 0 embryonated eggs
= 8.1.% fertile

Immature field collected female larvae were reared to the adult stage and individually mated with normal laboratory males. For determining the type (i.e. F_1 or wild female) of unknown parent egg masses from these matings they must be held for hatch. F_1 females when mated with normal males produce highly embryonated egg masses indistinguishable from normal egg masses, except when they do not hatch. Due to the necessity of satisfying embryonation and diapause requirements this evaluation (ca. 180 days) is not complete at this time.

To monitor adult male mating ratios, females were deployed throughout the flight period. Monitors were only placed in areas with known wild insects (i.e. 25 monitor stations in the core area and 5 additional sites around LO-3 where wilds were found based on chromosome analysis results). Virgin F_1 females, 1-day old, were placed as early in the morning as possible and recovered the following morning. If oviposition had started at the time of pickup this was noted and the female and any portion of an egg mass was recovered as well. Females were held for an additional 48 hours for oviposition. All egg masses were shipped to the OMDC for evaluation. In all, 615 monitor females were placed through the season. Of these we failed to recover egg masses in 190 cases. The remaining 425 egg masses were evaluated and separated for mating type using the following criteria:

1. Those egg masses which contained all unembryonated eggs were separated according to when oviposition had been initiated as follows:

    A. if oviposition had been initiated at the time of recovery they were considered sterile.
    B. if oviposition was initiated in the 48 hours after recovery they were considered the result of no mating.

2. Those egg masses which contained at least one embryonated egg were evaluated for mating type based on the percent embryonation. This observed embryonation was compared statistically with control values for the two possible mating crosses (i.e. F_1 male X F_1 female or fertile male X F_1 female). This procedure results in three classifications:

    A. sterile
    B. fertile
    C. unknown.

The "unknowns" are those which could not be statistically separated from one or the other mating type. Percent embryonation of egg masses from this study that fell into the unknown category ranged from 13.4 to 32 percent. The results of this evaluation follow:

1. egg masses containing no embryonated eggs

    A. sterile n = 96
    B. no mating n = 143

2. egg masses containing at least one embryonated egg

    A. sterile n = 124
    B. fertile n = 36
    C. unknown n = 26

An estimate of overflooding rate based on all egg masses considered sterile (i.e. 96 + 124 = 220) is 6.1 sterile:1 wild. However, if this ratio is based on only those egg masses which contained some embryonated eggs the estimate is lower, 3.4 F_1: 1 wild. The seasonal occurrence of egg masses classified in the different categories, described above, is printed in Figures 4,5 and 6. For comparison, numbers of males trapped throughout the season are plotted on the same charts.

If the two classifications of egg masses containing no embryonated eggs are compared with each other and with male trap captures (Figure 4) it can be seen that egg masses classified as sterile (i.e. originating from females initiating oviposition with in 24 hours of placement) occurred throughout the male flight period and those egg masses classified as resulting from no mating occurred at periods of low male activity particularly at the end of the flight season.

Fertile and sterile mating data are presented on Figure 5; generally both curves follow the data from the male capture distribution. Also, both mating curves hold a fairly constant relationship over the course of the season with no apparent asynchrony in the pattern of F_1 and wild emergence. Finally, Figure 6 presents a comparison of the two groups of matings classified as sterile.

In all, 4,621 males were trapped in our monitoring grid (36 traps/ sq. mi.) in 1986; 4,300 of these before the "traditional" July 4th cut off date for separation of native and migrant males. In 1986, observations by Bill Westcott indicated that the influx of males began earlier, June 26th. Using this date to calculate the number of local males captured results in a slightly lower number, 4097. The pattern of distribution of male captures was as expected, with the highest numbers being captured from the core area, and smaller numbers captured only a few trap lines beyond the release area.

Determining the exact impact of the F_1 release is nearly impossible. Factors mentioned previously, poor estimate of the native population, asynchronous hatch and difference in observed overflooding ratios, all complicate any estimate.

Computed overflooding ratios all vary with sampling technique. Of the overflooding ratios from male larval dissections and chromosome analysis which were used for most larvae collected before they had reached the 5th instar the highest was 30.2 sterile:1 fertile. The ratio computed for mostly late instar male larvae and pupae using egg mass evaluation was 14:1 and the sterile:fertile overflooding ratio computed using the monitor female data was the lowest at 6.1:1. Using a pairwise comparison, the results of these three techniques is presented in Table 2.

Table 2. A comparison of three evaluation techniques used for estimating overflooding ratios in Dare County, North Carolina, 1986.

	n	Percent fertile	Overflooding ratio 1/	
male dissection - chromosome analysis	156	3.21	30.2:1	a
male mating - egg mass evaluation	449	6.68	14:1	a
monitor female - egg mass evaluation	256	14.06	6:1:1	b

1/ Treatments followed by the same letter are not significantly different according to chi square analysis at the 5% level of significance.

A possible explanation for the differences in these overflooding ratios is that there was a gradual decay in the numbers of F_1 insects as the season progressed and this may be a factor. The overflooding ratio estimated by using the monitor female data is, however, intentionally biased. Monitor females were only placed within areas of known wild gypsy moth occurrence while larval sampling was conducted throughout the release site. The overflooding ratio established by this bias is helpful in projecting a "worst case" scenario.

Assuming that the 4097 males captured in the trapping grid represented roughly 34 percent of the male population (earlier studies with random releases of males in grids of 32 traps/sq. mi. captured a mean of 34.02%) then the actual male population was 11,979 or ca. 12,000. Furthermore, using the lowest estimate of overflooding ratio, 6.1 sterile:1 fertile, then the male population consisted of 1,690 wild males and 10,310 F_1 sterile males. Assuming a 1:1 sex ratio in the wild population, of the estimated 1,690 wild females, 238 and 1452 would have been mated with wild fertile and F_1 sterile males, respectively. To achieve a 100 sterile:1 fertile overflooding ratio in 1987 would require approximately 60,000 egg masses. It should be noted that if the wild male population was 1,690 in 1986, then there was nearly a 10 fold increase from our estimated 176 males in the 1985 population.

If a more optimistic estimate of overflooding ratio (i.e. 14:1) is used to make the previous calculations, then, of the 12,000 males in the population, 800 were wild. Again (assuming a 1:1 sex ratio) 800 wild females would have mated with 53 wild males and 747 F_1 males. Again, to achieve the 100:1 overflooding ratio would require 13,250 F_1 egg masses.

Due to the wide disparity in the above estimates, our intent in 1987 is to release a large enough number of egg masses in 1987 to achieve eradication based on our most conservative estimate (i.e. greater than 60,000 F_1 egg masses).

Figure 1.

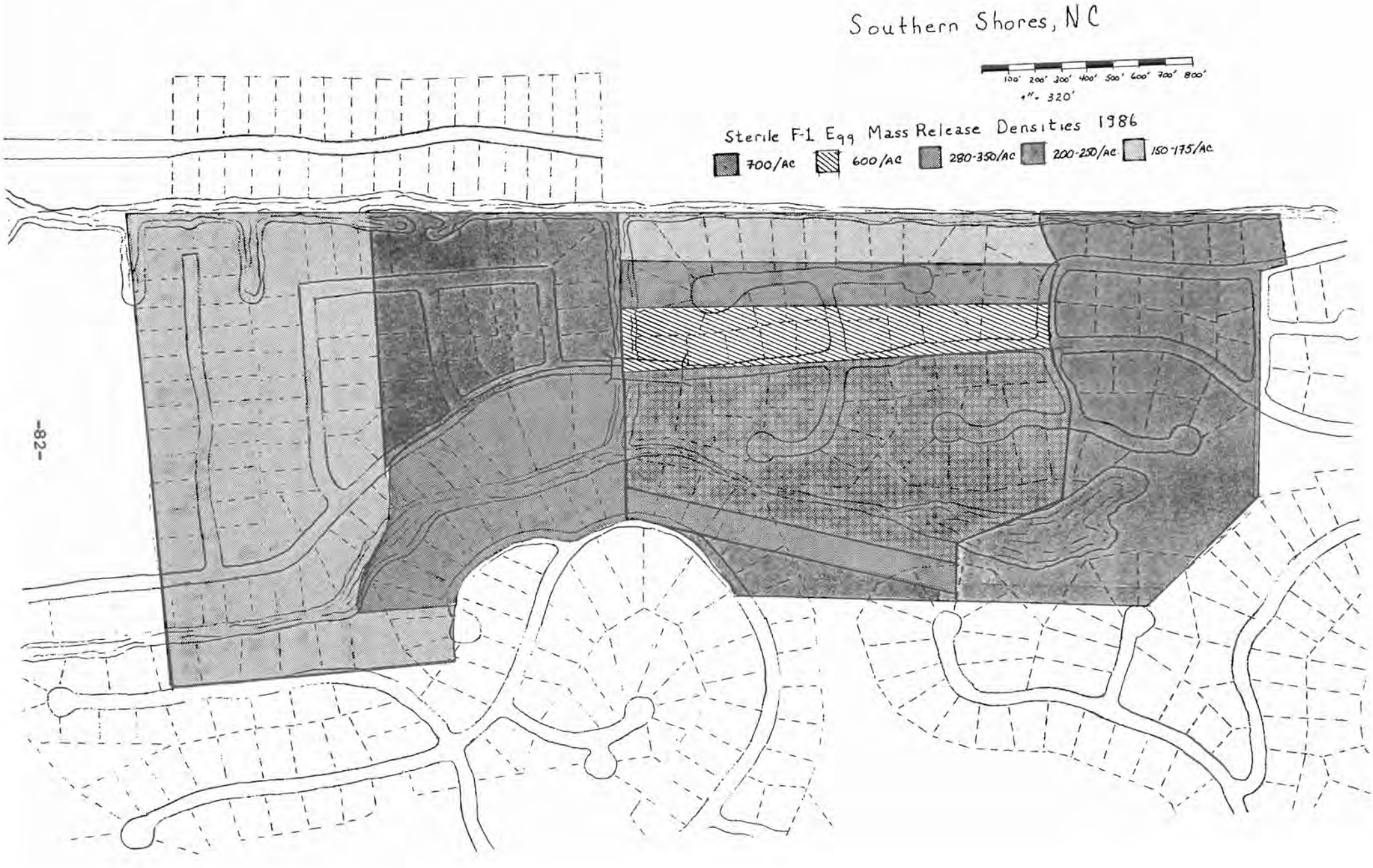

Southern Shores, NC
100' 200' 300' 400' 500' 600' 700' 800'
1" = 320'
Sterile F-1 Egg Mass Release Densities 1986
700/AC
600/AC
280-350/AC
200-250/AC
150-175/AC

Figure 2.

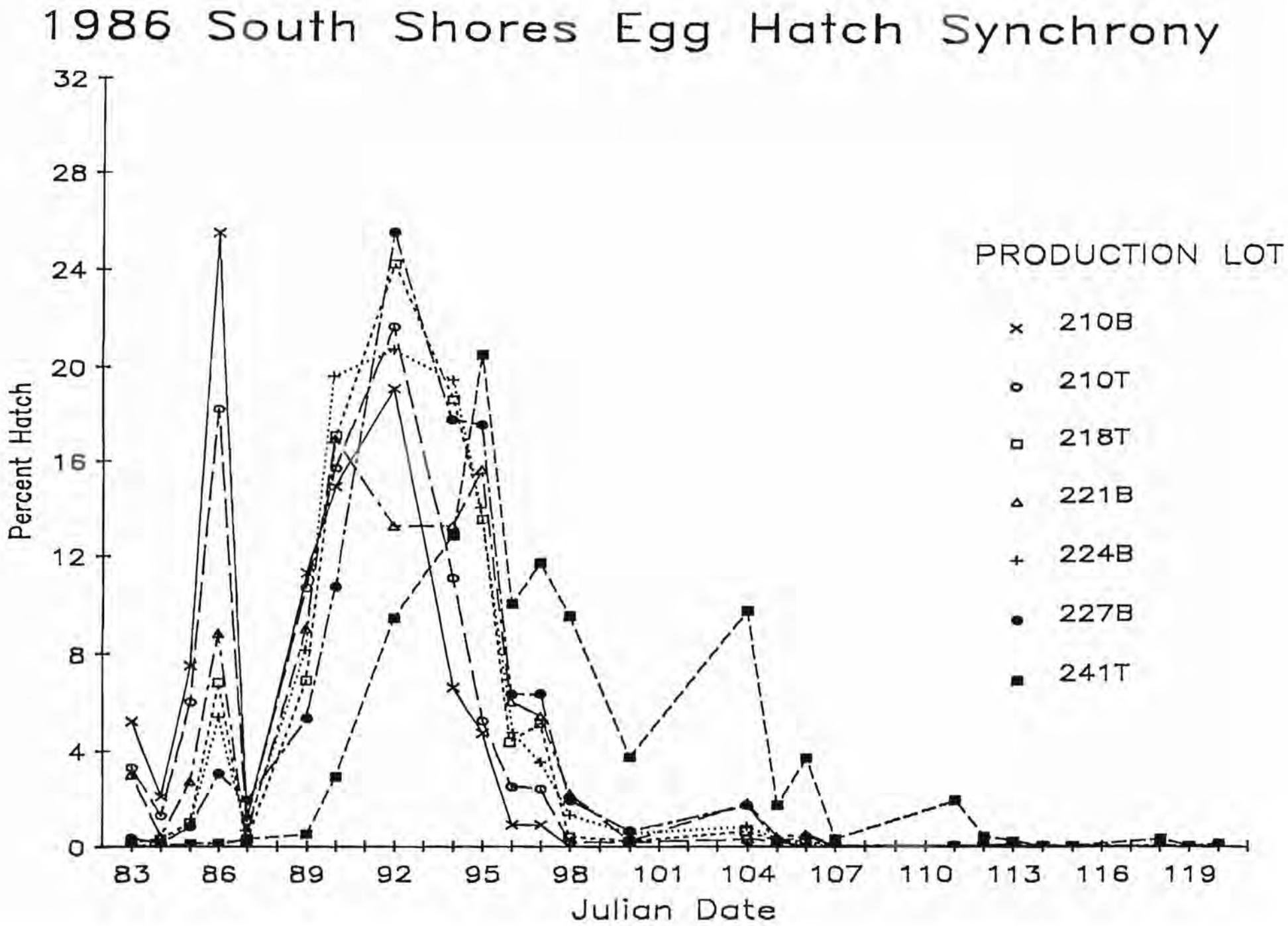

1986 South Shores Egg Hatch Synchrony
Percent Hatch
0
4
8
12
16
20
24
28
32
83
86
89
92
95
98
101
104
107
110
113
116
119
Julian Date
PRODUCTION LOT
210B
210T
218T
221B
224B
227B
241T

Figure 3.

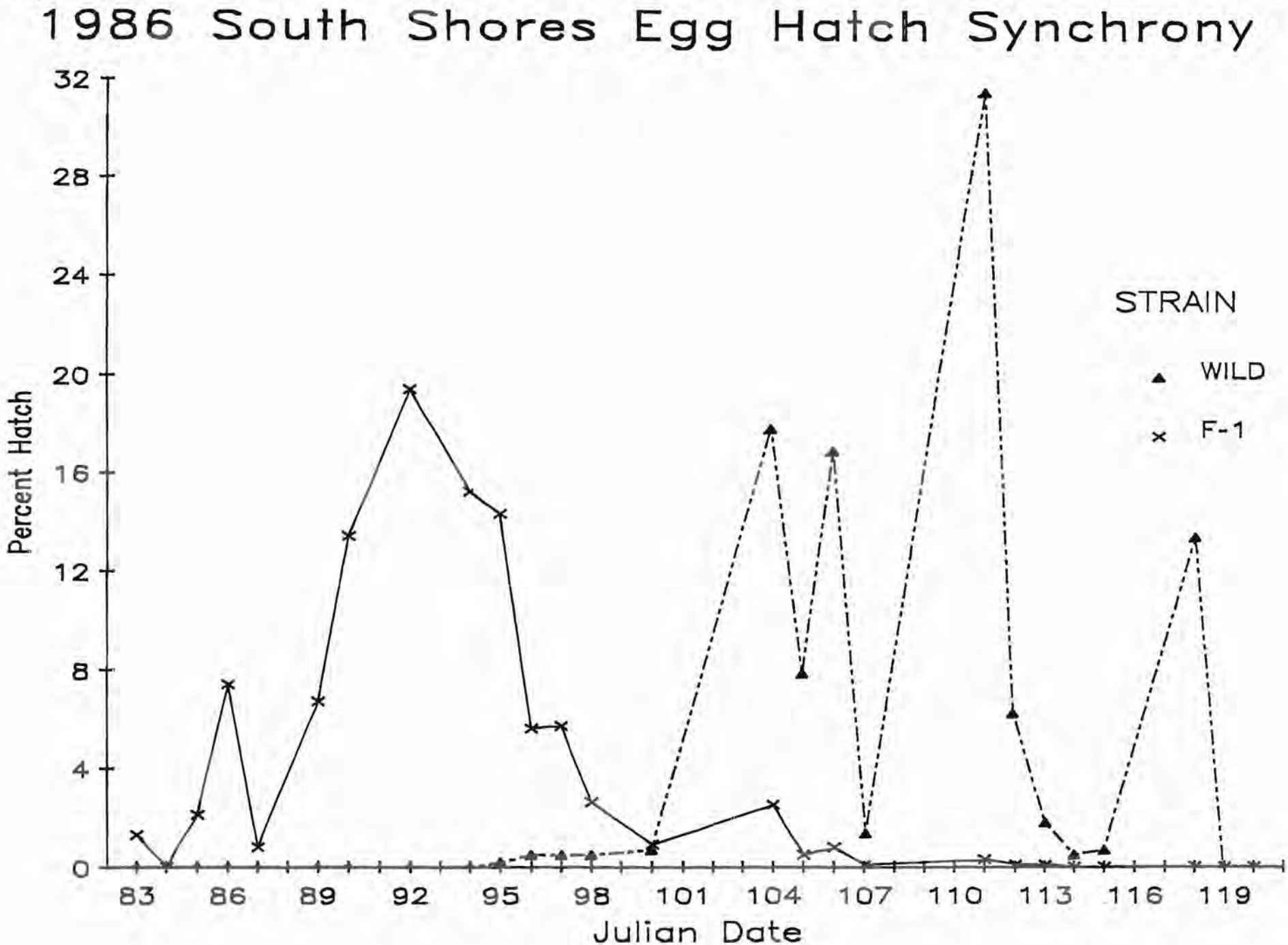

1986 South Shores Egg Hatch Synchrony
Percent Hatch
32
28
24
20
16
12
8
4
0
83
86
89
92
95
98
101
104
107
110
113
116
119
Julian Date
STRAIN
WILD
F-1

Figure 4.

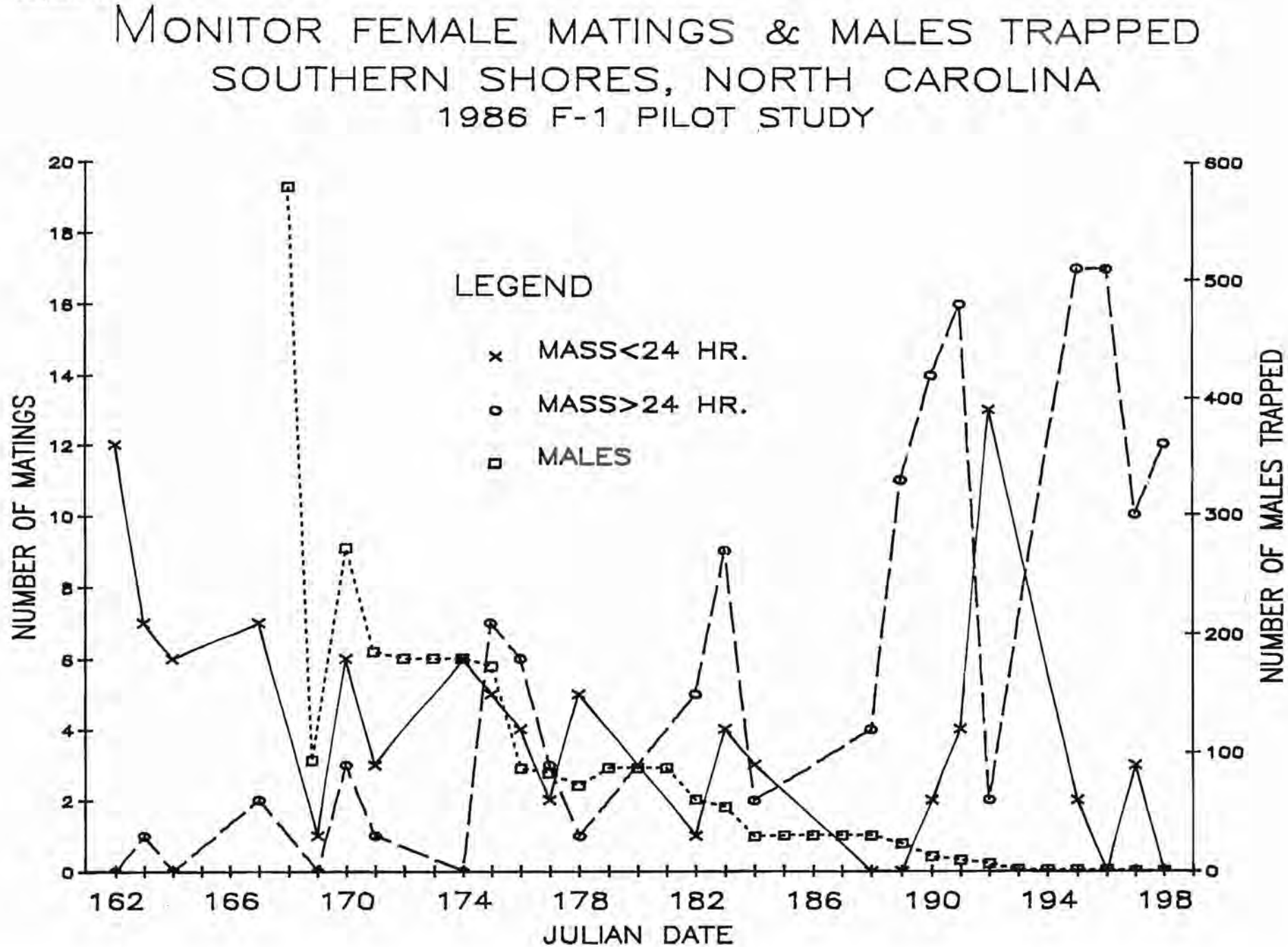

MONITOR FEMALE MATINGS & MALES TRAPPED
SOUTHERN SHORES, NORTH CAROLINA
1986 F-1 PILOT STUDY
LEGEND
MASS<24 HR.
MASS>24 HR.
MALES
NUMBER OF MATINGS
NUMBER OF MALES TRAPPED
JULIAN DATE
0
2
4
6
8
10
12
14
16
18
20
0
100
200
300
400
500
600
162
166
170
174
178
182
186
190
194
198

Figure 5.

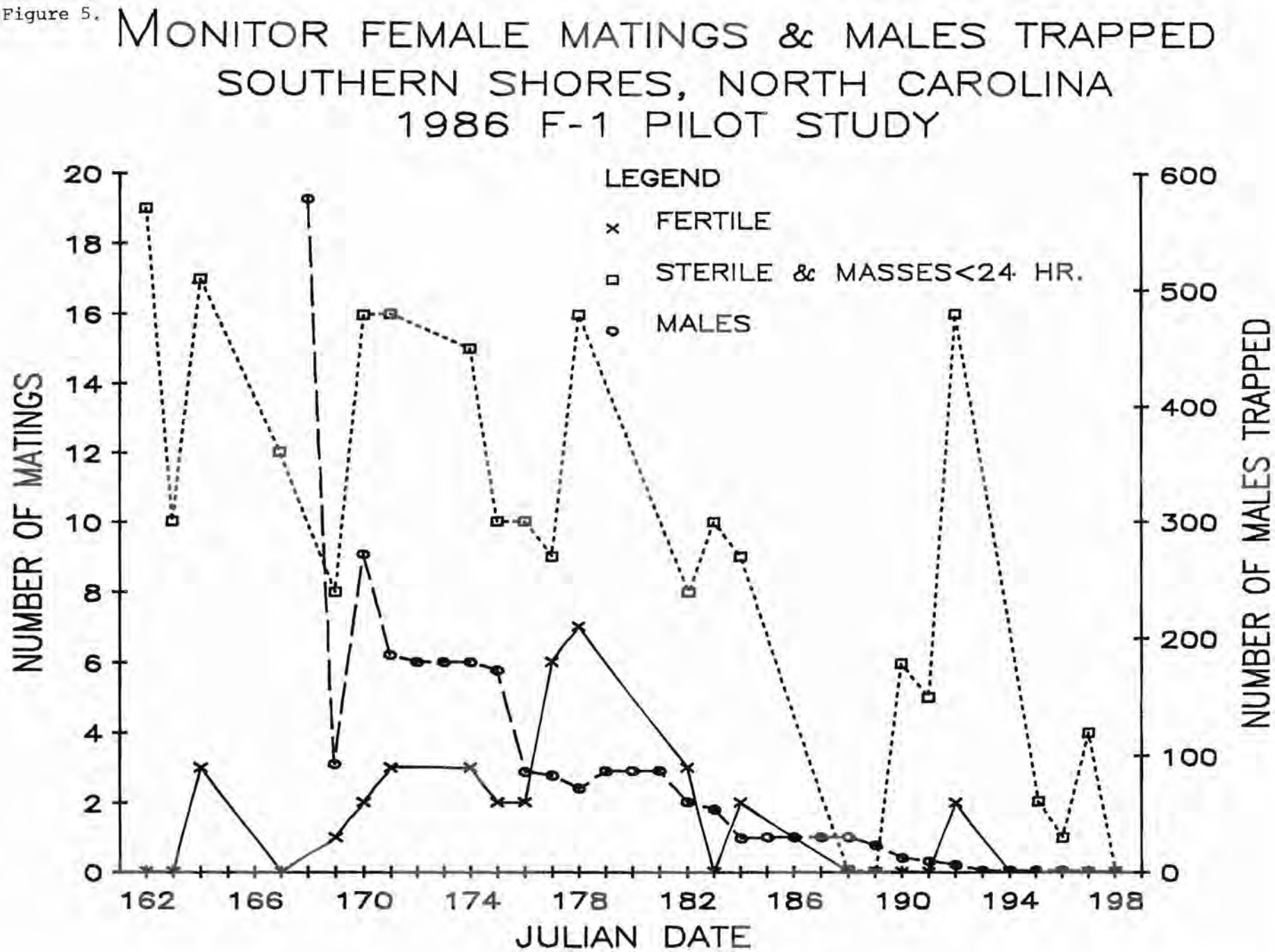
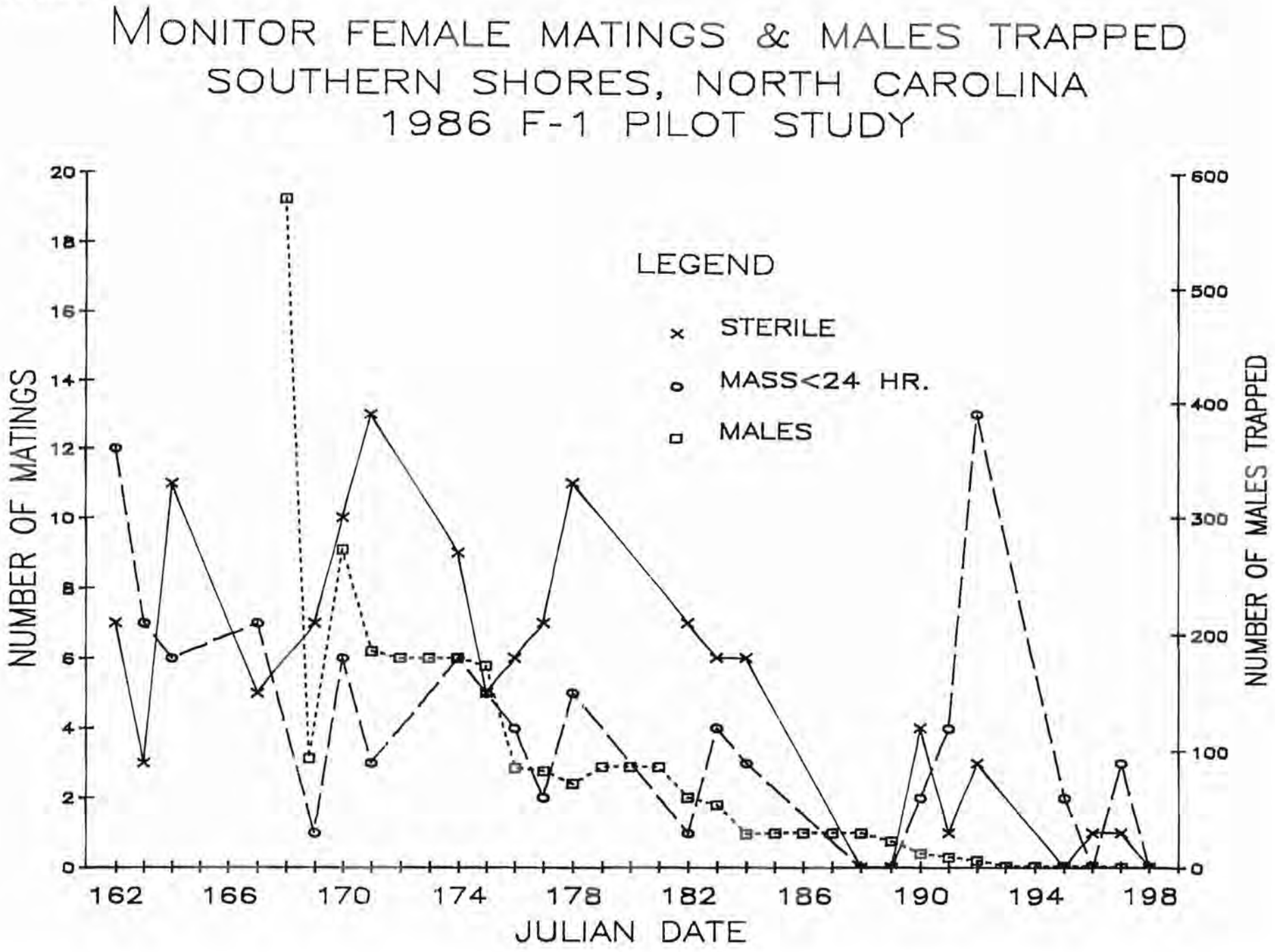

MONITOR FEMALE MATINGS & MALES TRAPPED
SOUTHERN SHORES, NORTH CAROLINA
1986 F-1 PILOT STUDY
LEGEND
FERTILE
STERILE & MASSES<24 HR.
MALES
NUMBER OF MATINGS
NUMBER OF MALES TRAPPED
JULIAN DATE
0
2
4
6
8
10
12
14
16
18
20
0
100
200
300
400
500
600
162
166
170
174
178
182
186
190
194
198

Figure 6


MONITOR FEMALE MATINGS & MALES TRAPPED
SOUTHERN SHORES, NORTH CAROLINA
1986 F-1 PILOT STUDY
LEGEND
STERILE
MASS<24 HR.
MALES
NUMBER OF MATINGS
NUMBER OF MALES TRAPPED
JULIAN DATE
0
2
4
6
8
10
12
14
16
18
20
0
100
200
300
400
500
600
162
166
170
174
178
182
186
190
194
198

Project Number: GM 6.2.2
Project Title: Sterile Male F_1 Demonstration Project - Allen County, Ohio
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: V. C. Mastro, C. P. Schwalbe, A. Pellegrini-Toole
Project Cooperators: D. Barth, K. Roach, B. Smith and D. Gerold

The infestation in Allen County, Ohio was first detected in 1984 when two single males were captured in two traps. In 1985 more intensive trapping was undertaken resulting in the capture of 20 males (9 singles, 2 doubles and one trap with 13 moths). Subsequent egg mass surveys near the site where the 13 males were captured succeeded in locating 5 egg masses. These egg masses were found in a grove of trees behind a farm house. The stand of trees (mainly black locust) was separated from wooded areas on all sides by at least 300-400 feet of field except on the north side where there was a small stand of mixed hardwoods. These two adjacent small groups of trees were treated with sterile F_1 egg masses in 1986. Protocols used in monitoring this site were similar to those used in other F_1 demonstration projects.

On April 14, 1986, 19,525 sterile F_1 egg masses were released in these two groves of trees and on trees surrounding the farm house. Estimating the density of the native population was difficult. The trapping pattern used in 1985 was not on a standard grid mainly because of the large areas of open fields. Traps were placed only along the major roads, approximating a trap density of 12/sq. mi. If traps were deployed in a grid system at this density a capture rate for males would be in the range of 5 percent. If this rate is used to calculate the native male density in 1985, then there were 400 males present. Assuming a 1:1 sex ratio and also assuming all females produced egg masses, 400 wild egg masses were present in the spring of 1986. Another estimate of the native density can be made by assuming that the egg mass survey succeeded in locating 10 percent of the native egg masses, resulting in a calculated native egg mass density of 50. Our calculated sterile:fertile (S:F) overflooding ratios were 156.2:1 and 19.5:1, respectively, based on egg mass survey and male trap capture estimates of native population density.

Egg hatch synchrony information is presented on Figure 1. Wild egg masses began hatching sooner and completed hatch before F_1 egg masses. In fact, wild hatch began the day after the F_1 egg mass release, whereas it was not anticipated before the week of April 20.

The mean proportion of F_1 eggs which hatched was 37.3%, close to the expected 40% hatch rate. Of the five wild egg masses observed, three expressed good hatch (i.e. 95.9%, 77.8%, 51.0%, X = 74.9) one had very low hatch (0.6%) and one egg mass failed to hatch and was probably an egg mass from the previous year.

Males collected as larvae and evaluated for sterility using the chromosome evaluation technique indicated the overflooding ratio was 34 sterile:1 wild (n = 70). A determination could not be made on 4 insects. Results of egg mass evaluations from males reared to the adult stage and mated with normal females were 118 sterile and 19 fertile which resulted in a much lower calculated overflooding ratio (i.e. 6.2 sterile:1 wild). A comparison between the two

evaluation techniques is presented on Figure 1. Generally, on a given sampling date the proportion of males identified as fertile using the chromosome evaluation technique was lower than the proportion established using mating evaluation. Why, the apparent disparity between these two evaluation techniques occured in this case is unknown. However, Chi square analysis of the data on a given date of collection or overall did not reveal any significant difference in the proportions of fertile males found using both techniques. Locations of wild male finds are listed in Table 1. The location of the majority of wild insects was on or near the trees where wild egg masses were foundin the preseason.

Table 1. Locations of immature gypsy moths determined to be fertile,Allen County, Ohio, 1986.

Tree no.	Tree type	Number of insects determined to be fertile: Chromosome analysis	Number of insects determined to be fertile: Mating evaluation
11	Willow		1
23	Black Locust	1	
24	" "		1
39	" "		1
41	Hackberry		1
53	Black Locust		1
55	" "		1
56	" "	1	
61	" "		4
64	" "		2
72	" "		1
74	Hickory		2
80	Honey Locust		1
114	Red Oak		1
124	Black Locust		2
TOTALS:		2	19

Results of placing monitor females for establishing sterile:fertile ratios during the adult flight period indicated that the mating ratio was lower than expected. In all, 409 monitor females were placed of which 211 produced egg masses for evaluation. Twenty-one of these egg masses were evaluated as fertile while 66 were evaluated as sterile, i.e. those which when compared statistically are not significantly different than the mean percent embryonation for a F_1 male X F_1 female mating (n = 36) and those producing unembryonated egg masses in less than 24 hours after placement (n = 30). In 14 cases the proportion of embryonated eggs in the mass was at a level which made it impossible to determine if it was a fertile (wild) or sterile F_1 mating. In an additional 110 cases, females oviposited egg masses containing all unembryonated eggs after the female was recovered and these masses were judged as the result of no mating. The calculated overflooding ratio on the monitor female data is 3.14S:1F and is much lower than the estimates based on sampling immatures.

A total of 389 males were captured, of which in traps; 306 were captured in the two traps placed within the release area. Distribution of capture generally indicated that the population was isolated except for a pattern of capture to the north following a stream. The first males were captured on July 8 with peak male capture occuring between July 13 and July 22 (Figure 3).

The number of males captured suggests that overall survival at this site was low. If a trap capture rate of 34 percent is used to estimate the actual male density in 1986, then a total of 1,143 males were present. Using the lowest estimate of overflooding (i.e. 3.14:1 - from monitor female data) the calculated number of fertile wild males present in the 1986 population was 276 and the number of F_1 sterile males was 867. Again assuming a 1:1 sex ratios in the wild population, of the 276 wild females, 67 would have mated with fertile males and 209 with F_1 males. If a more optimistic estimate of overflooding ratio is used to calculate these values (i.e. 8.9:1 - from male dissection and chromosome data) then of the 276 wild females, 28 would have mated with wild males and 248 mated with F_1 males.

Assuming that there were 67 fertile matings in 1986, then, overflooding in 1987 at a 100:1 ratio would require the release fo 16,750 egg masses. More optimistically, assuming that there were 28 fertile matings in 1986, to overflood at a 100:1 rate in 1987 would require 7,000 egg masses. We intend to overflood at a rate in 1987 which will provide a 100:1 ratio based on the most pessimistic estimates of results in 1986.

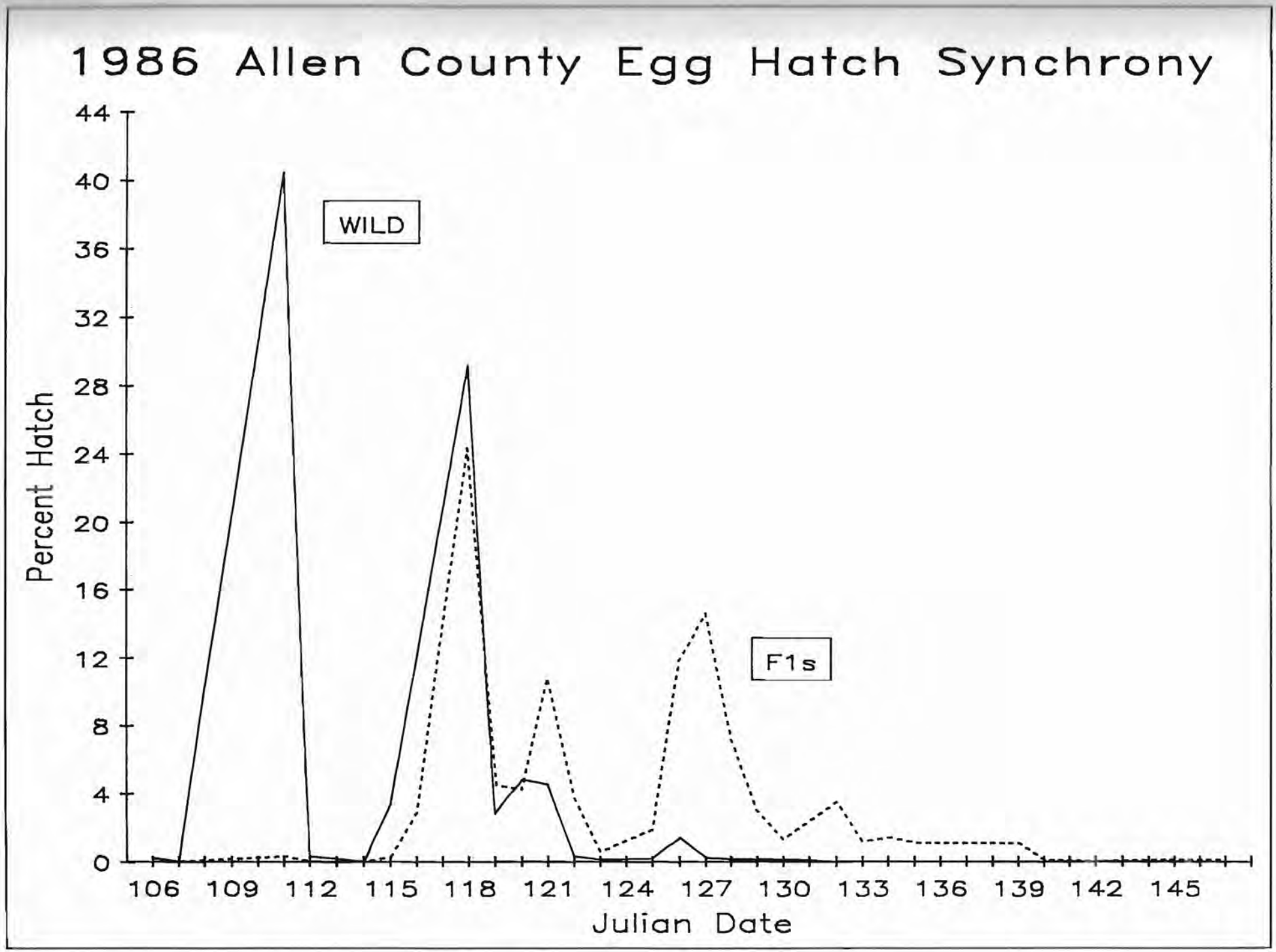

1986 Allen County Egg Hatch Synchrony
Percent Hatch
44
40
36
32
28
24
20
16
12
8
4
0
WILD
F1s
106
109
112
115
118
121
124
127
130
133
136
139
142
145
Julian Date

Figure 2.

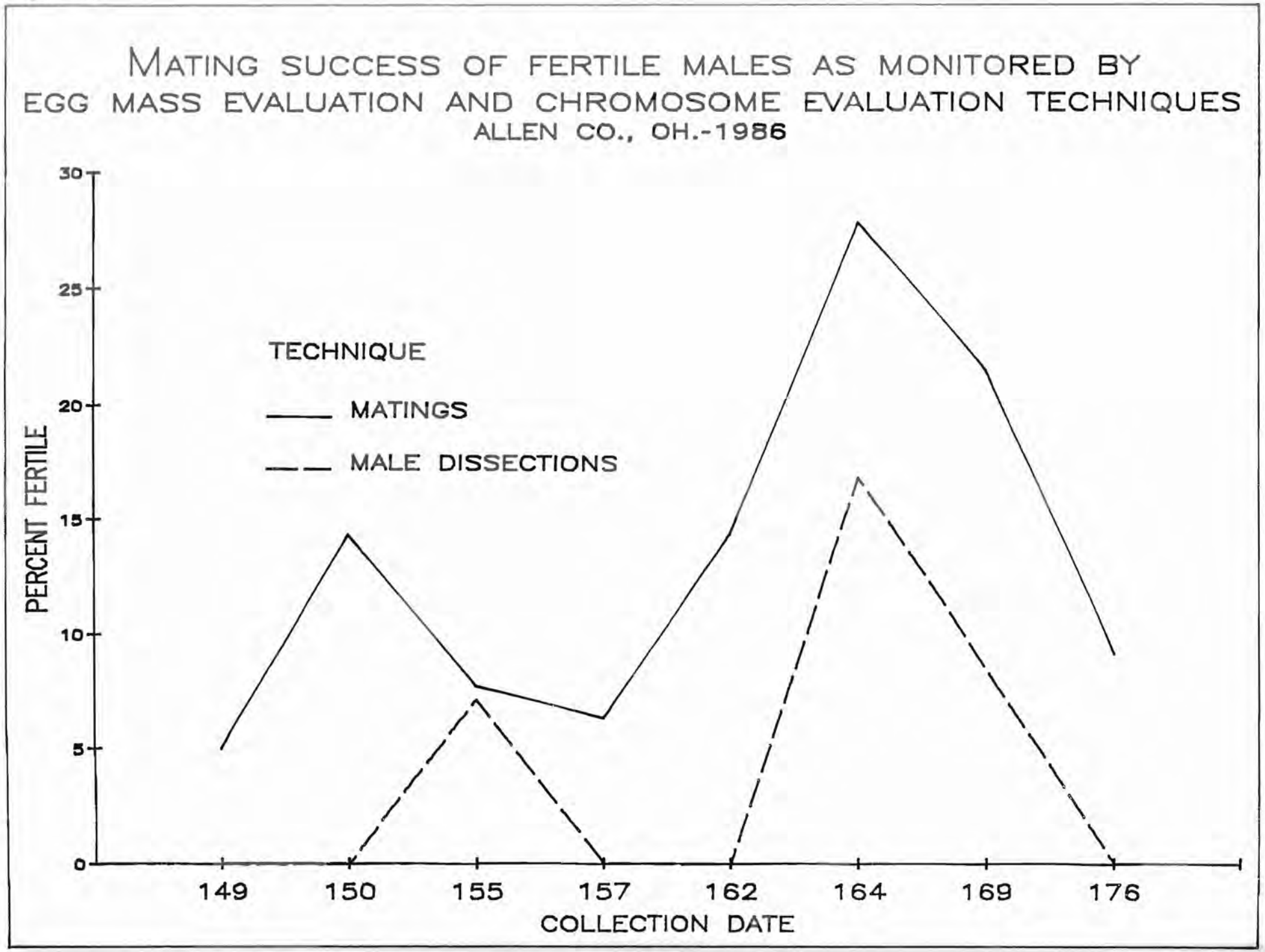
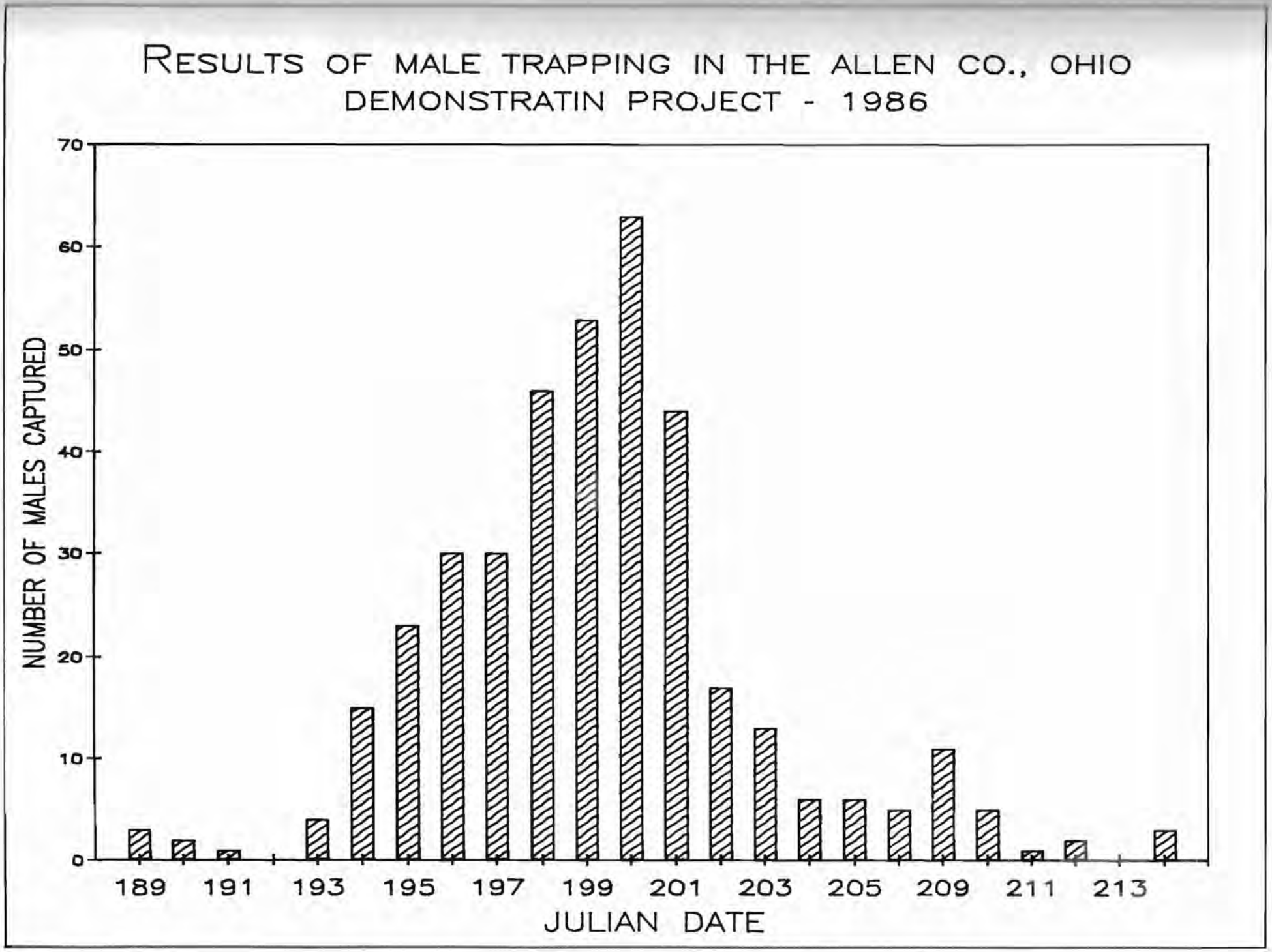

MATING SUCCESS OF FERTILE MALES AS MONITORED BY
EGG MASS EVALUATION AND CHROMOSOME EVALUATION TECHNIQUES
ALLEN CO., OH.-1986
TECHNIQUE
MATINGS
MALE DISSECTIONS
PERCENT FERTILE
30
25
20
15
10
5
0
149
150
155
157
162
164
169
176
COLLECTION DATE

Figure 1.


RESULTS OF MALE TRAPPING IN THE ALLEN CO., OHIO
DEMONSTRATIN PROJECT - 1986
NUMBER OF MALES CAPTURED
0
10
20
30
40
50
60
70
189
191
193
195
197
199
201
203
205
209
211
213
JULIAN DATE

Project Number: GM: 6.2.3
Project Title: Sterile F_1 Demonstration Project, Manor, Washington
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: V. C. Mastro, A. Pellegrini-Toole and C. P. Schwalbe
Cooperator: E. Lagasa, Washington State Department of Agriculture

The objectives of this project were similar to other F_1 demonstration projects described in this report. The Manor, Washington site has some unique characteristics; almost all of the area treated is covered by native vegetation, with an overstory of Douglas fir on the higher areas, and red alder along a creek, bisecting the plot. Of the approximately 120 acres treated there are only a few residences with ornamental plantings. Trap catches in 1985, which were concentrated in the area of one of these residences, totaled 94 males. Subsequent egg mass surveys succeeded in locating 3 viable egg masses and the remnants of one egg mass from a previous year. All egg mass finds were on red alder in the creek bottom near the sites of high male trap catches in 1985.

Estimating the native male density that was present in 1985 is difficult. Initially, males were captured in a trap and that was placed ca 800 feet from the site of the egg mass finds. After the initial male captures, additional traps were added on two occasions and no consistent grid system was used for trap placement. Because of the close proximity of some of the traps to where egg masses were found we suspected that the male capture rate was high and may have been a great as 50% of the male population.

Based on our estimate of 200 overwintering native egg masses, on April 2nd we released a total of 47,867 F_1 egg masses at the site. If the estimate of 200 egg masses in native populations was accurate this would have produced approximately a 96 F_1:1 wild ratio in the adult stage.

Samples of each lot of F_1 egg masses released at the site were checked daily for hatch (Figure 1). Wild hatch was evident on the day of release (one egg mass on the side of a tree had 12-15 neonates, light colored, not sclerotized). Samples from F_1 egg masses indicate the hatch rate was lower than expected, averaging 29%, about 11% lower than expected, 40%. Therefore, numbers of neonates available for establishment were about one quarter lower than expected. This difference, if all other estimates are accurate, would reduce the expected overflooding ratio to 72 F_1:1 wild. The three samples of wild egg masses indicated that hatch for the wild population was also low: one egg mass failed to hatch and the hatch of the remaining two egg masses averaged only 18.5% (84% was expected). However, it is possible that some hatch occured before the samples were collected.

Samples of immature males collected from this site were treated similarly to samples collected from other F_1 release sites except that a larger proportion of early collections were held for mating and egg mass evaluation as opposed to dissection and chromosome analysis. Of the male larvae dissected, chromosome analysis showed 86 were F_1's and 3 were wild, a 28.7:1 ratio. Egg mass evaluation from the males which were held to the adult stage and mated with normal females, produced similar results. Ninety-three pairs produced egg masses with the characteristics of a F_1 male x normal female cross and three pairs produced egg masses with the characteristics of a normal mating, resulting in a 31 F_1:1 wild overflooding ratio.

Results of placing monitor females produced the following results. Twenty-four females oviposited egg masses with some embryonation, all of which were classified as steriles and 175 females produced egg masses which contained no embryonated eggs. A large number of females (n=148) failed to produce an egg mass. No monitor females produced an egg mass which was evaluated as the result of mating with a fertile male. A summary of the results of three techniques for estimating overflooding ratios is presented in Table 1.

In all, 528 males were captured in disparlure-baited milk carton traps placed in the area. This represented a very low male recovery rate compared to the results from other F_1 sites. Trap density at this site was very high and in the area of release approximated 240 traps/sq. mi. (0.38 traps/acre). Past studies done at this laboratory suggest that the proportion of males captured at this trap density should approach 45.0 percent (0.304 traps/acre = 43.5% recovery and 1.012 traps/acre = 52.2% recovery). If 45 percent is a realistic capture rate then the male population was approximately 1173.

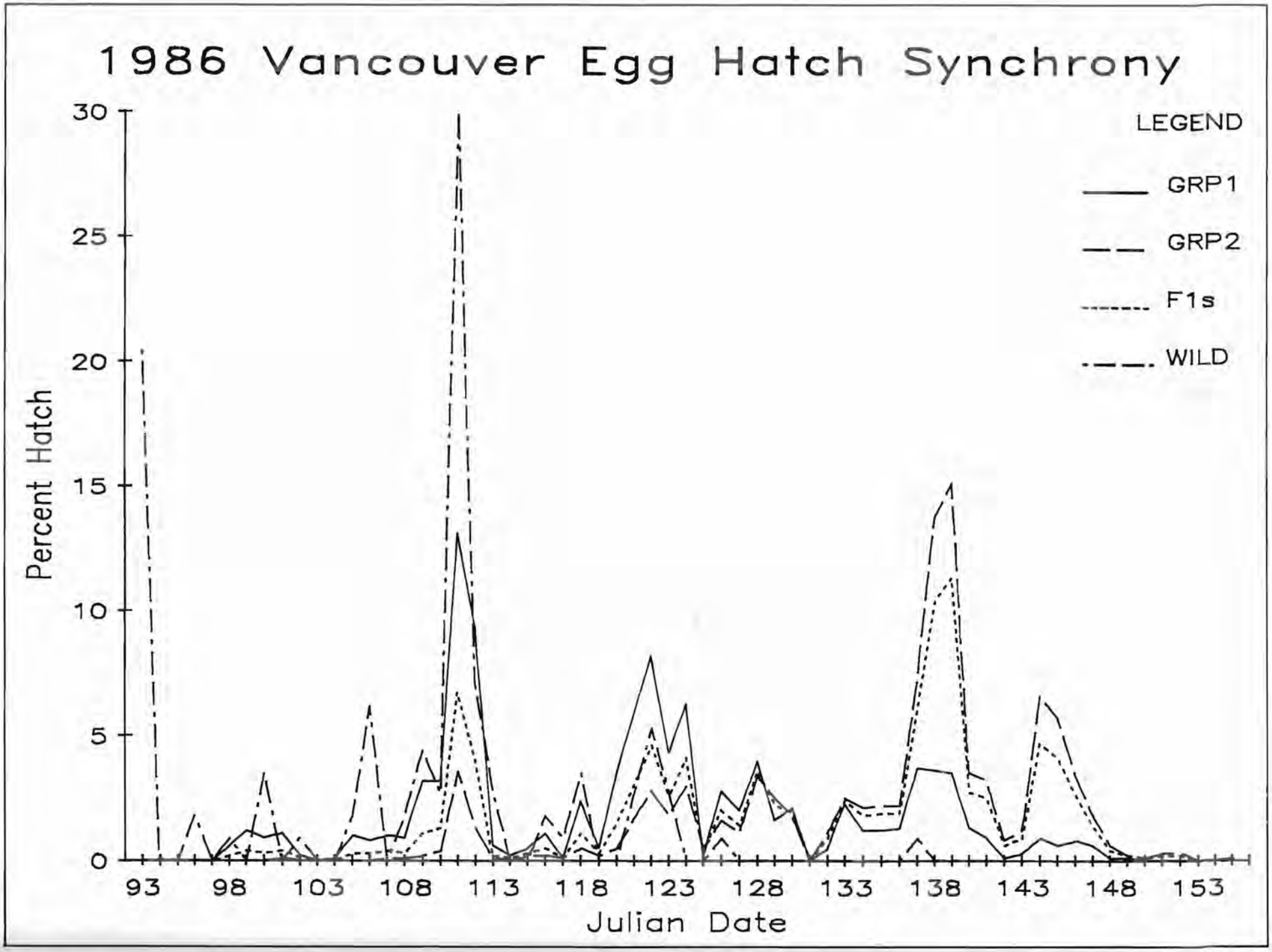
If we use the most conservative estimate of overflooding ratio (i.e. 28.7:1 calculated from male chromosome analysis) to evaluate the impact of the F_1 release, then the following calculations can be made. Of the estimated 1173 males present in 1986 39.5 (ca 40) were fertile males. Also assuming a 1:1 sex ratio for the wild population, there were 40 feral females available for mating. If the adult mating ratio is conservatively estimated at 28.7:1, then 1.4 wild females should have mated with wild males. If these estimates are accurate, then, the fertile egg mass population in 1987 should be very small. It should be noted that the estimated wild male population in 1986 was actually lower than our estimate for 1985, and so are estimates of wild egg masses. However, even if it is assumed that the wild population in 1986 was the same as the estimate used in 1985 (i.e. 200 males, females and egg masses) and if the 28.7:1 overflooding ratio is applied, only 6.7 wild females would have mated with wild males.

In 1987 we plan to release a sufficient number of F_1 egg masses to produce an overflooding ratio which will result in eradication.

Table 1. Results of monitoring overflooding rates in Manor, Washington, 1986.

Monitoring technique	n	Sterile	Fertile	Ratio
Male chromosome analysis	89	86	3	28.7:1
Male mating & egg mass evaluation	96	93	3	31:1
Monitor female egg mass evaluation	199	199	0	199:0

Figure 1.


1986 Vancouver Egg Hatch Synchrony
LEGEND
GRP1
GRP2
F1s
WILD
Percent Hatch
30
25
20
15
10
5
0
93
98
103
108
113
118
123
128
133
138
143
148
153
Julian Date

Project Number: GM 6.2.4
Project Title: Sterile F_1 Demonstration Project - Portland, OR
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Report Leaders: V. C. Mastro, C. P. Schwalbe, and A. Pellegrini-Toole
Cooperators: Alan Mudge, Oregon Department of Agriculture
Gary Smith, USDA, APHIS, PPQ

The objective of this project, similar to other sterile male demonstration projects, was to evaluate the utility of the sterile F_1 technique for treatment of isolated gypsy moth infestations. Techniques used for release and monitoring at this site were similar to other sites so a lengthy discussion of these will not be undertaken here.

In 1985 the Portland infestation was delimited using pheromone baited delta traps. In all, a total of 131 males were captured. Trap spacing in 1985 was not a uniform grid and after the first males were captured additional traps were added to aid in pinpointing the infestation. After an egg mass survey was completed, 28 viable native egg masses and 7 spent egg masses, probably from the previous year, were found. All egg masses were found on two adjacent properties.

Because of trap spacing and timing of placement, it was difficult to estimate the native insect density in 1985. A rough estimate was that 50 percent of the males had been captured because of the dense cluster of traps at the site of egg mass finds or that 10 percent of the viable egg masses had been located. Estimates of the native density derived from using trap data and egg mass survey data are, respectively, 262 males and (assuming a 1:1 sex ratio and that all females were mated) 262 or 280 egg masses.

In the spring of 1986, in preparation for the F_1 release, the approximately 75 acre area considered for release was surveyed for available hosts on a property by property basis by the Oregon Department of Agriculture. A total of 265 properties were surveyed and the hosts classified by type (i.e. primary, secondary or non-host) and size. F_1 egg masses were released only on those properties that had hosts and where home owner permission was granted. Egg mass release was weighted by two factors, 1) the predicted amount of host foliage (i.e. number and DBH of host) and 2) the proximity to the site of the native egg mass finds. Approximately 40,600 F_1 egg masses were released. Calculating projected overflooding ratios was difficult because of the poor estimates of the native density. If the native population did consist of 280 native egg masses the release would have been expected to produce an overall $60F_1$:1 wild male ratio in the adult stage. However, the expected ratio where natives were concentrated (on the two properties with native egg masses) would be lower (i.e. ca. 5:1).

Release was conducted on April 4. As in other sites, samples of F_1 production lots and samples of wild egg masses were placed in a cage for determination of hatch synchrony. Hatch from wild masses had begun at least four days prior to release of F_1's. Hatch synchrony of F_1 egg masses and wild egg masses was poor (Figure 1). The peak hatch of F_1 eggs occurred 14 days after the peak hatch of wild eggs. The impact of the late hatch on the survival and synchrony of development of F_1s at this site is unknown. However, other studies have demonstrated that as leaves mature gypsy moth neonates have a much lower probability of establishment.

Collections of larvae and pupae were made throughout the release area to provide estimates of overflooding ratios. To facilitate collections of late instar larvae and pupae, all host trees around the site of native egg mass finds and two host trees on every fifth property over the remainder of the area were banded with burlap. Collections were initiated on April 29 and completed on August 13. As the season progressed, most insects were collected from the two adjacent properties where wild egg masses had been found.

As in other sites, a portion of the males collected as larvae before the 5th instar were dissected for chromosome analysis while the remainder were held until the adult stage for mating-egg mass evaluation. Types of all males collected after the 4th/5th instar ecdysis were determined using the mating-egg mass evaluation technique. Chromosome analysis was used to make determinations on 154 males. One hundred and twenty-one were typed as F_1s and 33 as wilds resulting in a $3.7F_1$:1 wild ratio. Table 1 presents the location and stage at collection of both types of males. As can be seen, all wild type males were collected from the same property that wild egg masses had been found on.

Table 1. Preliminary summary of field collected male dissections from 1986 F_1 sterile insect pilot study, Portland, Oregon.

Collection Date (Julian)	No. of F-1 Males Dissected	Instar at Collection	No. of wild Males Dissected	Instar at Collection
119	4	4-1st 1/	0	--
120	7	7-1st 1/	0	--
122	15	15-1st 1/	0	--
125	3	3-1st 1/	0	--
126	9	9-1st 3/	0	--
127	1	1-1st 4/	3	1-1st, 2-2nd 4/
128	2	2-1st 5/	0	--
132	4	4-1st 6/	0	--
134	3	3-1st 4/	7	7-2nd 4/
136	6	5-1st, 1-2nd 7/	0	--
141	5	3-1st, 2-2nd 4/	5	1-2nd, 4-3rd 4/
143	2	2-2nd 4/	0	--
147	2	2-1st 8/	0	--
149	3	2-1st, 1-2nd 4/	0	--
150	2	1-2nd, 1-3rd 4/	0	--
157	2	1-1st, 1-2nd 4/	1	1-2nd 4/
160	4	2-2nd, 2-3rd 4/	2	1-2nd, 1-3rd 4/
161	2	2-3rd 4/	0	--
163	6	3-2nd, 3-3rd 4/	2	2-3rd 4/
167	7	203rd, 5-4th 4/	2	1-3rd, 1-4th 4/
169	7	2-3rd, 5-4th 4/	2	1-3rd, 1-4th 4/
170	1	1-4th 4/	7	3-3rd, 4-4th 4/
171	6	3-3rd, 3-4th 4/	0	--
174	10	1-2nd, 2-3rd, 7-4th 4/	1	1-5th
177	5	4-3rd, 1-4th 4/	1	1-4th 4/
183	3	3-4th 4/	0	--
TOTALS	121		33	

Location of Collections:

1/ Park - W. end of Alder
2/ Park - W. end of Yamhill (11 larvae), Park - W. end of Alder (1 larva), 11130 SE Yamhill (2 larvae) and 11024 SE Yamhill (1 larva)
3/ Park
4/ 11010 SE Yamhill
5/ Park - South end
6/ 11010 SE Yamhill (2 larvae), 11024 SE Yamhill (2 larvae)
7/ 11010 SE Yamhill (4 larvae), Park - South end (1 larva), 11024 SE Yamhill (1 larva)
8/ 11024 SE Yamhill (1 larva), 11130 SE Yamhill (1 larva).

Males evaluated using the mating-egg mass evaluation technique totaled 529; of these, 192 were determined to be fertile while 246 were determined to be sterile. Sixty-two pairs produced egg masses containing all unembryonated eggs. If only those pairs producing an egg mass with at least one embryonated egg are used to calculate an overflooding ratio, the resulting sterile:fertile estimate is 1.3:1 (n=438). If pairs producing unembryonated egg masses are included in the estimate, the ratio is 1.6:1 (n=500). Both ratios are lower than ratios computed from male chromosome analysis and significantly different from that ratio. This difference may be because a larger proportion of the males evaluated using the mating egg mass technique were collected from the two core properties or possibly because of a gradual decay in the ratio.

The results of monitoring male overflooding ratios using the two techniques are nearly parallel (Figure 2). Initially, it appears that there is a decay in the overflooding ratio during the period of the first three sampling weeks (e.g. largely when 1st or 2nd instars were collected). Data from other studies have shown that a higher mortality of F_1 larvae in the early instars is to be expected after which the mortality rate of F_1s should be similar to wilds and the ratio should remain fairly constant. When the data from both types of male evaluations (mating-egg mass evaluation and chromosome analysis) are combined and then separated on the basis of collection site (e.g. core areas, 11010 + 11024 SE Yamhill Street vs. all other areas) the seasonal overflooding ratios present a somewhat different result (Figure 3). Again, in the core area there was a rapid decay in the ratio during the first three sampling weeks, however, there also appears to be some additional decay in the ratio in the last three sampling weeks (these samples contained mostly 4 instar males or older).

Monitor females were placed on the Portland release site to provide estimates of male mating ratios throughout the male flight period. A total of 492 monitor females were placed but only 289 egg masses were recovered for evaluation. Egg masses which were categorized as having a fertile male parent totaled 13 while those characterized as having a sterile male parent totaled 54. In addition, 212 egg masses contained all unembryonated eggs. Unembryonated egg masses can result from an F_1 male mating or the result of no mating. Usually, monitor females which deposit unembryonated egg masses after recovery, are assigned to the "no mating" category and those initiating ovipostion before recovery (i.e. in the field) are considered the results of a mating with an F_1 male. In this case, perhaps because of the cooler conditions, all females whose egg masses were ultimately considered to be the result of mating with fertile males (and, indeed, most females) did not begin oviposition until after recovery and therefore, this separation cannot not be used. A calculated overflooding ratio based only on those egg masses which could be assigned to a mating type is 4.2S:1F; (n=67). If unembryonated egg masses are considered the result of F_1 male x normal female matings then a ratio 20.5S:1F (n=279) would apply. Ten egg masses could not be assigned statistically to either mating type. The proportion of embryonated eggs in all of these egg masses was low (i.e. ranged from 13.69% to 34.15%).

Undoubtedly, the actual ratio in the field was lower than expected. The mating ratio at the core (two properties where egg masses were found) was lower than the expected 5S:1F. Of the 13 egg masses produced by monitor females which were classified as fertile, 5 were from females placed on either of the two core properties and 4 were from females placed within the adjacent park area.

Table 2. Monitor females which produced egg masses characteristic of a mating with a wild male.

Female no.	Placement date	Location
26	192	T113 P43 S. Park
198	209	Upper S. Park
235	211	11011 S.E. Yamhill
251	217	Upper S. Park
254	217	11024 S.E. Yamhill
255	217	11010 S.E. Yamhill
273	218	Upper S. Park
280	218	11010 S.E. Yamhill
292	218	1233 S.E. 112th
306	223	11123 S.E. Salmon
324	223	113th - Park
359	227	11024 S.E. Yamhill
442	239	S. Park

Large capacity milk carton traps were placed on a grid (36 per sq. mi. - 880 ft. inter-trap spacing) over an area which extended 1.5 miles to the north, east and west of the release site. The trapping grid was extended ca. 2.75 miles further south and included a small area which was mass trapped (at 3-9 traps/acre). Delta traps were substituted for milk carton traps in the southern part of this grid. Surrounding the approximate 13.5 square miles trapped at 36 traps/sq. mi. were at least 2.5 miles on all sides trapped at 4 traps/sq. mi. In addition, at several sites within the trapped area a trap was placed on a property which had been associated with a recent household move.

Within the area trapped at 36 traps/sq. mi. 341 males were captured and an additional 13 males were captured in the surrounding area trapped at 4/sq. mi. There was a strong pattern of male capture extending from the release area to the southeast which included captures in the area trapped at 4/sq. mi. There were also three traps which captured a single male to the northeast, and two traps to the west-southwest of the release area. The pattern of capture to the southeast strongly suggests that there was dispersal (either neonate or adult) from the F_1 release site.

The 8 traps within the sterile release area, which were checked on a regular basis, accounted for 249 of the total males captured. If only those males within this area are used to estimate the male density in 1986 and a 34.02% capture rate is used, then an estimate of the total male population would be 732 males or, if the total males (341) captured within the area (trapped at 36 traps/sq. mi.) is used to estimate the male population, then 1,002 males were present in 1986.

If a 4:1 sterile to fertile ratio is applied to the estimate of 1,002 males then 200 males were wild and 802 were F_1's. Assuming the sex ratio of the wild population was 1:1 in the adult stage, 200 native females would have been present in 1986 and if they freely mated with wild and F_1 males, then 40 females paired with wild fertile males and 160 with F_1 males. Note that the estimate of the wild population in this case is actually smaller than either estimate of the 1985 population, however, even if it assumed there was a 2x growth in the wild population (i.e. 600 wild males and females), and if 1:1 overflooding ratio (nearly the ratio at the core) is applied then 300 wild x wild matings would have occurred. In this case the impact of the release would have been only to maintain the same population level.

Final results of evaluation of collections of female larvae and pupae and post season egg masses from the site are not available at this time but will be presented in the next annual report. Because the pattern of male capture to the southeast of the release site suggests dispersal from the site or, more seriously, additional wild populations, no further releases of F_1 egg masses will be made in 1987. Plans are to intensively monitor the whole site and to mass trap the core area. Based on the preliminary 1986 results this trapping should, in 1987, yield data that suggest that the density of the wild population is no larger than the 1985 estimates.

Undoubtedly, the release failed to establish the desired overflooding ratio. Possible and probable reasons are: poor hatch synchrony of F_1s and wilds resulting in poor establishment of steriles; inaccurate estimates of the wild population; poor synchrony of development; and possibly some unexplained decay in the later part of the development period.

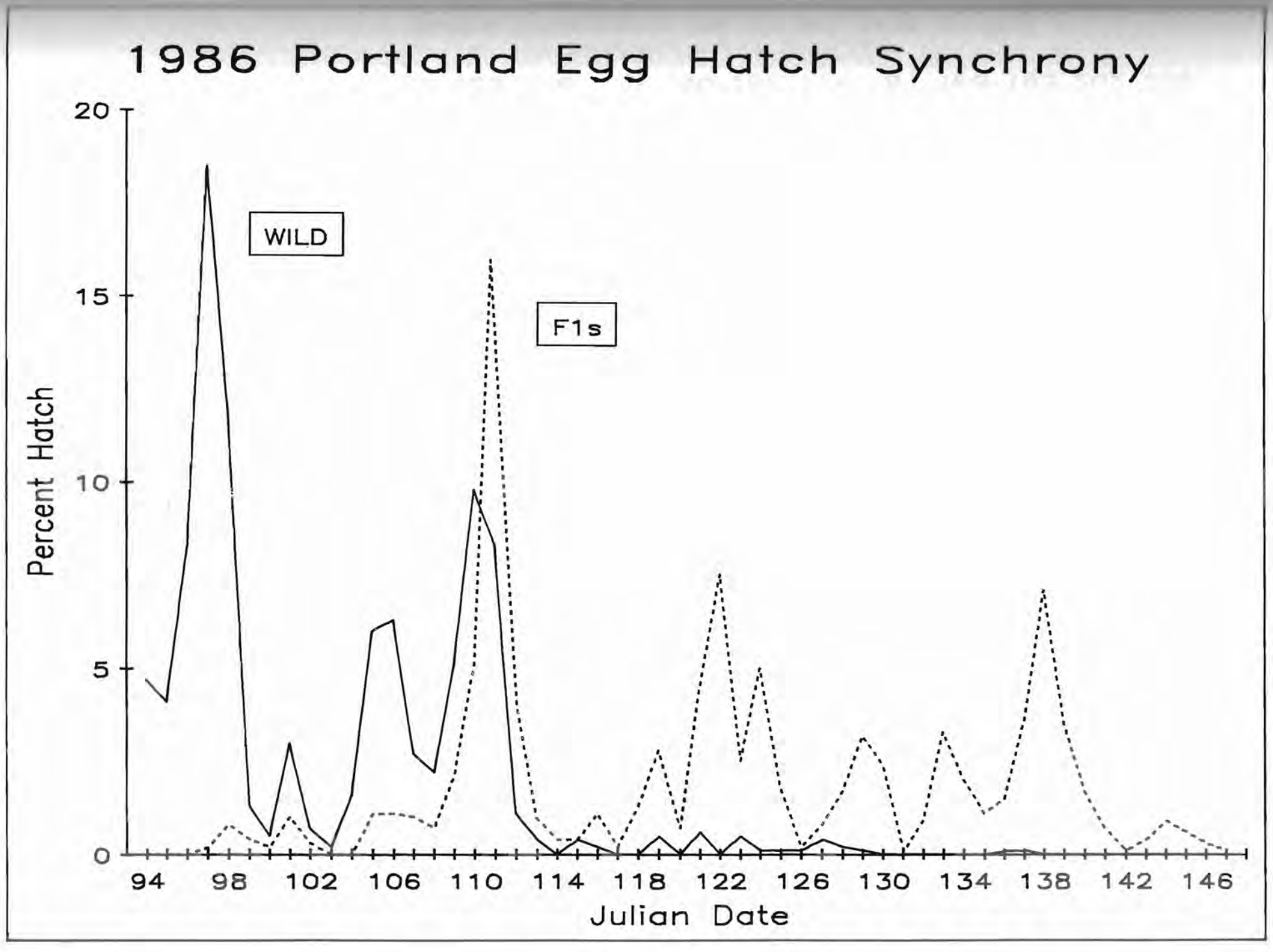

1986 Portland Egg Hatch Synchrony
WILD
F1s
Percent Hatch
20
15
10
5
0
94
98
102
106
110
114
118
122
126
130
134
138
142
146
Julian Date

Figure 2.

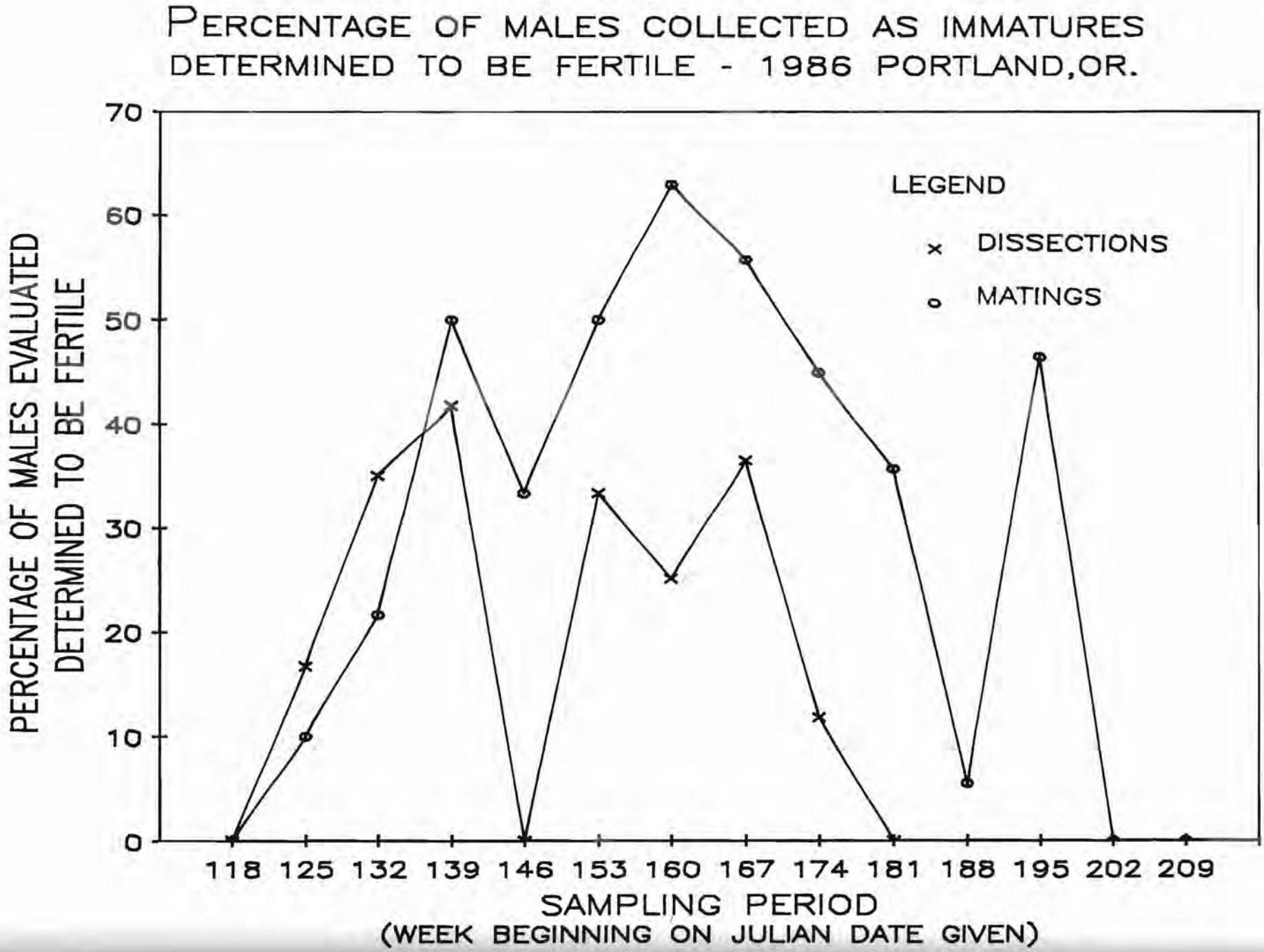
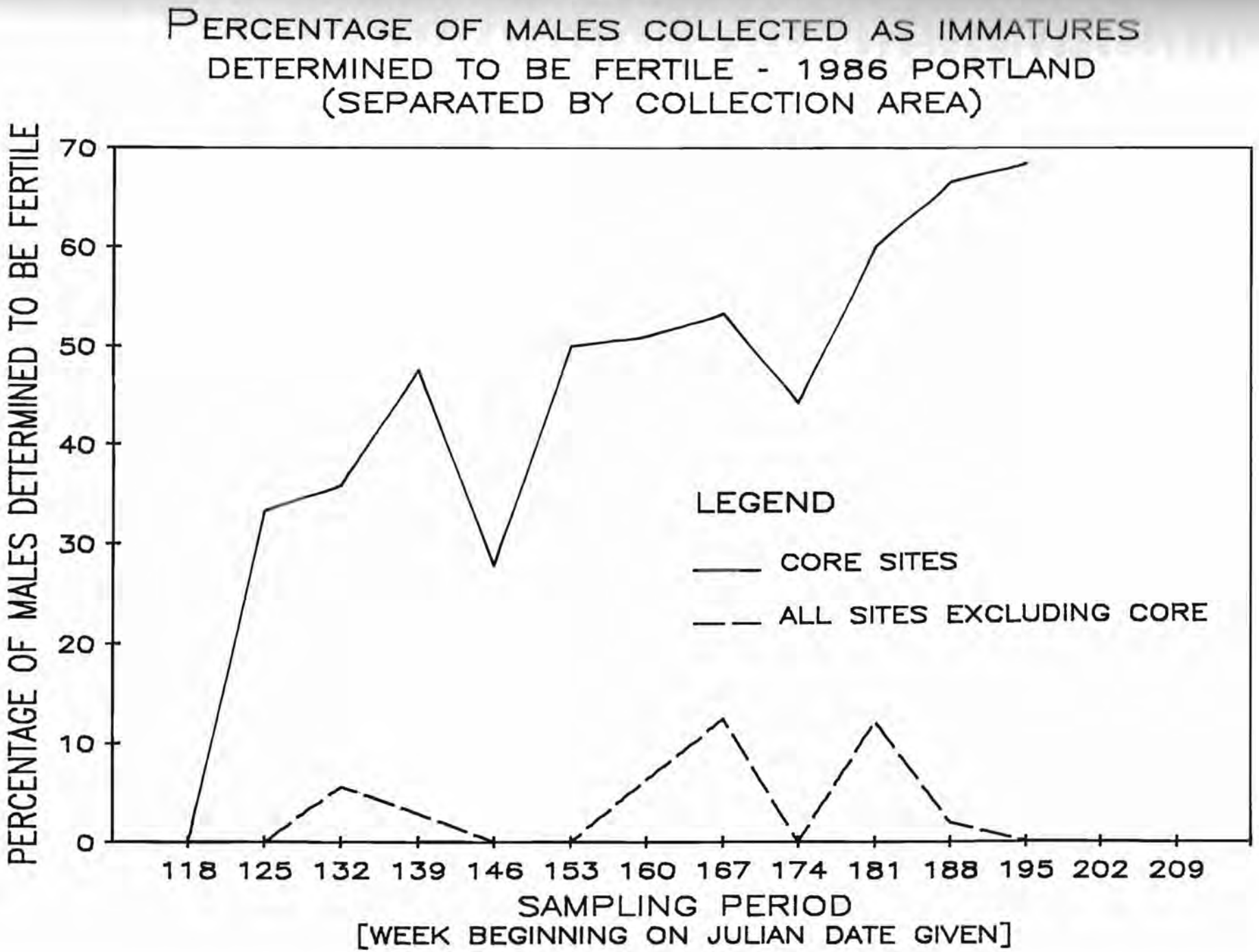

PERCENTAGE OF MALES COLLECTED AS IMMATURES
DETERMINED TO BE FERTILE - 1986 PORTLAND,OR.
PERCENTAGE OF MALES EVALUATED
DETERMINED TO BE FERTILE
70
60
50
40
30
20
10
0
LEGEND
DISSECTIONS
MATINGS
118 125 132 139 146 153 160 167 174 181 188 195 202 209
SAMPLING PERIOD
(WEEK BEGINNING ON JULIAN DATE GIVEN)

Figure 9.


PERCENTAGE OF MALES COLLECTED AS IMMATURES
DETERMINED TO BE FERTILE - 1986 PORTLAND
(SEPARATED BY COLLECTION AREA)
PERCENTAGE OF MALES DETERMINED TO BE FERTILE
0
10
20
30
40
50
60
70
LEGEND
CORE SITES
ALL SITES EXCLUDING CORE
118 125 132 139 146 153 160 167 174 181 188 195 202 209
SAMPLING PERIOD
[WEEK BEGINNING ON JULIAN DATE GIVEN]

Project Number: GM: 6.2.5
Project Title: Comparative Larval Behavior of Gypsy Moths
Report Period: October 1, 1985, September 30, 1986
Report Type: Interim
Project Leaders: V. C. Mastro, C. P. Schwalbe

The objective of these studies was to compare the larval behavior of F_1 progeny of irradiated males with the normal laboratory strain (NJSS) and P-1 progeny of wild gypsy moths. Larval behavior and survival of F_1 steriles, if somehow aberrant, could potentially negatively impact on their competitiveness. In a preliminary study we released three strains of males (F_1, NJSS and P-1 "wild") on fourteen trees and attempted to observe their behavior throughout the day. Because of rapid losses in the number of larvae, very few were available for observation and the following two studies were designed and conducted.

In the first study all three strains of larvae were released on each of seven trees. Seven larvae of NJSS and F_1 strains and either five or four of the P-1 strain (i.e. total respectively 49, 49, and 33) were released on each tree. Prior to release, insects were reared on artificial diet in the insectary. Insects for identification were marked by applying a dot of paint to the dorsal side of the thorax: NJSS - yellow, F-1 - blue and P-1 (Sleepy Creek, W.V. strain) - green. Trees used in the study were banded with burlap at ca. 4 ft. to provide larval resting sites and also were wrapped at ground level with double sided tape (Repelem II) in an attempt to restrict larval movement off the tree. Trees were white oaks, Quercus alba, 3-5 in, DBH, the crowns of which were open above to sunlight. Larvae were released on 7/14 between 1900 and 2000. General observations at this time indicated nearly all larvae began to ascend the trees immediately. Observations on larval position were initiated at 0700 on 7/15 and done regularly until 1833 on the 16th. All remaining larvae were collected on the 18th and held in an insectary on oak foliage for observations on survival. The percent recovery for each strain was, 79.6% for the NJSS strain, 48.9% for the F-1 strain and 84.8% for the P-1 strain. Of the insects recovered 61.5%, 19.0% and 60.7% survived to the adult stage, respectively for the NJSS, F-1 and P-1 strains. Parasitism rates for the three strains were roughly comparable, again, respectively, 20.5%, 28.6% and 14.3%. The major category contributing to low survival for F-1 larvae was in the unknown larval mortality category (i.e. 17.9%, 52.4% and 25.0%) for the NJSS, F-1 and P-1 strains. Generally, the double sided sticky tape did not restrict larval movement off of the tree. Larvae were observed either crawling over the tape or simply dropping from the crown of the tree. Generally, higher proportions of both the NJSS and, particularly, the F-1 strain were found off of the tree than the P-1 strain. Undoubtedly, this tendency to disperse contributed to the low F-1 recovery. However, the reason for this tendency is unclear. Figures 1-4 compare the relative activities of the three strains over time.

In preparation for the second study a white oak, Quercus alba, (5 1/2" DBH) was selected for the observations. The tree crown was part of the overstory canopy and was isolated from the crowns of adjacent trees by pruning branches. To restrict movement of larvae, a barrier of corrugated plastic garden edging was positioned just beyond the drip line of the tree crown. The bottom of the edging was sunk into the soil and the top edge was coated with tack trap. Ladders were positioned on two adjacent trees to provide access for observation of all parts of the study tree's crown. Burlap swatches were placed on the bole of the tree to provide resting sites, and additional resting sites were provided by small wooden A-frames placed around the base of the tree.

Three "strains" of larvae were selected for the comparison: the standard laboratory strain, (NJSS); an F-1 strain which are progeny of irradiated males (10 krads) and normal females, and a "wild" P-1 strain (N. Mountain), field collected as eggs and reared in the laboratory. All larvae were reared on the standard B-4 diet from egg hatch until five days before the initiation of the study. The NJSS and F-1 insects were reared collectively on diet in large cups (X-E6) and the wild N. Mountain strain were reared individually in small 1 1/2 oz cups (Thunderbirds).

Five days prior to the study, all strains of larvae were changed to individual rearing containers (16 oz. cups) provisioned with white oak foliage. Foliage was changed daily and the degree of feeding noted. Also, frass from these cups was saved and the number of pellets later counted and recorded.

Forty insects from each strain (i.e. 120 total) were released at 2000 on July 22. All of the NJSS and F-1 larvae were in the 5th instar and, although a few of the N. Mountain strain were in the 6th instar, they were predominately 5ths. To distinguish between strains, insects were marked with water based acrylic paint (Pelikan) on the dorsal side of the thorax; NJSS - orange, F-1 - yellow and N. Mountain - pink. Observations were initiated at 0620 on 7/23 and repeated at 3 or 4 hour intervals until 1530 on 7/25. During an observation period the position and activity of all larvae were noted and recorded. During the first night after release observations were restricted to only those insects on the bole or resting on the ground. During the second night, observations were also made in the tree crown. After the conclusion of the tests all remaining larvae were recovered and returned to the laboratory where they were either reared to the adult stage or, if they died, held for possible parasite emergence.

By the conclusion of the test fewer of the F-1 larvae (n=27) were recovered alive than either the NJSS strain (n=35) or the N. Mountain strain (n=34). Most of the F-1 which were not recovered alive were simply not found (n=10) and only 3 larvae were found dead. Similarly 4 and 2 larvae of the NJSS and wild P-1 strain, respectively, were found dead. The remaining larvae were simply missing.

Activity of all three larval strains appears to be comparable with some exceptions (figures 5-8). F-1 larvae during daylight (resting) hours appear to be found in a larger proportion on the ground as opposed to on the tree bole or under burlap. This behavioral aberration was first noticed in the preliminary tests. The impact this proclivity to disperse from the trees on which they were originally placed is unknown. Potentially, it could decrease the competitiveness of the F-1 strain; either by increasing the opportunity for parasitism or increasing mortality due to the failure of the larvae to find another host.

At the conclusion of the observations all larvae were recovered and placed on foliage in an insectary. Survival to the adult stage was 50% for the NJSS-strain (n=34), 32.1% for the F-1 strain (n=28) and 43.3% (n=30) for the P-1 (N. Mountain) strains. In this study an elevated proportion of F-1 larvae recovered were parasitized (50%) than the NJSS strain (30%) or the P-1 strain (14.7%). However, unknown larval mortality accounted for higher portions of NJSS larvae 32.3%, and P-1 larvae 20.0% than F-1 larvae 7.1%. Death during the pupal stage also appeared to be slightly higher for the F-1 strain (10.7%), than for the NJSS (2.9%) or the P-1 strain (6.7%).

Larval behaviors during the course of both of these studies indicate that a proportion of the F-1 strain appears to have a greater tendency to leave the tree and disperse. Also, there generally appears to be a lower survival to the adult stage. Earlier studies on caged and cut foliage essentially confirm this second observation. Generally, earlier studies suggest that only ca. 40%-50% of F-1 survive when compared with wild or NJSS strains. In the first study only ca. 20% of the F-1s survived to the adult stage after recovery, about a third the survival rate of NJSS or wild. In the second study, survival of the recovered F-1 was higher and was about 64% the survival of the wild P-1 and 74% of the NJSS strain.

POSITIONS OF THREE STRAINS OF GYPSY MOTH LARVAE AFTER PLACEMENT ON SEVEN OAK TREES

Figure 1

GROUND AND ADJACENT TREE

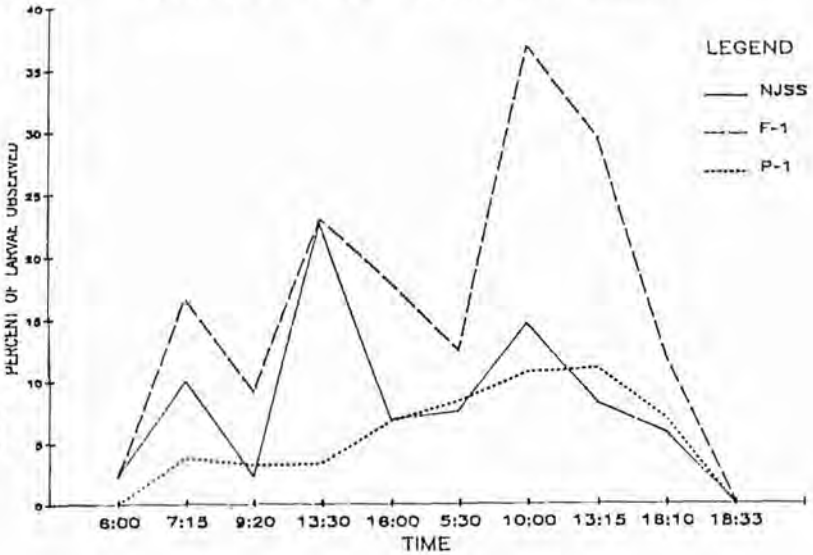


Figure 2

BOLE AND BURLAP

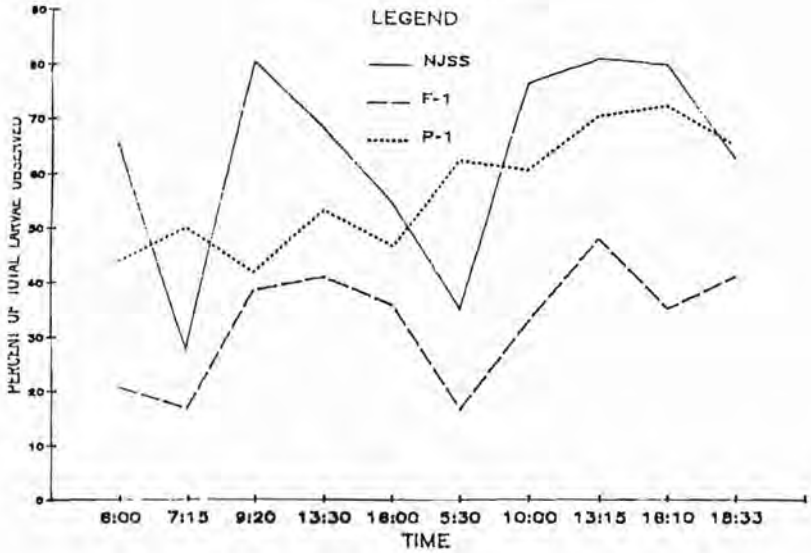


Figure 3

LEAVES

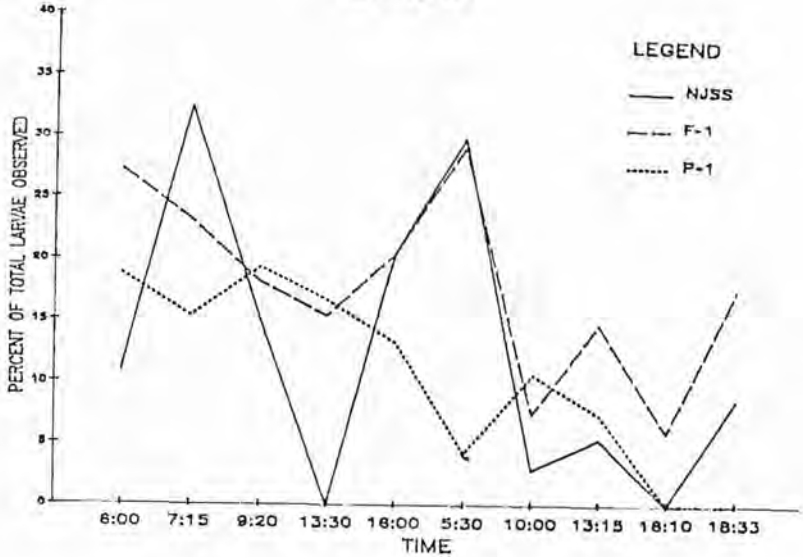
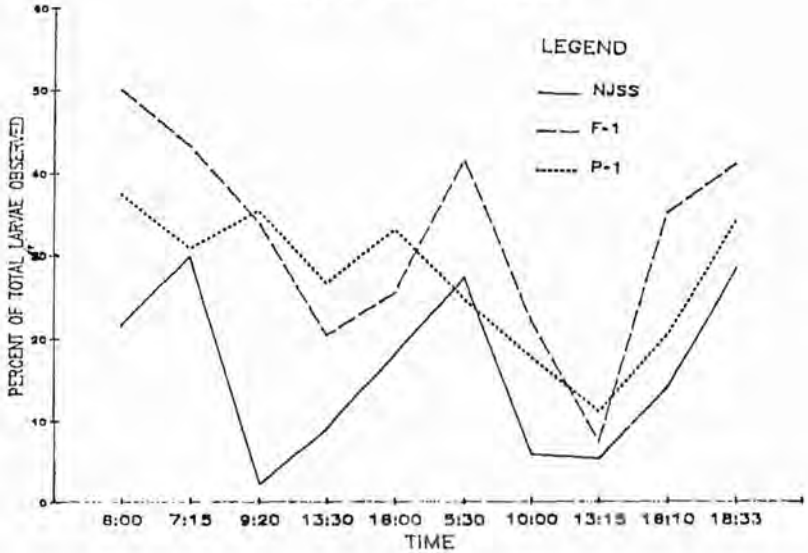


Figure 4

LIMBS AND TWIGS



POSITIONS OF THREE STRAINS OF GYPSY MOTH LARVAE CONFINED TO A SINGLE WHITE OAK TREE

Figure 5

GROUND RESTING

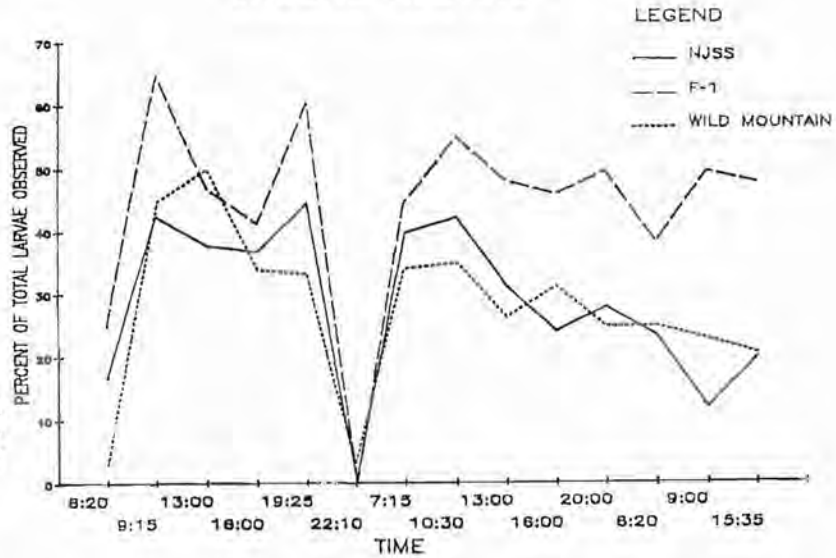


Figure 6

BOLE AND BURLAP

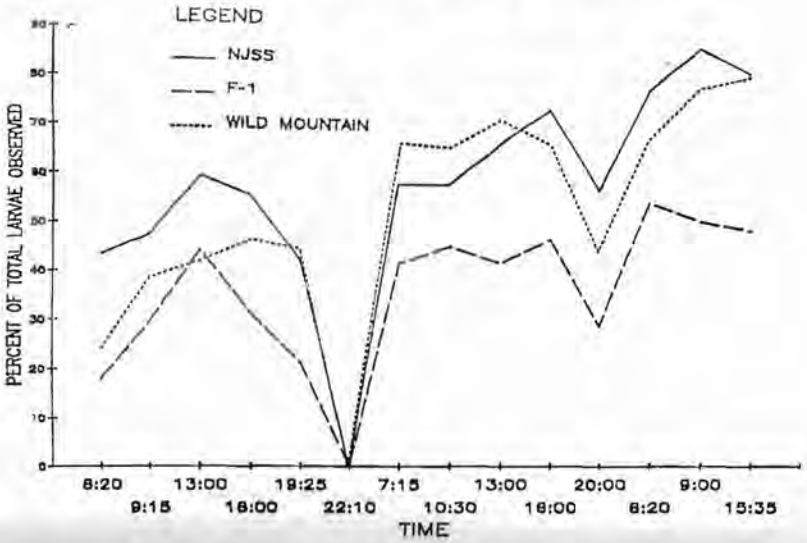


Figure 7

LOWER CROWN

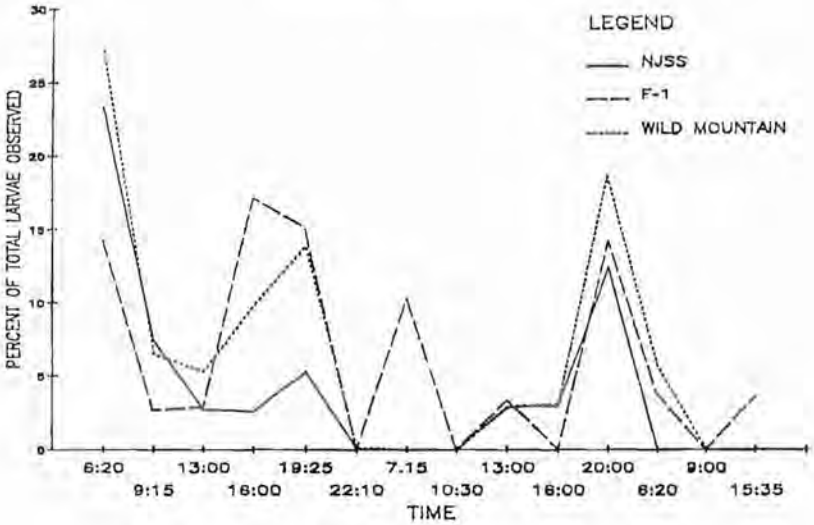
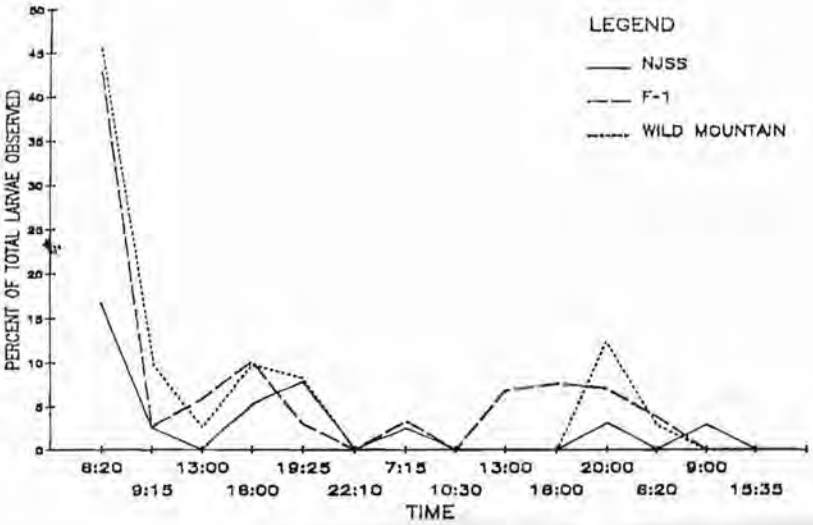


Figure 8

UPPER CROWN



Project Number: GM 6.2.6
Project Title: 1986 Disparlure Comparison Studies
Report Type: Final
Report Period: October 1, 1985 - September 30, 1986
Project Leaders: E. C. Paszek, C. P. Schwalbe, V. C. Mastro, B. A. Leonhardt

Hercon[R] Gypsy Moth Lure Tape containing (+) disparlure is used exclusively in gypsy moth survey traps. From 1981-84 companies submitted the following disparlure dispensers which were bioassayed: Zoecon - 8 rubber septa formulations, Albany - 6 hollow fibers and 2 PVC formulations and Sinclair Rush - 1 rubber septum formulation. The above commercial dispensers captured significantly fewer moths than the laminated Hercon dispensers.

In 1986 Trece Co. submitted five types of (+) disparlure pheromone dispensers for evaluation. These dispensers (manufactured by Consep, Inc.) are constructed of a pheromone reservoir sandwiched between a polymeric membrane and an impermeable backing. The polymeric membrane is semi-permeable and, theoretically, depending on the type of membrane used, the rate of release can be altered. The five types of dispensers submitted for evaluation were labeled types A, B, C, D and E. Although these types varied in the type of semi-membrane used in construction, all contained the same loading of (+) disparlure (i.e. 2.7 mg/dispenser). The objective of the study was to determine how the five types of Consep dispensers performed in comparison to the standard 1986 Hercon dispenser (lot D0426). The dispenser manufactured by Hercon is of a sandwich construction with two outside layer of 2 mil PVC plastic covering a reservoir containing 0.5 mg of (+) disparlure. The dimensions of this dispenser are approximately 1/8 inch x 1 inch.

Dispenser Test 1

Two methods were used to evaluate and compare the dispensers: a field bioassay and a chemical analysis of the residual content and emission rates of the lures. To determine how "fresh" and "aged" dispensers would perform, lots of each type were placed in a green house 1, 3, 5, 7 and 11 weeks before the field bioassay was conducted. A randomized complete block design was used for the field comparison. In all, 36 treatments (i.e., 6 wicks types x 6 aging intervals - 5 described above and fresh wicks) were compared in five blocks. USDA high capacity milk carton traps were used for testing all dispensers. Traps were placed on lines 50m apart and with a 50m spacing between traps. All traps were checked daily, cleared of moths and rerandomized 5 times (7/29 - 8/3/86). To complement the field bioassay, a chemical analysis of the residual content of a separate set of the six wick types was undertaken after they had been aged for the same six periods. Also, the lures used in the field bioassay were retrieved after completion of the test and analyzed for residual lure content. The emission rates from two formulations, Hercon (D0426) and Consep A, aged in the greenhouse for 0, 2, 4, 8 and 12 weeks were also measured using a laboratory air collection technique. Volatiles were collected for 6 hrs from individual wicks at a constant air temperature (35°C) and air flow (100 ml/min).

Dispenser Test II

Based on data from the field bioassay, a second test was conducted to further compare the "best" Consep formulation (A) with three Hercon formulations (1985, 1986 and a pre-1987 sample), and a standard 100 ug loading of Plimmer-Schwartz (+) disparlure on a cotton wick. The three Hercon formulations were all similar in wick construction (2 mil PVC sandwich), size (1/8" x 1") and loading 0.5 mg of (+) disparlure. The only difference was the source of the (+) disparlure. Again, to compare the effect of aging, the four formulations were held in a greenhouse prior to the test. The 1985 Hercon, 1986 Hercon and Consep A were aged for 2, 4, 6, 8, and 12 weeks and the 1987 Hercon was aged for only 2, 4, and 6 weeks. A similar randomized complete block design (5 blocks) was used for the field bioassay. In all, 24 treatments were tested which included an unbaited control trap. Because of the release characteristics of cotton wicks they were replaced daily. Traps were checked and rerandomized on four consecutive days.

Results and Discussion

Table 1 presents the results of the first field bioassay. Unaged 1986 Hercon dispensers captured significantly more males than any of the five Consep formulations of any age. Indeed, only when Hercon wicks which had aged 6 weeks are compared with similarily aged Consep dispenser (A and D) are differences not significant. A clearer picture of the results of this test are given on Figures 1, 2, 3, 4 and 5. Presented are means and S.E. for each dispenser of each age. Traps baited with Hercon dispensers consistently captured more males than traps baited with any of the five types of Consep dispensers. Regression analysis of the data demonstrates that there is not a significant difference between how the five types of Consep dispensers performed and that all were different from the Hercon formulation.

Chemical analysis of the residual content of unaged and aged lures (greenhouse for 1, 3, 5, 7 and 11 weeks) and lures removed from traps following the field biossay (aged 2, 4, 6, 8 and 12 weeks) revealed that the Consep wick formulations were all releasing at very similar rates. Respectively, for the Consep A, B, C, D and E formulations the release rates in mg/week were 0.15, 0.15, 0.13, 0.15 and 0.15 and 0.20. The release rate for the Hercon wick was 0.026 mg/week. From past studies, the release rates of the Consep formulation are known to be too great to maximize trap catch. Also, apparently the release rate from these five types of dispensers is not altered appreciably by changing the membrane type. Catch in Consep-baited traps only approached that of Hercon-baited traps when the reservoir of lure was nearly depleted and the release rate decreased.

Chemical analysis of effluent from the unaged and aged Hercon and the Consep A dispenser also supported trap catch and residual analysis data. The emission rate for the unaged Hercon was initially 0.12 mg/hr and decreased to 0.032 mg/hr for wicks aged 12 weeks. The unaged Consep A dispenser released at a rate of 1.14 mg/hr and, after aging for 12 weeks, the emission had decreased to 0.24 mg/hr.

Results of the second field bioassay are presented in Table 2. Traps baited with the three Hercon wick formulations performed similarly, however, traps baited with the unaged (0 week) 1985 formulation captured significantly fewer males than either the 1986 or 1987 Hercon formulations. This anomaly in performance of unaged 1985 Hercon dispensers was demonstrated earlier and described in the last annual report (GM 5.2.1). Trap catch in traps baited with the 1986 dispenser aged for 6, 8, and 12 weeks also captured significantly fewer males than traps baited with similarily aged 1985 dispensers. The evaluation of the preproduction sample of the 1987 Hercon formulation, although it was not possible to include lures aged for the longest periods, indicates that its performance is acceptable. Although catches for traps baited with the Consep A dispensers were generally lower than any of the other treatments, those baited with 6, 8 and 12 week aged lures did not capture significantly fewer males than the 1986 Hercon dispenser (Figure 2).

1986 Disparlure Comparison Test #1

Mean No. of males captured per trap per reading

Dispenser Type	Dispenser Age In Weeks 0	1	3	5	7	11
1986 Hercon	71.60 a	53.12 abcd	50.24 abcd	60.72 ab	51.52 abcd	54.88 abc
Consep - A	13.40 ij	19.84 ghi	33.16 e	41.52 bcde	33.36 e	38.28 cde
Consep - B	12.24 j	22.32 fgh	32.88 ef	32.60 e	37.24 cde	37.68 cde
Consep - C	14.92 hij	21.32 ghi	28.24 efg	34.88 de	38.56 cde	39.84 cde
Consep - D	13.80 ij	11.84 j	29.88 efg	40.36 bcde	41.68 bcde	42.76 bcde
Consep - E	15.48 hij	20.56 ghi	32.52 ef	38.80 cde	38.44 cde	37.88 cde

1./ Means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

2./ Five complete replicates were checked and randomized five times = 25 observations per treatment. Analysis was for a randomized complete block design using transformed data [log (n + 1)] actual means are presented.

1986 Disparlure Comparison Test #2

Mean Numbers Of Males Captured Per Trap Per Reading

Dispenser Type	Dispenser Age In Weeks 0	2	4	6	8	12
1985 Hercon	23.95 i	116.25 ab	139.45 a	120.80 ab	108.05 abc	108.70 abcd
1986 Hercon	107.55 abc	80.93 cdef	113.15 ab	74.50 efg	77.53 def	61.20 fg
1987 Hercon	110.20 abc	113.15 ab	91.65 bcde	92.95 bcde	-	-
Consep A	41.60 h	38.15 h	53.45 g	67.20 efg	64.75 fg	69.10 efg
Cotton 1/ wick	76.85 def	-	-	-	-	-
Blank	0.80 j	-	-	-	-	-

1/ Cotton wick charged with 100 ug of (+) disparlure (Plimmer-Schwartz standard) and replaced daily.

2/ Means followed by the same letter were not significantly different at the 5% level according to Duncan Multiple Range Test. Analysis was for a Randomized Complete Block using log (n+1), actual means are presented. Five complete replicates were read and randomized 4 times = 20 observation per drop.

Figure 1. DISPARLURE DISPENSER COMPARSION 1986

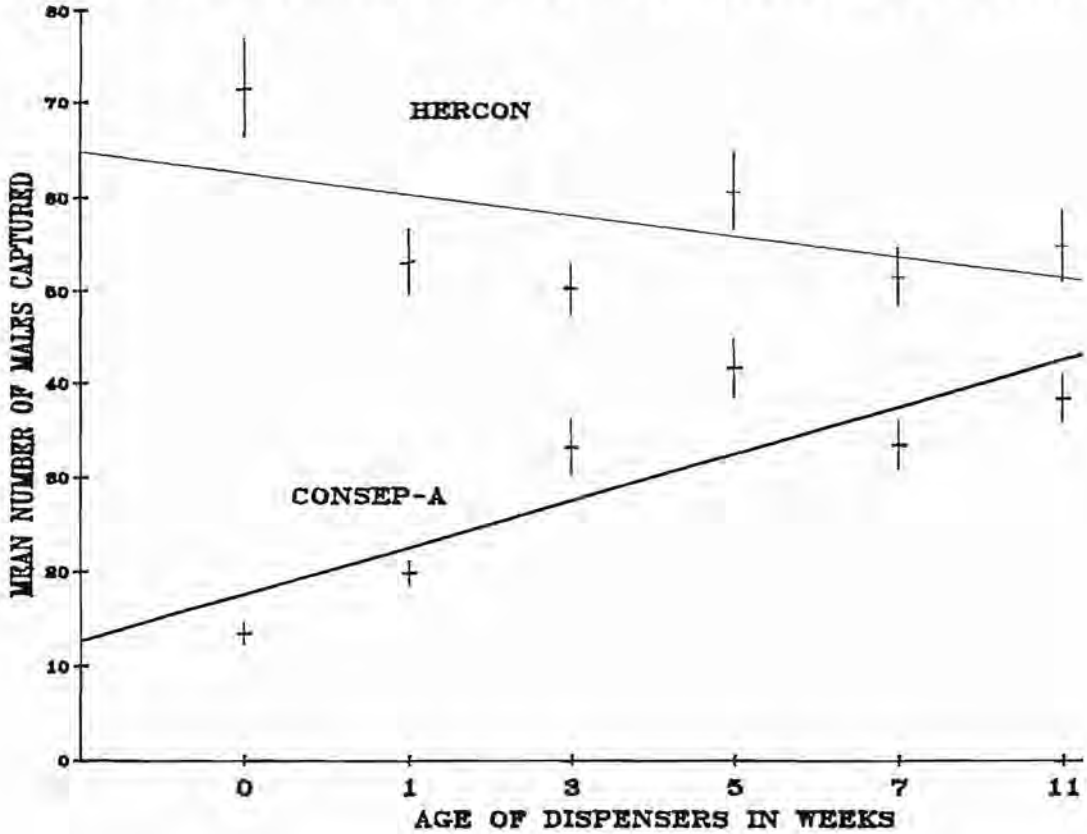


Figure 2.

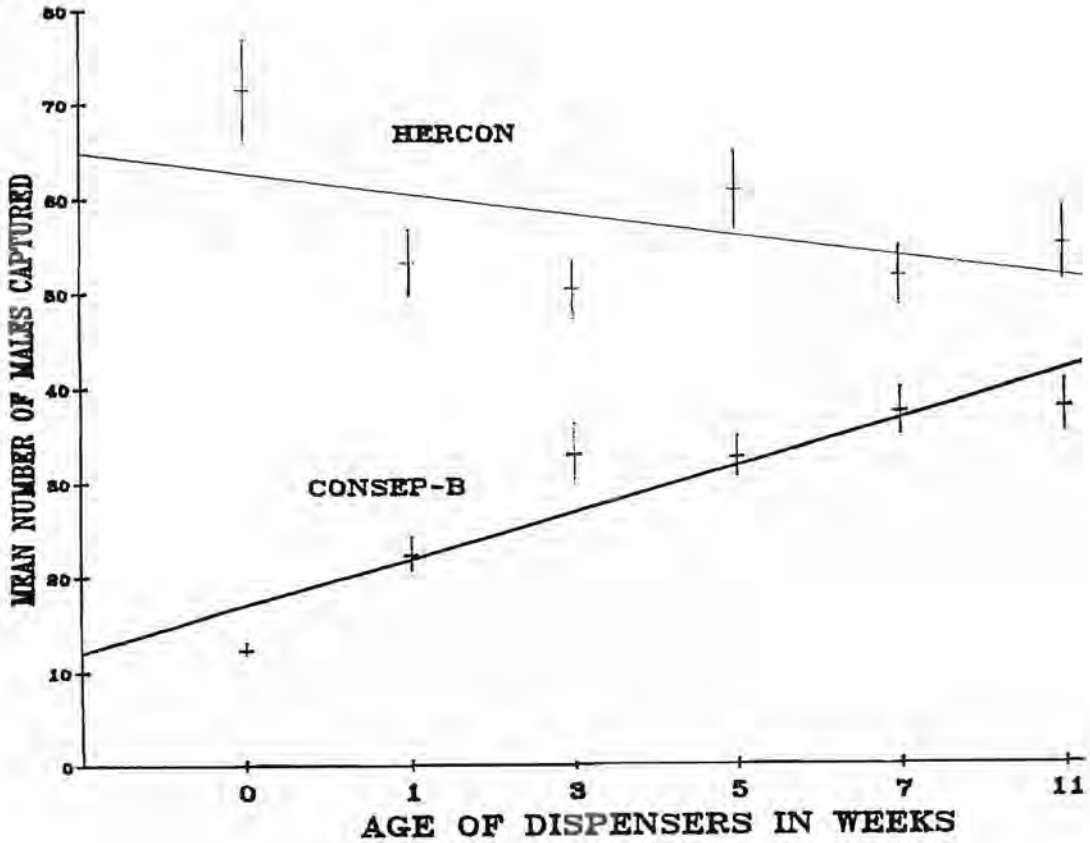


Figure 3. DISPARLURE DISPENSER COMPARSION 1986

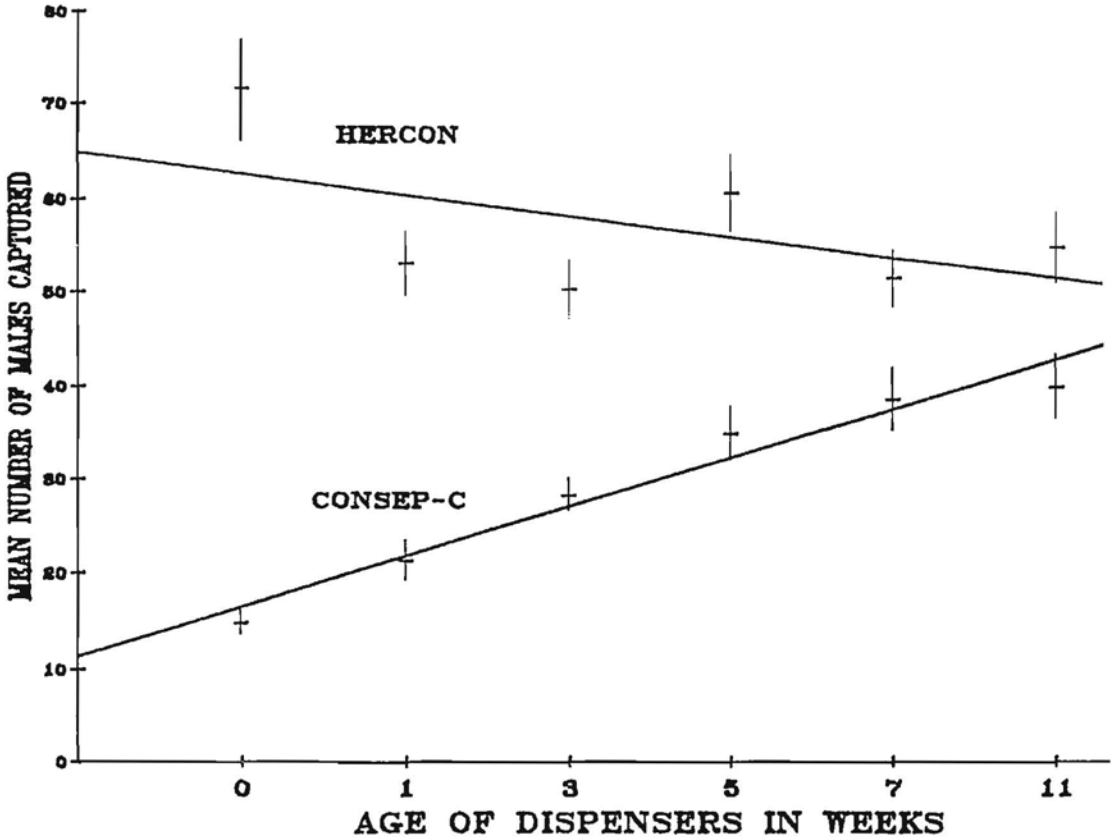


Figure 4.

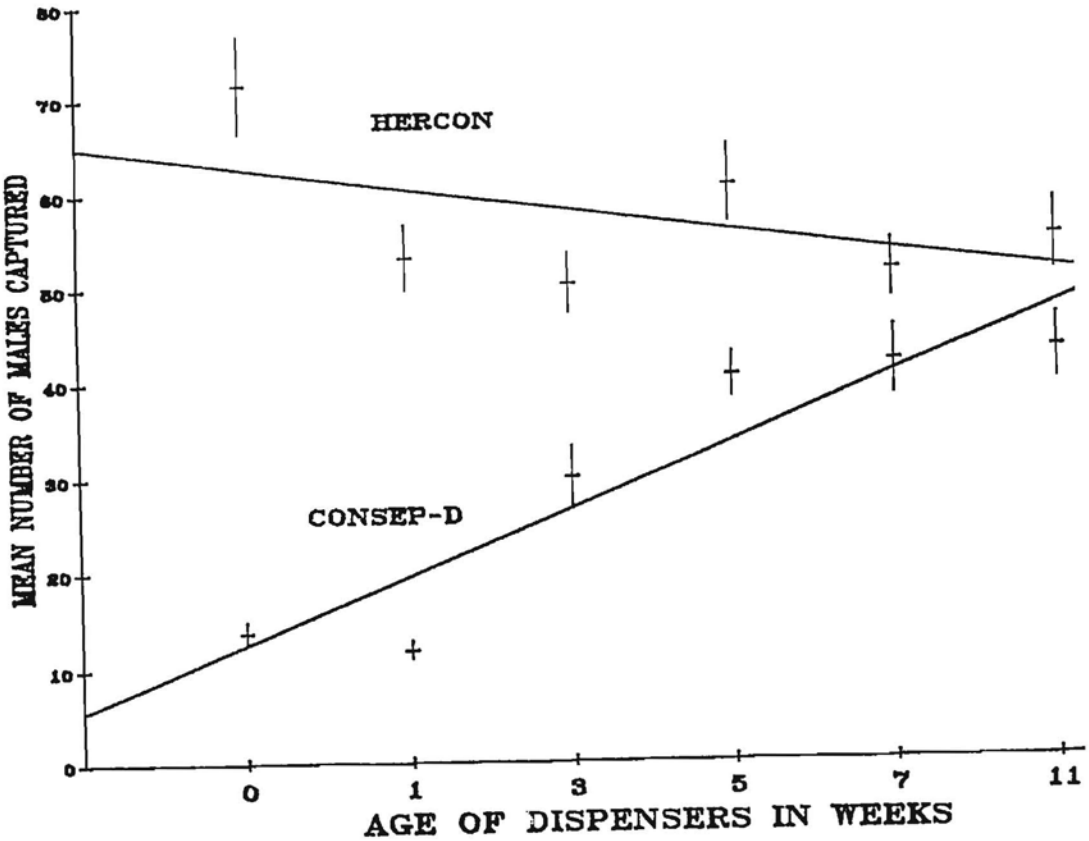
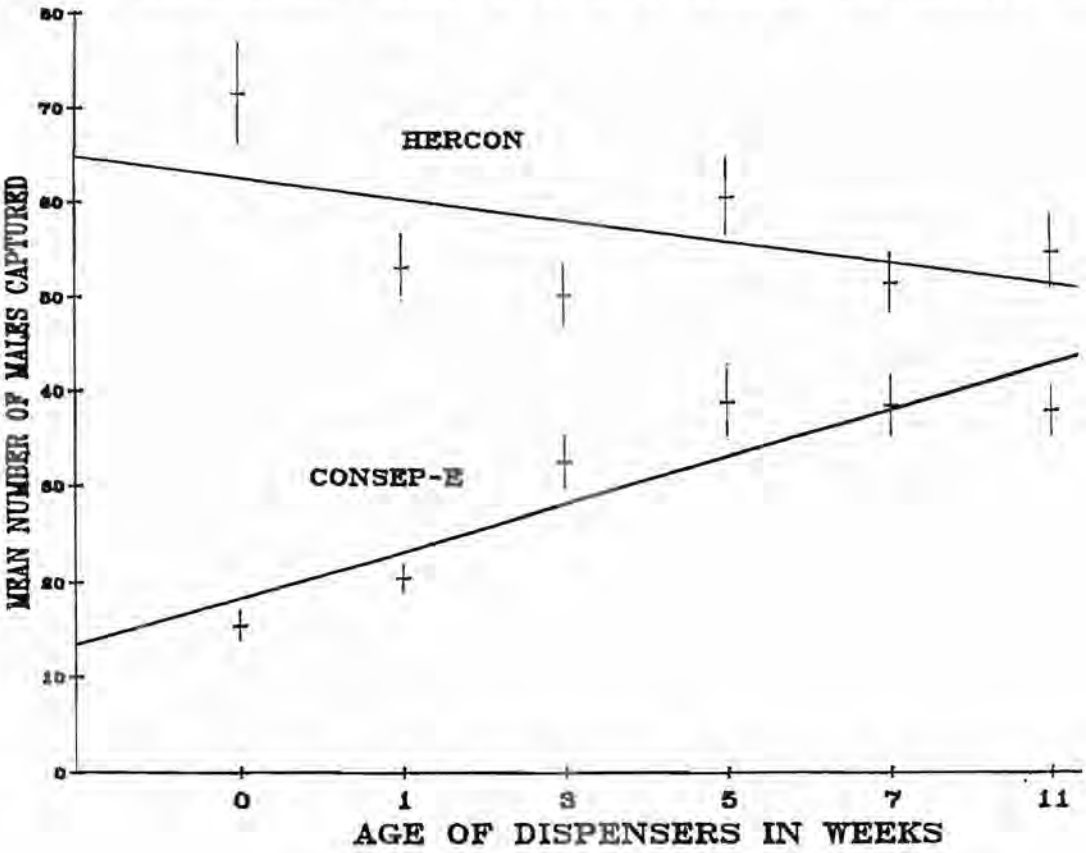


Figure 5. DISPARLURE DISPENSER COMPARSION 1986



Project Number: GM 6.2.7
Project Title: Delimination Trapping - Calibration of Trap Grids Using Releases of F_1 Steriles
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: V. C. Mastro and C. P. Schwalbe
Cooperators: R. Barth, Ohio Department of Agriculture; K. Drobnik, J. Carroll and B. Smith, APHIS, PPQ

Six sites were selected to study male capture patterns which result from a release of F_1 egg masses in delimitation trapping grids. Three sites were located in Ohio: Columbus, Reynoldsburg and Springfield, two in Indiana: Hanna and Rushville and the sixth site was located in Illinois: Elwood. At each site, five packets containing F_1 eggs (progeny of irradiated [10 krads] males x normal females) were stapled to host trees. Packets were then covered with simulated bark flaps and left in place until egg hatch was complete. Packets at the three Ohio sites contained 4,000 eggs, or a total of 20,000 eggs (i.e. 5 packets). All eggs placed in Ohio were from one production lot; QC-279. At the three remaining sites, (Hanna and Rushville, Indiana and Elwood, Illinois) 8,000 eggs were placed in each packet for a total of 40,000 eggs per site (i.e. 5 packets). Eggs for the last three sites were taken from another production lot, QC 305. Packets of non-dehaired eggs were prepared at the Otis Methods Development Center by weighing out the appropriate number of eggs (1 g. = 1,403.5 eggs) and placing these inside packets constructed of standard nylon window screening (18 mesh). Egg mass packets were left stapled in place until all hatch was complete when they were returned to the Otis Methods Development Center for counting of unhatched, embryonated and unembryonated eggs. Prior to shipment of packets, samples of each production lot were taken and held at the OMDC for comparison of hatch with those placed in the field.

Centered over each egg mass release site was a grid of (+) disparlure-baited delta traps with an inter-trap spacing of 350m (i.e. 36 traps/mi^2). Traps were placed before adult flight was anticipated and removed after flight was over.

Based on the counts of eggs remaining in the packets which had been placed in the various field sites, the numbers of neonates hatching at each site can be estimated (Table 1). As can be seen from the table, hatch was low. Normally, hatch from F_1 egg masses should be in the range of 35-40% ($\bar{X}$=41.8%). To project the number of adult males that would result from those neonates at each site, some assumptions have to be made and some known values have to be used.

1) An intrinsic rate of increase has to be estimated or, conversely, a larval and pupal mortality rate has to assigned. Past experience has shown that the rate of increase for various populations can range between a negative value and 15 or 20 X increase. Most often, however, the value for isolated populations appears to be approximately 3 X and, therefore, we will use this value in our calculations.

2) F_1 larval survival is ca 49% of the survival rate of wild, normal larvae. In laboratory and field feeding studies, this was the

lowest survival rate observed (worst case). The remaining 51% are either insects that die or do not develop in synchrony with the wild larvae.

3) Laboratory and field studies have shown that insects developing from F_1 egg masses are predominantly males (i.e. 2 males:1 female) while normal egg masses produce nearly a 1:1 sex ratio in the adult stage.

4) An "average" wild egg mass contains 600 eggs, 84.3% of which hatch (i.e. 506 neonates). This is based on samples from four healthy populations.

To calculate the number of males that should have been captured at any site, the following calculations apply. For example, in the Elwood study site, a total of 6,811 neonates hatched from all packets. This is equivalent to the number of neonates produced by 13.46 wild egg masses.

$$\text{No. of wild egg mass equivalents} = \frac{\text{Total neonates hatched}}{\text{average number of neonates produced by one wild egg mass}} = \frac{6{,}811}{506} = 13.46$$

If the intrinsic rate if increase of 3x is applied, 13.46 wild egg masses should have resulted in 40.4 males and 40.4 females, or a total of 81 adults.

$$13.46 \times 3 = 40.4$$

However, compared to the wild strain, survival to the adult stage of F_1 is only ca. 49%.

$$81 \times 0.49 = 40.38\ F_1 \text{ adults}$$

And the sex ratio of F_1 adults is skewed in favor of males (2 males:1 female or 66% males). Therefore, the following caluclation is made:

$$40.38 \text{ (adults)} \times .66 = 26.89 \text{ or ca. } 27 \text{ males.}$$

From previous field studies in which releases of males were made into the center of grids of 36 traps per square mile, a mean of 7.8% of them are captured. Applying this to the calculated total number of males, we can calculate the expected number of males captured:

$$27 \text{ (total males)} \times .078 = 2.1 \text{ expected number captured.}$$

From data presented in Table 1, observed male capture was very similar to that expected, indicating a high degree of predictability in male recoveries following F_1 release. Additionally, these results add credibility to the practice of estimating insect density based upon trapping results.

Table 1. Expected and observed numbers of males captured at six locations after releasing sterile F_1 eggs in the center of a grid of 36 delta traps per square mile.

Release Sites	Source of Eggs (Prod. lot)	Eggs Remaining in Packets		Estimated no. of Neonates	Percent Hatch	Estimated no. males Surviving	Expected no. males Trapped	Observed no. males Trapped
		Embryonated	Unembryonated					
Elwood, IL	QC 305	25,935	7,254	6,811 1/	17.03	27	2.1	1
Rushville, IN	QC 305	23,355	9,632	7,013 1/	17.53	27	2.1	1
Hanna, IN	QC 305	22,311	6,919	10,770 1/	26.93	41.7	3.3	2
Columbus, OH	QC 279 4/	5,863	2,203	6,556 2/	32.78	25	2.0	2
Reynoldsburg, OH	QC 279 5/	6,451	2,225	7,450 2/	37.25	29	2.3	2
Springfield, OH	QC 279	9,672	3,943	6,385 2/	31.93	25	2.0	1
Preshipment								
lab sample	QC 305	1,549	958	335 3/	11.79	--	-	-
	QC 279	664	354	380 3/	27.18	--	-	-

1/ Number neonates = 40,000 - (no. embryonated + no. unembryonated eggs)

2/ Number neonates = 20,000 - (no. embryonated + no. unembryonated eggs)

3/ Actual counts lot QC 305: Sampled twice at 110 and 129 days of chill
lot QC 279: Sampled once after 115 days of chill.

4/ Samples from only 3 of the 5 packets available for evaluation

5/ Samples from only 4 of the 5 packets available for evaluation

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, 1985 - September 30, 1986
Report Type:	Interim
Project Leaders:	J. A. Tanner, J. J. Baker, H. A. Hamilton and B. A. Fontes

The purpose of this project is to monitor the development of the laboratory strains and to determine if it falls within acceptable ranges. Data are collected for each strain/generation and are used to detect changes from normal development so that corrective action can be taken.

Budgetary restraints have forced us to limit collection of developmental data to those insects used to maintain each strain (colony). Currently, only the New Jersey strain is being maintained, but in the near future, we plan to add several wild or near wild strains.

Developmental data are collected only on larval development, female pupal weights, sex ratio, pupal yields, adult female emergence and deformity, and female fecundity. The data are collected using the methods described by Tanner, et. al. in the 1983 and 1984 Annual Reports.

The yield of colony eggs/colony mating was also calculated. This gives a reasonable estimate of the fecundity of the colony. It reflects not only the ability of the adults to emerge and mate, but also the number of eggs the females can deposit.

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)(Tanner and Baker, 1985) of 11 day old larvae. The larvae were hatched from egg mass core samples taken 21 days prior to their scheduled use for colony maintenance. Core samples were taken weekly from 100 masses and based on the highest MLS's, 18 masses were selected for use in colony maintenance.

This method was first used in 1985 with New Jersey F_{28} egg masses. Figure 1 shows the distribution of sampled masses among the different MLS groups for New Jersey F_{28} and F_{29} egg masses (see Tanner and Baker, 1985 for the description of each group). The figures show a reduction in the percentage of masses producing "straggling" larvae (MLS 1.00 - 1.25) from 8% in the F_{28} to 1% in the F_{29}. The figure also shows that more F_{29} egg masses gave rise to higher MLS than F_{28} egg masses. The higher MLS indicates that more larvae are developing at a fast rate.

The performance and reproductive data for F_{28} and F_{29} colony insects are presented in Table 1 and 2 respectively. Selecting for "non-straggling" larvae did not seem to have any overt effect on the growth and reproduction of F_{29} insects. The F_{29} tended to develop a little faster, have a slightly higher adult female emergence and deformity, and produce a higher number of eggs per colony mating than F_{28}, however, data must be collected over several generations to identify any trend.

Table 1. Performance of New Jersey Colony Insects

Parameter	Generation[1/] 28 (N=640) mean	SE	29 (N=647) mean	SE
Larvae per cup	9.8	.06a	8.8	.04b
Mean larval stage[2/]	2.9	.01a	2.8	.01a
Female pupal weights (GM)	2.2	.03a	2.2	.04a
% survival neonate to pupae	92.5	1.2a	91.2	1.2a
Developmental time to 50% pupation (DI50)				
female	27.0	.05a	26.6	.05b
male	29.0	.05a	28.7	.04b
Sex ratio, M:F	1.0:1	.02a	1.0:1	.02a
% adult female				
emergence	88.6	2.4b	95.6	0.8a
deformity	4.4	0.8b	6.6	0.8a

1/ Generation 28 - started March 25, 1985 through November 20, 1985.
Generation 29 - started November 27, 1985 through August 13, 1986.

2/ 9th Post-neonate infest date.

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1/ Generation 28 - started March 25, 1985 through November 20, 1985.
Generation 29 - started November 27, 1985 through August 13, 1986.

2/ 9th Post-neonate infest date.

Table 2. Reproductive data of New Jersey colony insects.

Parameter	Generation[1/] 28 (N=640) mean	SE	29 (N=647) mean	SE
% of females depositing egg masses	80.9	3.0a	84.7	2.7a
# eggs - deposited per female	95.8	22.4a	99.6	19.1a
# colony eggs per colony mating	762.0	24.2a	873.0	23.5a

1/ Generation 28 - started March 25, 1985 through November 20, 1985.
Generation 29 - started November 27, 1985 through August 13, 1986.

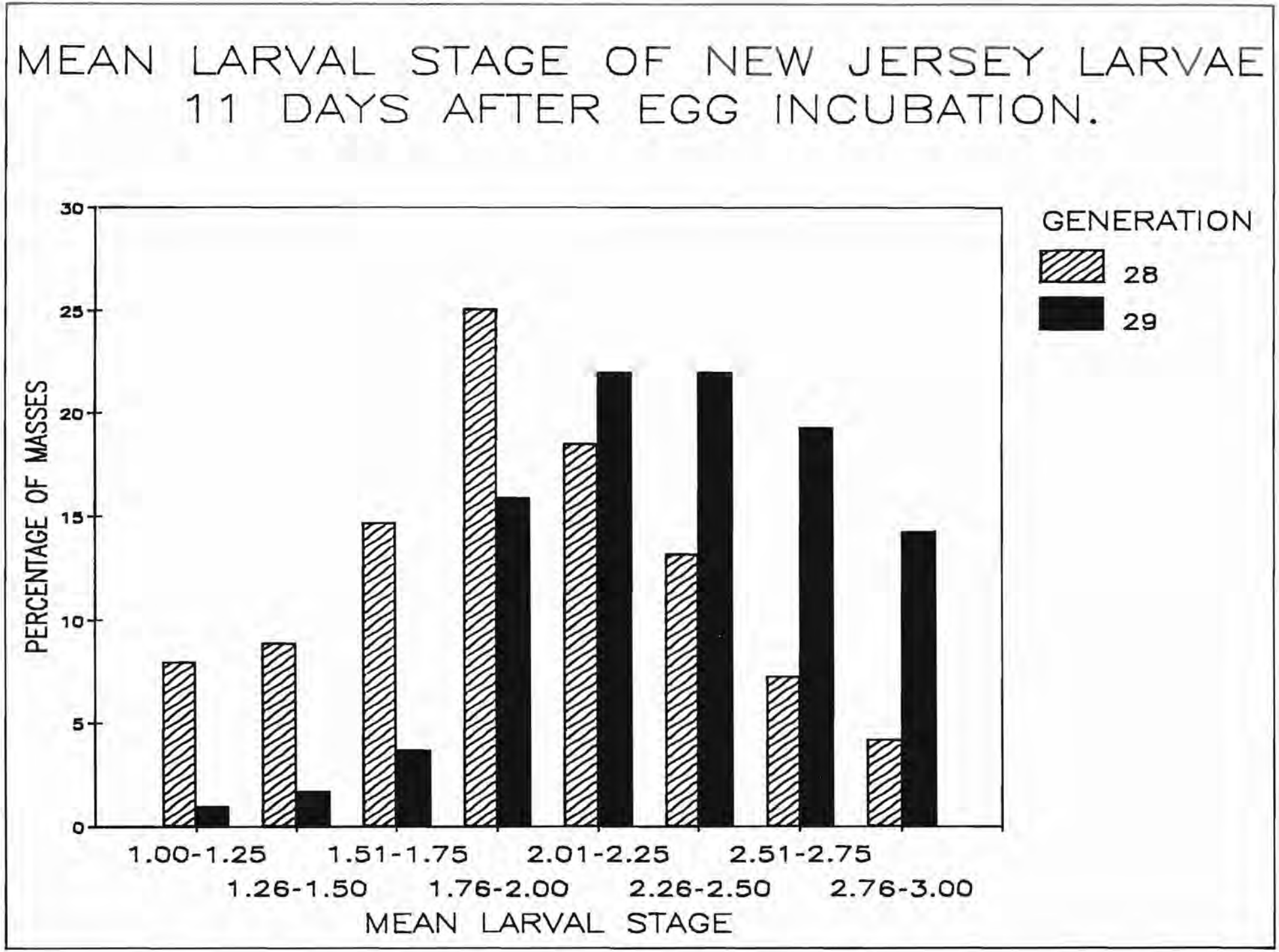
References Cited

Tanner, J. A., B. P. Weeks and M. Palmeri. 1983. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1982 - September 30, 1983:127-135.

Tanner, J. A., J. J. Baker and M. Palmeri. 1984. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1983 - September 30, 1984:77-79.

Tanner, J. A., and J. J. Baker. 1985. Evaluating the development and reproduction of insects produced in the Otis Metnods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1984 - September 30, 1985:117-121.

Figure 1.


MEAN LARVAL STAGE OF NEW JERSEY LARVAE
11 DAYS AFTER EGG INCUBATION.
GENERATION
28
29
PERCENTAGE OF MASSES
0
5
10
15
20
25
30
1.00-1.25
1.26-1.50
1.51-1.75
1.76-2.00
2.01-2.25
2.26-2.50
2.51-2.75
2.76-3.00
MEAN LARVAL STAGE

Project Number: GM 3.3.1
Project Title: Development and Evaluation of Improved Rearing Techniques
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: J. A. Tanner, J. G. R. Tardif, J. J. Baker, H. Hamilton and B. A. Fontes

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

The F_1 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.

In the last report (Tanner, et. al., 1985), we began to evaluate the possibility of replacing the standard size grocery bag, used as the mating container in the F_1 sterile male program, with a compact Rigid box (32"L X 3"W X 3"H, 040 plain chipboard). This box would enable us to place 16,800 matings on a mating cart (5'6"h X 3'10"d X 2'11"W) compared to only 3600-4800 mating now possible with the bags. Fewer mating carts would be needed, thereby freeing up rearing area for larval production.

In the last report we showed that matings done in the boxes produced a considerably higher number of eggs/mating (based on weight), and that the percent embryonation was similar for the two containers. The percent hatch was not available at the time, but is now presented in Figure 1.

Mating F_1 sterile male adults in the Rigid boxes resulted in eggs that had a slightly lower percent hatch than adults mated in bags. The reduction in hatch was 5% for eggs chilled 120 or 180 days and only 1% for 150 day chilled eggs, however, this loss in hatch would be more than offset by the increased number of insects that could be reared in the rearing facility. These Rigid mating boxes were used during the 1986 F_1 program.

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°C, hatch coincidentally with wild eggs; F_1 eggs held longer than 180 days will hatch within the refrigerator.

Wild eggs begin hatching 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 1985 laboratory report (Tanner, et. al.), test results were presented that indicated that the F_1 production "window" could possibly be lengthened by either extending the chilling period or extending the embryonation period.

The normal maximum chilling period is 180 days at 7°C. The chilling period could be extended to 210 days if the eggs were acclimated for 30 or 60 days at 7°C then chilled 180 or 150 days at 5°C. The percent hatch was similar to eggs chilled 150 to 180 at 7°C and there was no premature hatch within the refrigerator that normally occurs when eggs are chilled longer than 180 days at 7°C. The hatch profile was not available in the 1985 report and is now presented in Figure 2. The hatch profile for the acclimated eggs was similar to the profile found with "normally" chilled eggs. Almost all the hatch occurred over a 3 to 4 day period in all the treatments, however, the time to initial hatch was 1 to 2 days later in the acclimated eggs. If this delay should be a problem, it can be eliminated by holding acclimated eggs at ambient temperatures for 1 or 2 days before release.

The second way to extend the production "window" is to increase the embryonation period while keeping the chilling period at 150 to 180 days. Present protocol requires the eggs to be embryonated 28 to 35 days at 55% relative humidity. Last years hatch data indicated that eggs could be embryonated up to 112 days with only a minor loss in hatch if the eggs were embryonated under high humidities (80%+). The hatch profile was not available in the 1985 report and is now presented in Figure 3. The hatch profile for eggs embryonated 28 to 112 days at 80%+ humidity was similar to normal embryonated eggs (28 days, 55% RH). Increasing the embryonation period to 112 days would increase the production "window" by 84 days.

In the previous tests, the standard New Jersey strain was used. These were preliminary tests to determine which treatments to evaluate with F_1 sterile eggs. This evaluation was done in 1986 using different combinations of the best embryonation and acclimation-chilling periods. The hatch of the F_1 eggs and the subsequent growth of the larvae were compared with F_1 eggs embryonated 28 days at 55% RH and chilled 180 days at 7°C (control). The different embryonation, acclimation and chilling periods used are presented in Table 1. The total length of the combined embryonation, acclimation and chilling periods are presented in Table 2. The lengths of the combined periods ran from a minimum of 206-208 days (control) to a maximum of 337 days. If we can hold the F_1 eggs for 337 days, the production "window" could be increased by 131 days to 222 days

Table 1. The different embryonation, acclimation and chilling periods evaluated to determine the maximum period of time that F_1 eggs can be held prior for field release.

	Days Embryonated (% RH)									
	28(55%)		28(80%)		56(55%)		84(80%)		112(80%)	
Acclimation-Chill Temperature (°C)	7	5	7	5	7	5	7	5	7	5
Acclimated eggs					30	120	30	120	30	120
					30	150	30	150	30	150
	30	180	30	180	30	180	30	180	30	180
	30	195	30	195	30	195	30	195	30	195
Non-acclimated eggs					150	0	150	0	150	0
	180	0	180	0	180	0	180	0	180	0
	210	0	210	0	210	0	210	0	210	0
					225	0	225	0	225	0

1/ Control - chilled 180 days at 7°C with no acclimation period.

Table 2. The combined length (days) of the embryonation, acclimation and chilling periods evaluated to determine the maximum period of time that F_1 eggs can be held prior to field release.

Total days chilled	Days Embryonated (% RH) 1/					
	28(55%)	28(80%)	56(55%)	56(80%)	84(80%)	112(80%)
150 (30-120, 150-0)2/			206	206	234	262
180 (30-150, 180-0)	208 3/	208	236	236	264	292
210 (30-180, 210-0)	238	238	266	266	294	322
225 (30-195, 225-0)	253	253	281	309	337	

1/ length of time in days = length of embryonation period plus the length of the acclimation-chilling period.

2/ (days at 7°C - days at 5°C)

3/ Control-chilled 180 days at 7°C with no acclimation.

At the present time, only the percent hatch has been determined and is presented in Table 3. The data indicate that if the total days of acclimation-chilling is less than or equal to 180 days, it is best to continually chill the eggs at 7°C. However, if the total length of chilling is to be longer than 180 days, it is best to acclimate the eggs 30 days at 7°C, then chill them at 5°C. The data also indicate that high humidity (80%) during the embryonation period becomes critical when the eggs are embryonated 56 or more days. Eggs embryonated 28 days are neither benefited nor harmed by the high humidity.

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

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. Non-acclimated eggs were chilled continuously at 7°C.
3/ Control

The minimum, acceptable, percent hatch of the F_1 eggs is now set at 20% but a 15% hatch may be acceptable in the future (Mastro, personal communication). If we accept the 20% minimum hatch level, the production "window" could be increased by 26 to 30 days. However, if we accept the 15% minimum hatch level, the production "window" could be increased by 45 to 58 days.

Table 4. Increase (days) in the F_1 sterile male production "window" by selected combinations of embryonation-acclimation-chilling periods.1/.

	Length (days)						
Embryonation (% RH)2/	28(55)3/	84(80)	56(80)	28(55)	28(80)	84(80)	56(80)
Acclimation (7°C)	0	0	0	30	30	0	30
Chill (°C)4/	180(7)	150(7)	180(7)	180(5)	195(5)	180(7)	180(5)
Total pre-egg incubation period5/	208	234	236	238	253	264	266
Increase in the F_1 production "window"		26	28	30	45	56	58
Percent hatch	25.1	30.1	27.1	25.1	17.0	15.7	16.7

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 F_1 production "window" = pre-egg incubation period of treatment - pre-egg incubation period of control.

The hatch profile and larval growth data must be evaluated before any of the treatments listed in Tables 4 and 5 could be recommended for use in the F_1 sterile male program. Those data will be presented in the next report.

The percent hatch of the F_1 partially sterilized eggs produced during the 1985 program was consistently higher for all chilling periods than the eggs produced during the 1984 program (Figure 4). The larvae used in the 1984 program may have been of lower quality because there was a higher percentage of "straggling" larvae (Tanner and Baker, 1985) than in the 1985 program. The eggs from the 1984 program were embryonated 28 days at 50-55% RH while the eggs from the 1985 program were embryonated 28 days at 75-80%, however, as the data from Table 3 indicate, the hatch of F_1 eggs embryonated 28 days is not effected by the relative humidity.

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. G. R. Tardif, J. J. Baker, and M. C. Flynn. 1985. Development and Evaluation of Improved Rearing Techniques. October 1, 1984 - September 30, 1985:122-135.

FIGURE 1. THE EFFECTS OF DIFFERENT MATING-EMBRYONATION CONTAINERS ON THE % HATCH OF EGGS DEPOSITED BY GYPSY MOTH FEMALES MATED TO MALES EXPOSED TO 10 KRADS.

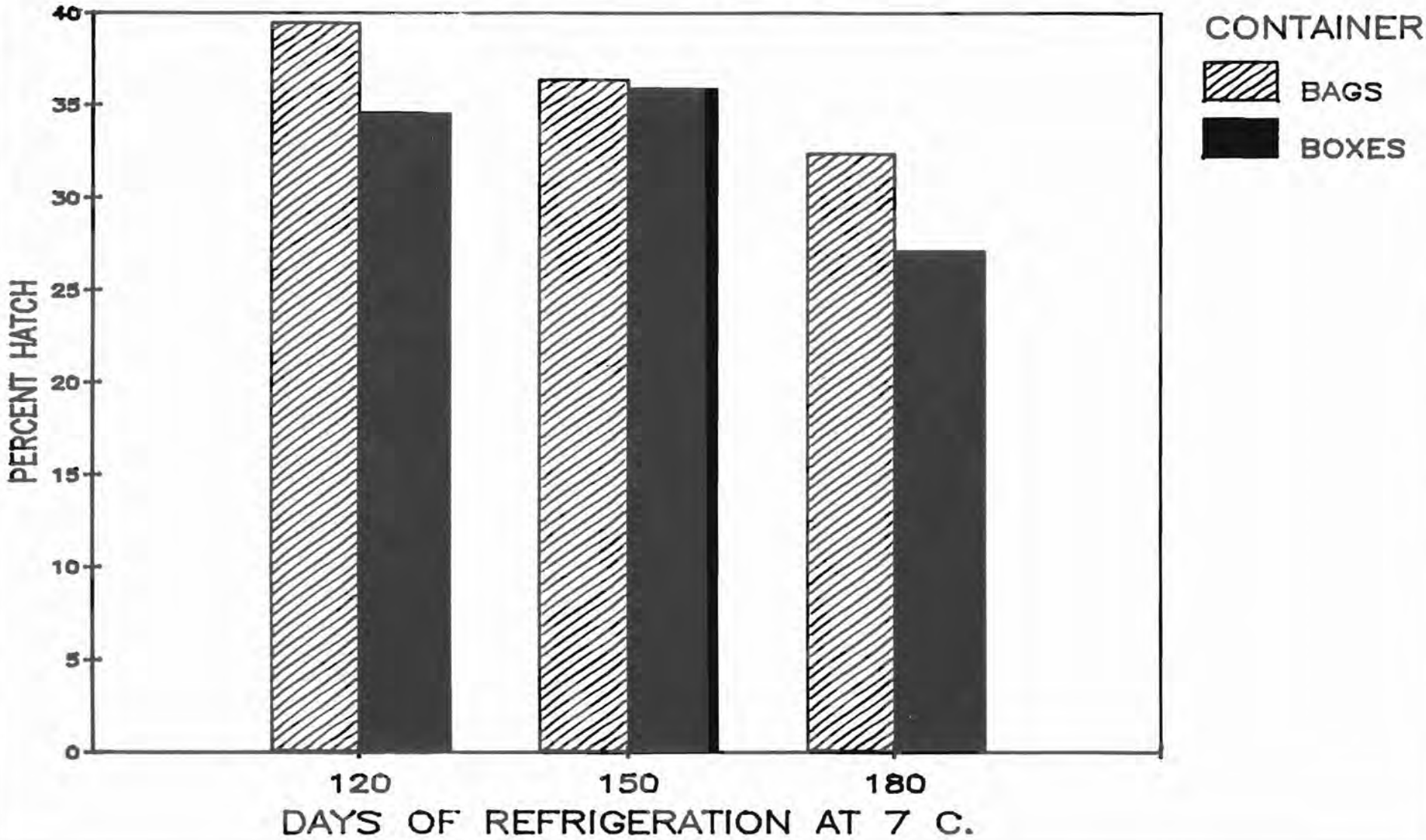


FIGURE 2. THE HATCH PROFILE OF NEW JERSEY EGGS CHILLED AT 5C. FOR 150 OR 180 DAYS AFTER A 30 OR 60 DAY ACCLIMATION PERIOD AT 7C.

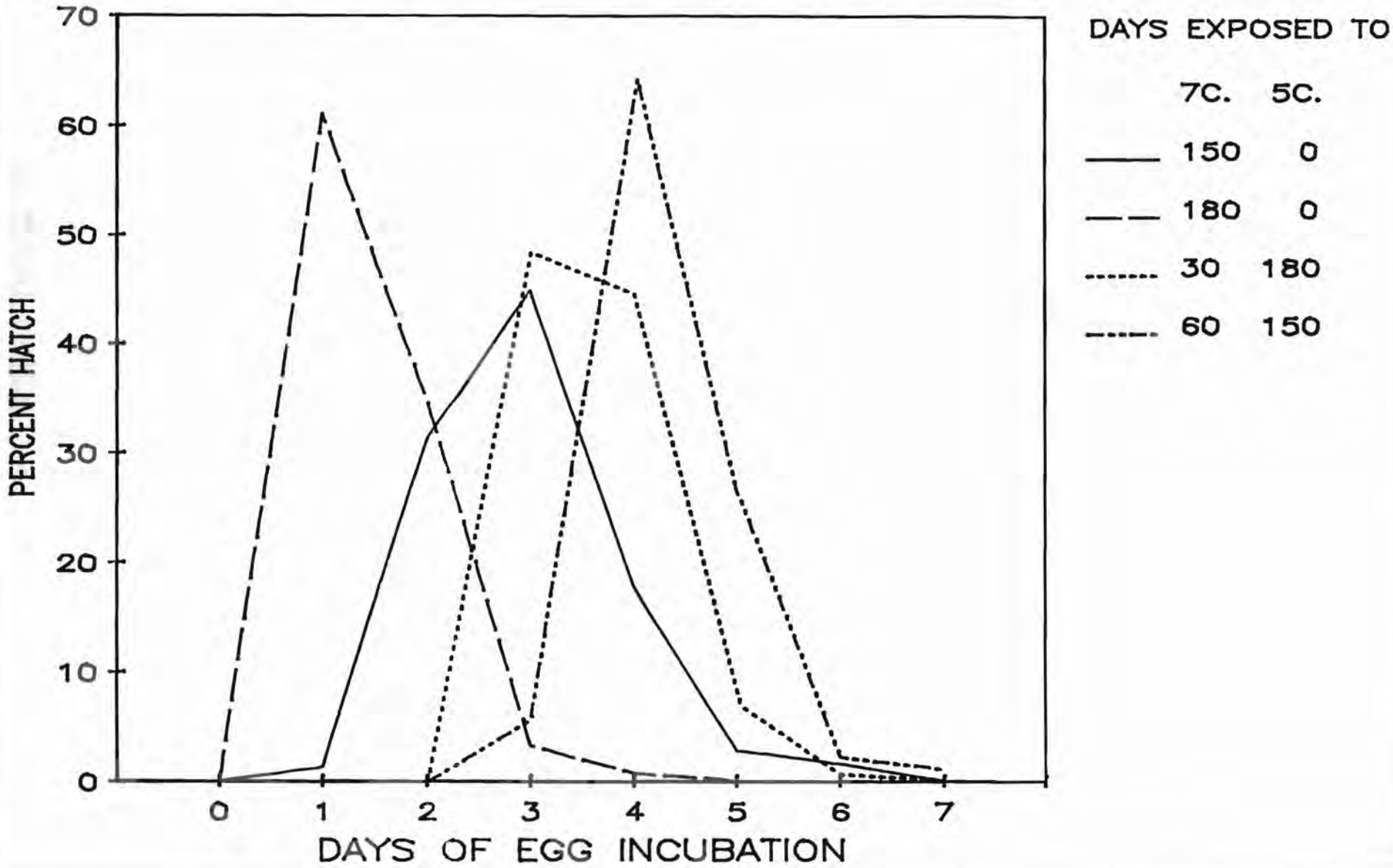


FIGURE 3. THE HATCH PROFILE OF NEW JERSEY EGGS EMBRYONATED 28 TO 56 DAYS UNDER HIGH HUMIDITY(80%+) OR 28 DAYS UNDER 55% HUMIDITY(CONTROL).

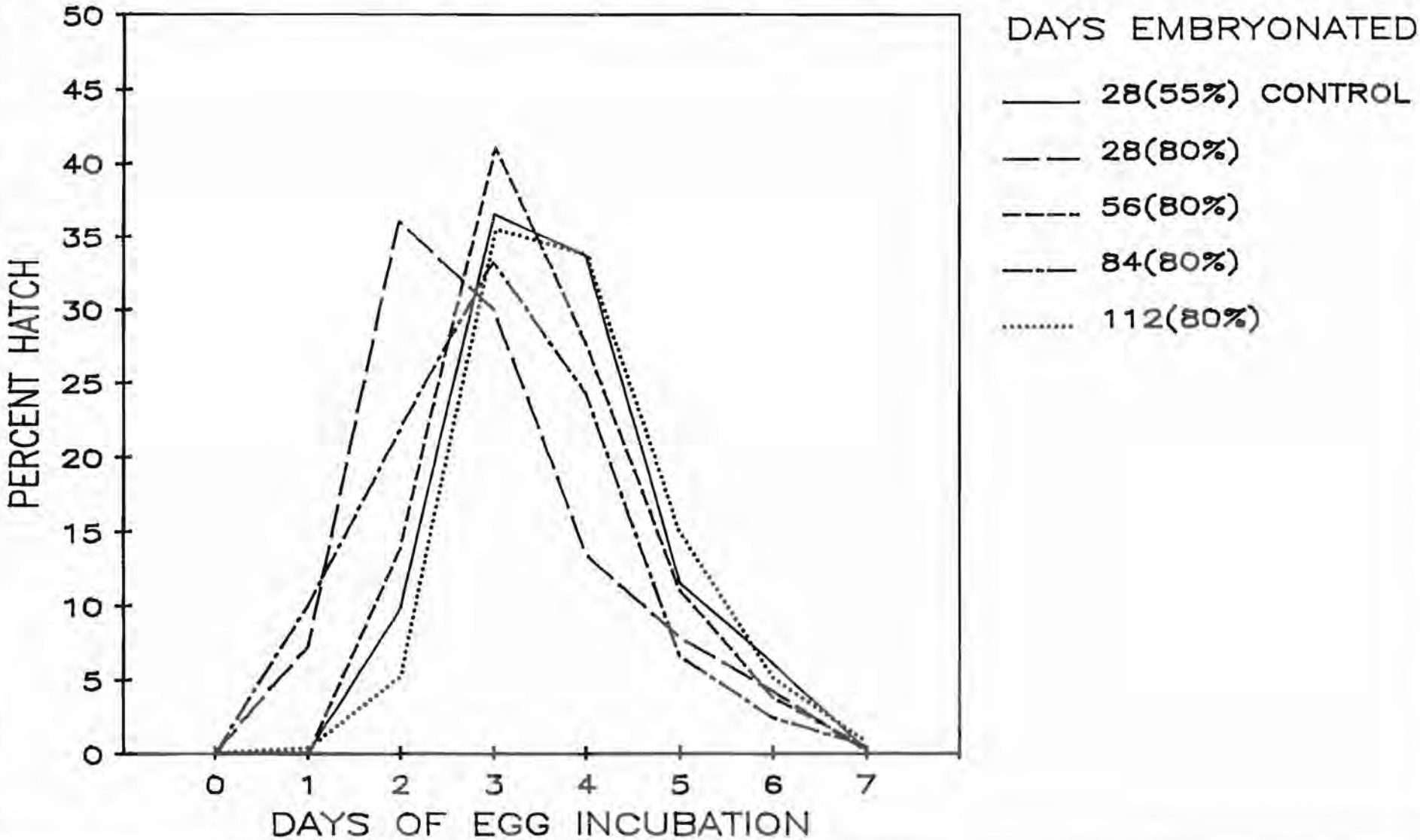
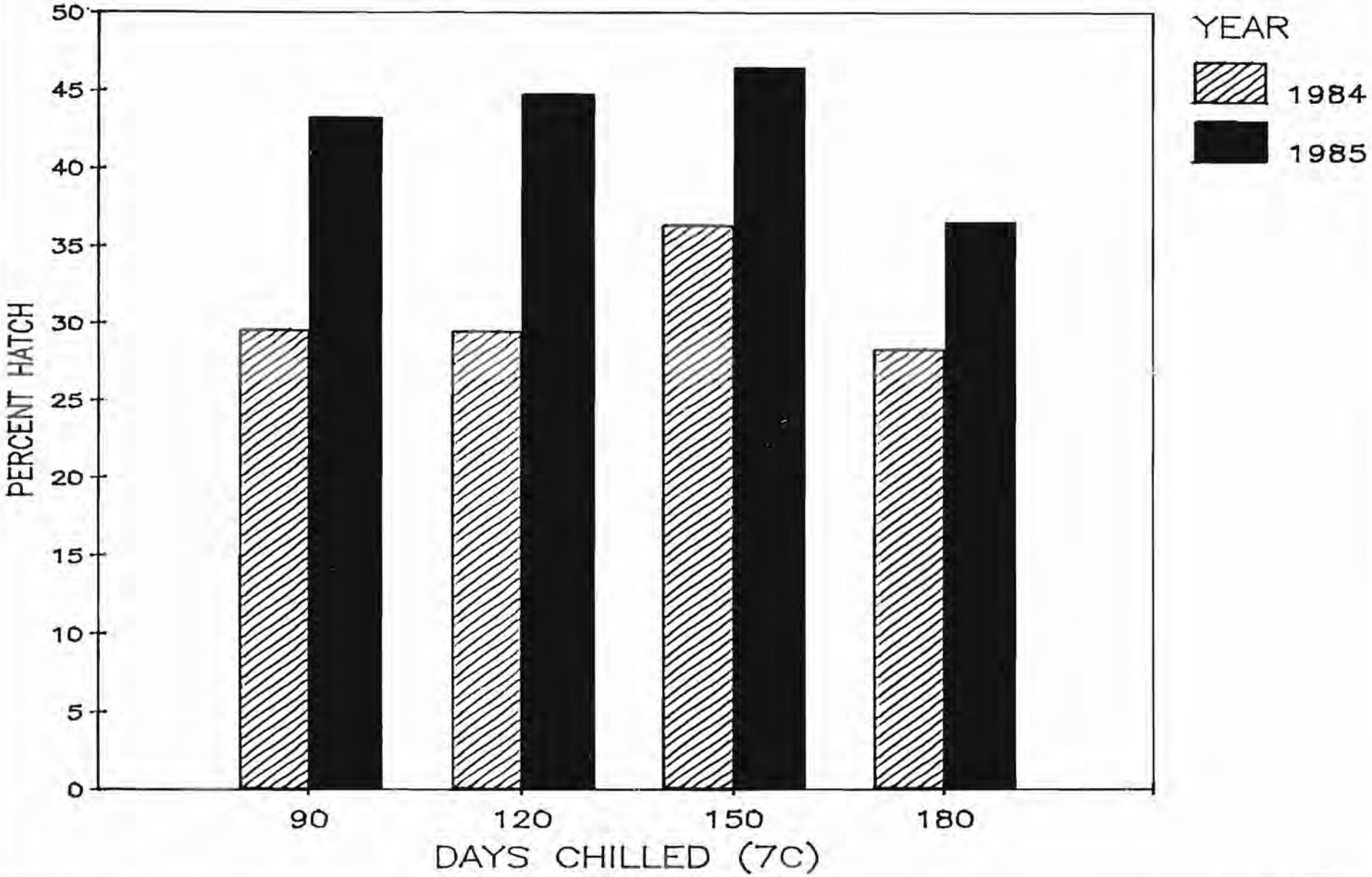


FIGURE 4. THE PERCENT HATCH OF F1 PARTIALLY STERILIZED EGGS PRODUCED IN THE FALL OF 1984 AND 1985.



Project Number: GM 4.3.1
Project Title: The Number of Instars in the New Jersey Standard Strain
Report Period: October 1, 1985 - September 30, 1986
Report Type: Final
Project Leaders: J.A. Tanner, J.J. Baker, and B.A. Fontes

Introduction

In the F_1 sterile male program, male and female pupae are routinely harvested four days after 10% male pupation has occured (Tanner and Weeks, 1981). Early in the 1984 rearing program, it was noticed that more female larvae (therefore, fewer pupae) were present at the time of pupal harvest than had been observed in the 1983 program. This resulted in a considerable reduction in the number of F_1 matings (since few females were available) and quickly forced the abandonment of the 10% male pupation plus four days" harvest trigger. In order to meet the production schedule, a time consuming "double harvest" technique was implemented (each container was harvested twice, at 7 day intervals).

The New Jersey laboratory strain used in the F_1 sterile male program was previously observed to produce few six-instar type female larvae (Bell, personal communication). However, in tests conducted in 1984, almost 33% of the female larvae were of the six and seven instar type (Tanner, et. al., 1984). Their developmental time to pupation was 8 days longer than the male larvae and the five instar female larvae.

Prior to the 1985 F_1 sterile male program, the colony was sampled to determine if the "double harvest" technique would be needed. This proved to be unnecessary because there were fewer six instar type female larvae (Tanner, et. al., 1985). However, it was not known as to what caused the shift to fewer instars.

Table 1 was unavailable for the last report. It shows the number of days for 4, 5 and 6 instar type larvae to go from neonates to the adult stage in 1985. Male and female 5 instar type larvae required a similar number of days. Male 4 instar type larvae required 2 fewer days than the 5 instar type larvae while 6 instar type female larvae required over 4 days more than the 5 instar type male larvae and over 6 days more than 4 instar type male larvae.

The New Jersey strain adult males are capable of mating up to 4 days after emerging under the current laboratory conditions (personal observations). It is evident from the data in Table 1 that adult females produced by from 5 instar type larvae could mate with adult males produced by either 4 or 5 instar type larvae, however, the over 6 day difference in the number of days to adult emergence between males produced by 4 instar type larvae and females produced by 6 instar type larvae indicate that the females produced by 6 instar type larvae would most likely mate only with adult males produced by 5 instar type larvae.

Table 1. The number of days required for 4, 5 and 6 instar type male and female larvae to go from neonates to the adult stage in 1985.

	Number of instars prior to pupation		
	4	5	6
Male	39.7 ± 1.7(48)2/	41.7 ± 2.1(276)	
Female		41.4 ± 1.9(276)	45.9 ± 3.7(30)

1/ ± S.E.
2/ ( ) = Number of observations

To determine if the numbers of instars in the offsprings was influenced by the number of larval instars in the parents, the following matings were made with adults from the 1985 sample:

Mating type	Number of larval instars	
	Male	Female
4 X 5	4	5
5 X 5	5	5
5 X 6	5	6

Newly laid egg masses were embryonated 35 days before being refrigerated 170 days at 7-10°C. Treated eggs were incubated in 6 oz fluted cups containing 50 ml of B-4 diet. Newly eclosed larvae were placed into individual 1.5 ounce cups containing 24 ml of fresh diet and reared through to adults.

Data in Table 2 show that the parent mating type did influence the distribution of 4, 5, and 6 instar type larvae in the next generation. The 4 X 5 parent mating type produced the highest percentage (25.1) of 4 instar male larvae and the lowest percentage (6.1) of 6 instar female larvae. Conversely, the 5 X 6 parent mating type produced the lowest percentage (13.2) of 4 instar male larvae and the highest percentage (11.6) of 6 instar female larvae. The 5 X 5 parent mating type had a distribution that was between the 4 X 5 and 5 X 6 parent mating type. In the male offspring the 5 X 5 parent mating type had a distribution similar to the 5 X 6 parent mating type distribution. In the female offspring the 5 X 5 parent mating type had a distribution similar to the 4 X 5 parent mating type.

Table 2. The percentage of 4, 5, or 6 instar male and female larvae produced by mating adults based on the number of instars they had in their larval stage.

Sex	Mating Type[1/]	Percentage of 4 larval instars	5	6
Male	4 X 5	25.1	74.9	
	5 X 5	15.7	83.7	0.6[2/]
	5 X 6	13.2	86.3	0.5[2/]
Female	4 X 5	0.6[2/]	93.3	6.1
	5 X 5	1.3[2/]	91.8	6.9
	5 X 6		88.4	11.6

1/ 4 X 5 = 4 instar male with 5 instar female
5 X 5 = 5 instar male with 5 instar female
5 X 6 = 5 instar male with 6 instar female

2/ Few larvae were of this instar.

It can not be determined if the higher number of 6 instar type larvae found in the 1984 F_1 sterile male program was due to a higher percentage of 5 X 6 matings in the parents compared to the parents of the 1985 program. There was one difference in the rearing method used to produce the 1984 and 1985 parents. The parents of the 1984 program were reared using the egg infest method (Tanner, et. al., 1983) while the parents of the 1985 program and also the 1983 program, which likewise did not have a problem with excessive numbers of 6 instar larvae, were reared using the old and new neonate infest method (Tanner, et. al., 1982 and 1985). In the egg infest method, pupae were harvested 38 days after the eggs were placed into the rearing container. In the neonate infest method the pupae were harvested 35 days after 1 to 3 day old neonates were placed into the rearing container.

Comparing the two mentods with the data from the above test shows that there would have been a considerable difference in the percentage of harvested adult females produced by 6 instar type larvae (Table 3). With the egg infest method, over 41% of the adult females produced by 6 instar type female larvae would have been harvested while only 16% would have been harvested with the neonate infest methods. This means that with the neonate infest method there would have been fewer 5 X 6 matings and possibly fewer 6 instar larvae in the next generation. Conversely with the egg infest method there would have been more 5 x 6 matings and possibly more 6 instar larvae in the next generation.

Table 3. The percentage of harvested adult females produced by 6 instar type larvae as effected by the colony infest method.1/

Mating Type	Total	Infest Method	
		Egg	Neonate
4 X 5	10	4	1
5 X 5	11	5	2
5 X 6	16	6	3
Total	37	15	6
Percent harvested		41	16

1/ Egg infest method - the pupae were harvested 38 days after the eggs were placed into the rearing container.

Neonate infest method - the pupae were harvested 35 days after 1 to 3 day old neonates were placed into the rearing container.

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Project Number: GM 7.3.6
Project Title: Insect Production and Distribution
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leaders: J. J. Baker and J. A. Tanner

The primary objectives of the rearing facility are to produce sufficient quantities of all gypsy moth life stages for the support of projects at this laboratory and at research institutions in the United States and abroad, and to produce an adequate supply of gypsy moth eggs of various strains for future projects.

This year, we completed over 1,142,112 matings for the F_1 sterile male program which ran from July 7, 1986 to January 7, 1987. All of the estimated 925,000 egg masses are presently in storage.

Table 1. The number of eggs, cups and liters of B-4 diet used to provide gypsy moth life stages for in-house and cooperative programs, FY 1986.

Program	Eggs Infested	Neonates Infested	B-4 Diet (Liters)	Number of Cups 6 oz.	Number of Cups 1.5 oz.
Cooperative Programs	251,088		1,867	15,693	15,165
Colony		224,640	2,386	28,080	
Insecticide	1,063,296		1,107	22,152	
Irradiation Study and Sterile Male QC Testing	1,518,592		8,245	94,912	5,880
F_1 Sterile Male Plain Diet	4,838,400		25,704	302,400	
Wild Strains		15,793	552		15,793
Monitor F_1 Female for 1986 Release Program	1,187,160		1,681	19,786	
Totals	8,858,536	240,433	43,082	501,143	36,838

Table 2. Distribution of reared insects, FY 1986.

Project	Egg Masses	L-2 L-3	L-4 L-5	Male Prepupae	Male Pupae	Female Prepupae	Female Pupae
W. McLane (APHIS)		76,320	26,340				
V. Mastro (APHIS)				40,307	22,934	20,173	58,017
R. Carde' (U. Mass)	280						
P. Barbosa (U. Maryland)	430						
C. Yin (U. Mass)	180						
R. Chianese (New Jersey)	3,225						
L. Rhoads (Pennsylvania)	665						
W. Yendol (Penn. State)	1,020						
G. Carrol (Oregon Univ.)	220						
D. Vogt (New York)		1,140	9,735				
G. Rosenberry (Maryland)	577						
D. Soper (Ithaca, NY)	180						
M. Ramos (Boyce,VA)	120						
K. Pittman (Annapolis,MD)	220						
J. Wissman (Champaign,IL)	60						
M. Raupp (U. Maryland)	168						
M. Ticehurst (Pennsylvania)	1,375						
D. Craun (Richmond,VA)	384						
J. Chunningham (Canada)	1,800						
G. Morris (U. Maryland)	82		200				
R. Rosander (Bakersfield,CA)					3,700		
J. Miller (U. Oregon)	60						
L. Venebales (Annapolis,MD)				9,401			8,595
T. ODell (Forest Service)	220	360			13,269		10,923
J. Brezner (U. New York)	45						
R. Bell (ARS)	25		300				
Totals	11,336	77,820	36,575	49,708	39,903	20,173	77,535

A total of 313,050 gypsy moth life stages were distributed in FY 1986.

Table 3. Summary of colony, sterile male and wild pupal production, FY 1986.

Program	Male Pupae	Female Pupae
Colony (NJF28&29)	123,783	106,898
F_1 Sterile Male Plain diet	1,342,100	1,142,112
P_1 Michigan Wild	275	375
P_1 North Mountain Wild	416	586
P_1 Sleepy Creek Wild	337	598
F_1 Ashumet Wild	393	484
F_1 Yard Creek Wild	346	545
F_1 Main Wild	387	412
Totals	1,468,037	1,252,010

Table 4. Number of colony eggs and wild eggs produced in FY 1986.

Program	Number of Eggs
Colony (NJF29&30)	71,289,262
F_1 Michigan Wild	118,500
F_1 North Mountain Wild	101,000
F_1 Sleepy Creek Wild	83,500
F_2 Ashumet Wild	215,000
F_2 Yard Creek Wild	87,000
F_2 Main Wild	187,500
Total	72,081,762

Project Number: CNPPSDP 4.1.1
Project Title: Pheromone-Based Survey Technology for Early Detection of Exotic Insect Pests
Report Period: October 1, 1985 - September 30, 1986
Report Type: Interim
Project Leader: V. C. Mastro, C. P. Schwalbe and P. C. Kingsley

A new survey manual was completed this past year and distributed to all cooperators. It was designed so that it can be updated periodically. To provide pheromone baits for servicing this program in 1987 the Otis Center produced and distributed approximately 22,000 lures for 12 species. Over the past year the design of the lure for three species was modified to either make them more species specific or more attractive. Current activities include developing new trap designs for two species. The objective of these tests is to develop traps which do not rely on a tacky surface for capture. Other ongoing projects involve the definition or optimization of the pheromone for two species.

Project Number: AP 6.1.1
Project Title: Artificial Diets for Predatory Coccinellids.
Report Period: October 1, 1985 - September 30, 1986
Report Type: Preliminary
Project Leader: O. T. Forrester

The objective of this study was to develop an artificial diet that would induce oviposition in overwintered and teneral F_1 adult C-7 prior to field release.

Fifty-six pairs of teneral F_1 C-7 from the Niles Biological Control Satellite Facility were divided into two groups. The sex of all adult C-7 was determined. This was facilitated by using a 14 cm x 7 cm beverage stirring stick with a coating of raisins, honey and wheat germ paste on 2 cm of the tip. This paste formula has enough tack to pick up and hold adult C-7, yet allows for easy removal; it is not toxic and did not injure the beetles.

In treatment I, individual pairs were held in cages constructed of 24 oz wax coated lily cups and 3 3/8" (1 qt) paper cans with the bottoms removed. Cages were closed with 100 mm plastic petri dish lids. Pea aphids were supplied on heavily infested fava bean plants.

In treatment II, individual pairs were held in cages consisting of 6 ounce plastic cups (Sweetheart XE-6) and Dixie 962T plastic lids with 3.2 cm holes covered with nylon organdy. A watering tube constructed of a 5 3/4" x 7 mm od Pasteur pipet with a sealed tip loosely plugged with dental cotton, was inserted through a 7 mm hole near the edge of the lid. Eight, 2-3 mm drops of artificial diet were placed around the edge of a 3.2 cm plastic feeding disc. Feeding discs and diet were changed daily. The artificial diet was prepared by grinding 2" squares of frozen raw pork liver in a No. 10 Cut Rite food grinder. The ground liver was homogenized in a 1 liter blender and mixed with 1 part powdered sugar to 5 parts homogenized pork liver. Aliquotes of 75 gm were stored at -30°C. Adults were held on artificial diet until oviposition was observed and then transferred to pea aphid if, after 41 days, oviposition did not occur. Cages in both groups were supplied with oviposition strips of black plastic trash bags 2"x 12". Cages were held at 23-25 degrees C, 50-60% RH and a 14 hr photoperiod.

In treatment I (pea aphid fed) pre-oviposition period varied from 4 days to 58 days ($\bar{x}$ = 16.5); 28.6 percent of the females did not oviposit during the 63 days of this test. One female died before ovipositing.

In treatment II (diet pre-fed) preoviposition period varied from 9 to 55 days. The mean was 36.3 days. Oviposition was observed in 28.6% of the females fed artificial diet alone. The remaining females, 53.6%, were transferred to pea aphid after 41 days on the artificial diet. The mean preoviposition time after being transferred to pea aphid was 6.1 days $\pm$ 3.5. Five females died before they oviposited.

Oviposition site preference was also observed. Fifty-eight percent oviposited on the oviposition strips, 37.2% on the side of the container, 2.3% on the pot, and 2.2% on the plants. Significantly more eggs were laid on the oviposition strips (Chi square = 142.4 at .001%).

Pre-feeding on artificial diet of raw pork liver and powdered sugar did not adversely affect the fecundity of adult C-7. Longevity, mean number of eggs per female, and percent hatch were similar to those fed on pea aphid (Figure 1, Table 1). The major difference was days to peak oviposition; 15 days for females on pea aphids and 47 days for diet pre-fed females then transferred to aphids. This could provide some flexibility in pre-release feeding or trigger for release, i.e. feed teneral adults on artificial diet for 20 days, aphid for 3 days and release. These parameters are estimates and will require confirmatory tests.

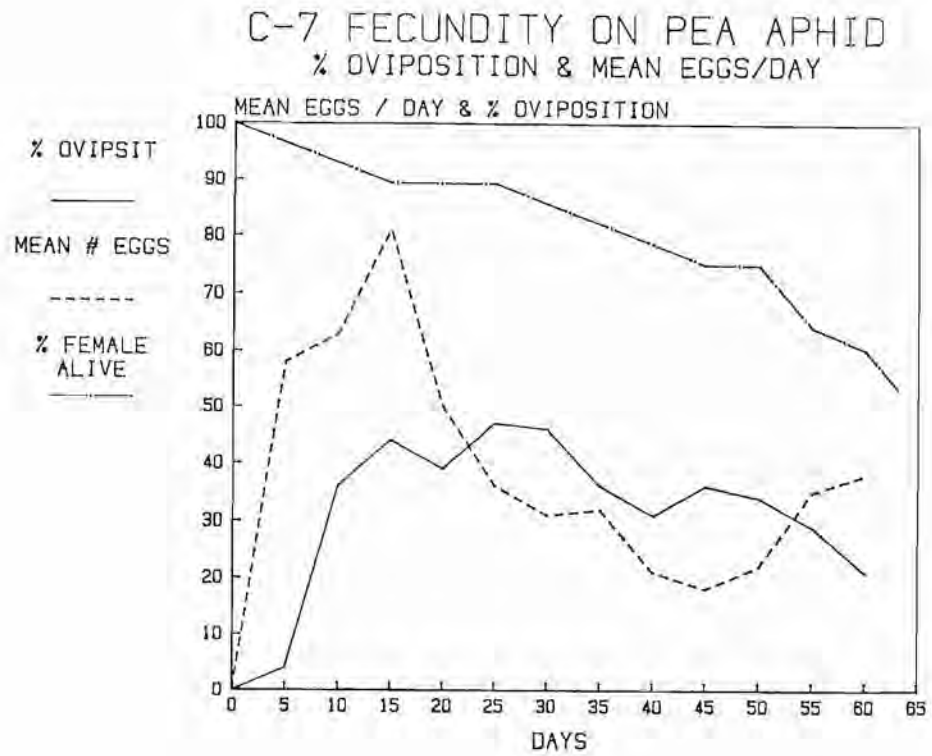
Preliminary tests were conducted on several other diet formulations. A variation of the liver diet, substituting high fructose corn syrup for powdered sugar and adding 10% (w) glycerol and 2 ml of either wheat germ oil, corn oil, olive oil or raw linseed oil, was fed to 5 groups of C-7 for 2 weeks. Dissection revealed good fat body production in all groups. Fat body color of the aphid control was bright yellow-orange. Fat body color of artificial diet groups ranged from brownish yellow to almost white. Ovigenisis was not observed in any of the dissected adults.

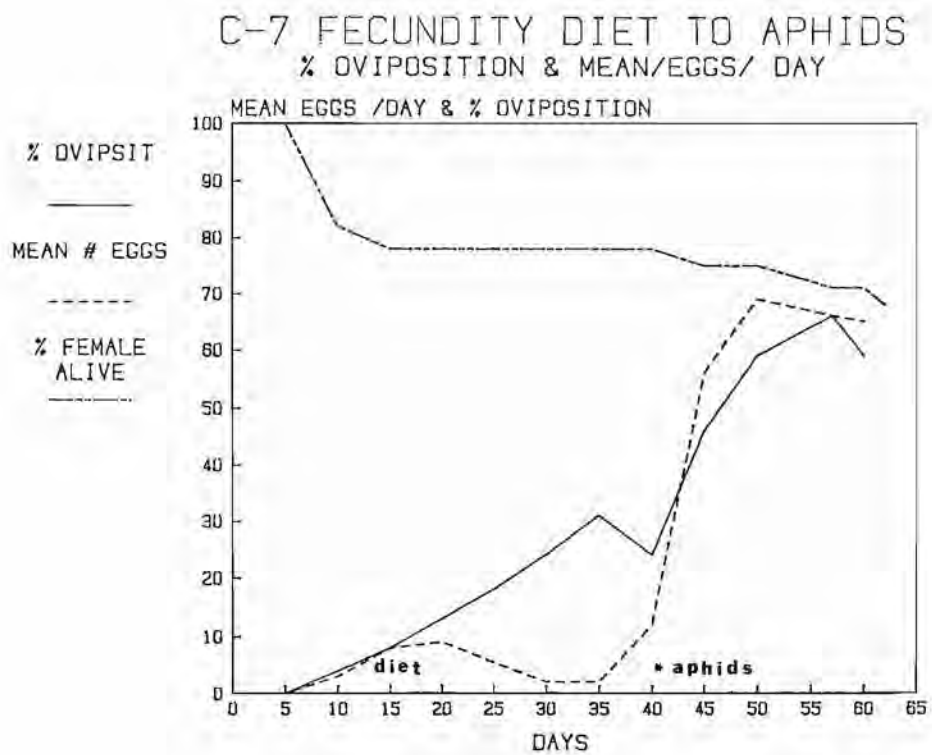
Catfood diets (9 Lives + sugar), fresh banana, baby food banana and strained carrots were screened as basic ingredients. All were acceptable as food by adults but did not stimulate ovigenesis.

Table 1.

	% Survival	Mean Pre-oviposition Period	Mean Eggs/ /Day	Days Oviposition	% Hatch	mean Eggs/
Pre-feed diet transferred to aphids	71.4	36.5	54.6	25	80.1	1248
Pea aphids	68.4	16.5	42.0	30	79.3	1292

Figure 1. Comparison of fecundity.





Project Number: CPB 5.1.1
Project Title: Laboratory and Field Tests with Edovum puttleri Controlling Leptinotarsa decemlineata.
Report Period: October 1, 1985 - September 30, 1986
Project Leader: P. C. Kingsley

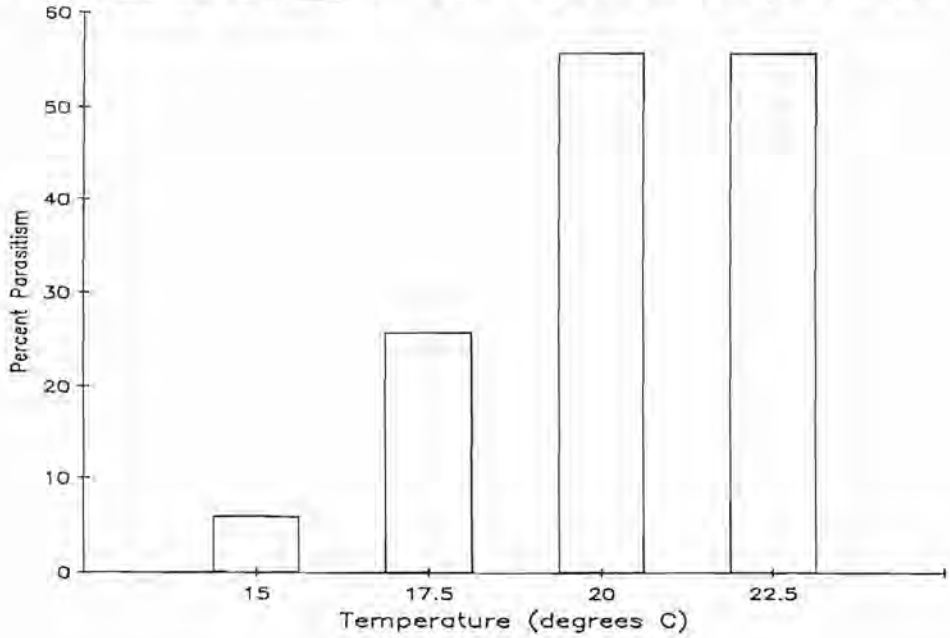
Laboratory Studies

Oviposition Threshold Test

Edovum puttleri is not active on potato plants early in the season, presumably because of cool temperatures. The objective of this experiment was to determine the lowest temperature at which E. puttleri will oviposit.

Approximately 100 CPB eggs were submitted to 50 wasps at each of four temperatures (15, 17.5, 20 and 22.5 degrees C) for 24 hours. Percent parasitism, determined by dissection, declined at temperatures below 20 degrees C indicating a threshold of oviposition between 15 and 17.5 degrees (Figure 1).

Figure 1. Percentage of CPB Eggs Parasitised by Edovum puttleri at four Oviposition Temperatures.



In an effort to produce a wasp with the ability to oviposit at lower temperatures, we are presently selecting for E. puttleri females ovipositing at 16 degrees C.

CO2 "Knockdown" Test

One difficulty in rearing small parasites is in transferring host material in and out of oviposition cages. CO_2 has been successfully used for the "knocking down" of several species of parasites. Here we tested for adverse effects of CO_2 exposure on parasitism rates.

Nine hundred E. puttleri, from the Mission Biological Control Laboratory, were divided into equal groups. Fifteen egg masses were submitted to each group for 24 hours for 3 consecutive days. One group was exposed to CO_2 for 25 seconds, or until knockdown, then ventilated with fresh air. The control group was placed under dark conditions for 1 hour, facilitating egg transfers (this method seems to work better than using the phototactic response, as dark induces the wasps to terminate oviposition and move to the top of the cage). The test was replicated twice and results were as follows:

	% Parasitism (N)	
	CO_2	Control
Day 1	29 (100)	57 (100)
2	37 (100)	59 (100)
3	21 (100)	70 (100)
Total	29 (300)	62 (300)

Overall, there was a 53% reduction in parasitism by those exposed to CO_2 for 25 seconds, although this reduction was not cumulative from day to day.

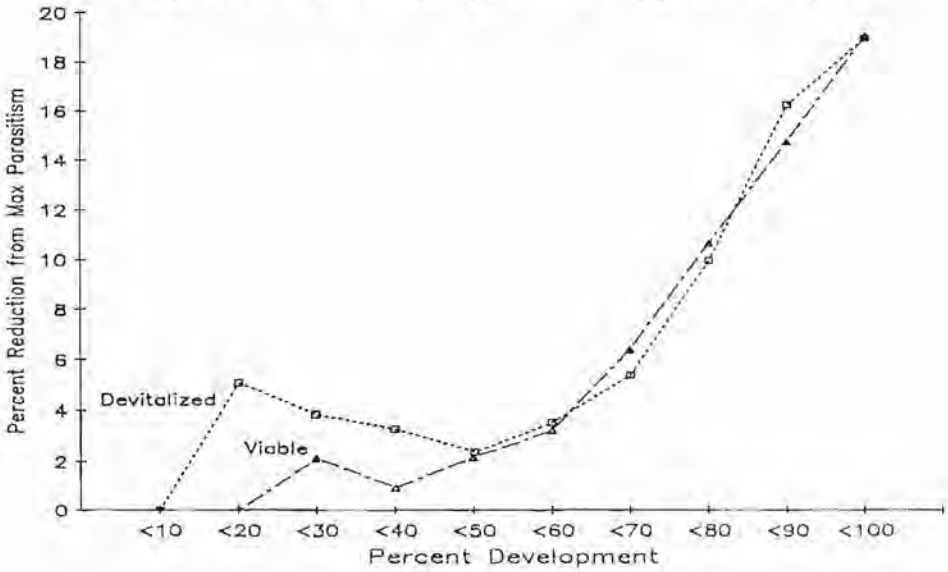
Host Age Study

The following test had two objectives: 1) to determine the maximal age of a CPB egg suitable for parasitism by E. puttleri, and 2) to test the effect of irradiated, devitalized eggs on this parameter. The hypothesis tested in the second objective was that E. puttleri might be able to utilize older eggs if CPB egg development was curtailed by irradiation.

Egg masses of ages varying from 1 hour old to hatching were each divided using a razor blade. Paired halves were marked for later identification. One set of egg mass halves was placed in a 25 degree C incubator where hatch was monitored every two hours. The other set was divided into two groups one pf which was devitalized by irradiation (20 KRAD cobalt source). Both groups of eggs were then parasitised for a four-hour period. This procedure allowed us to know the precise age of an egg mass at the time of parasitism. An additional 12 freshly laid egg masses were also held at 25 degrees C to determine the overall developmental period. This way the percentage of complete development for each egg mass could be calculated.

CPB eggs became less suitable with age, but large reductions in parasitism did not occur in eggs less than 60% developed (Figure 2).

Figure 2. Reduction in Parasitism Rates by Edovum puttleri as a Function of Host Age (Percentage of Total CPB Egg Development). (N=468 Egg Masses)



There was an overall difference of 4% between devitalized and viable eggs.

	All egg masses			
	Mean % Parasitism (SE)	N	t-value	Sig.
Devitalized	39.5 (1.90)	225	1.53	0.127
Viable	43.5 (1.94)	243		

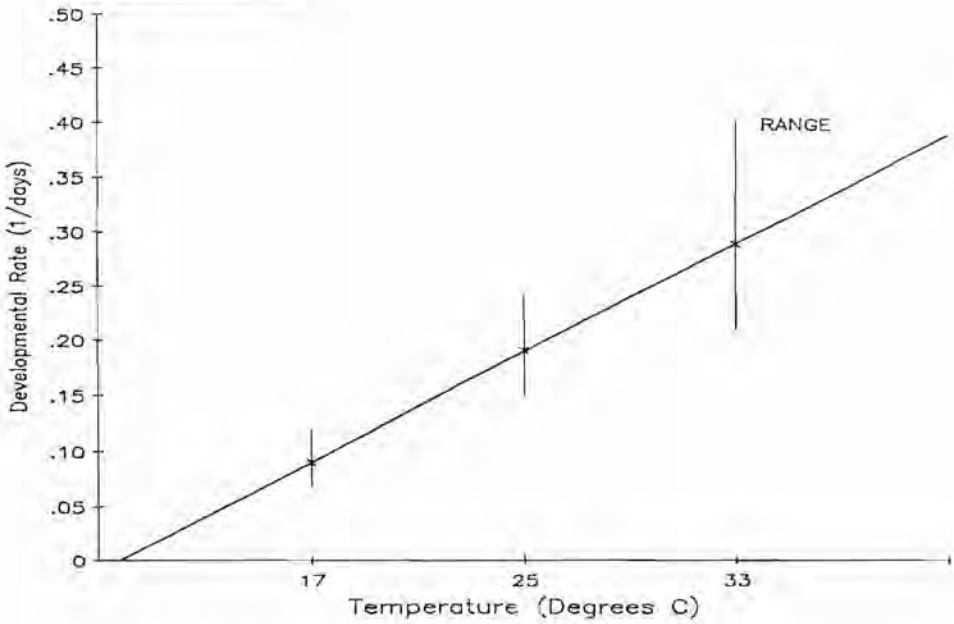
In eggs that were greater than 70% developed however, devitalizing increased the ability of E. puttleri to successfully parasitize CPB eggs.

	Greater than 70% developed eggs			
	Mean % Parasitism (SE)	N	t-value	Sig.
Devitalized	15.8 (0.01)	82	-4.16	.001
Viable	4.1 (0.02)	59		

In a separate experiment to describe the relationship between CPB egg development and temperature, 233 egg masses were reared at three temperatures (17, 25 and 33 degrees C). This linear relationship is represented in figure

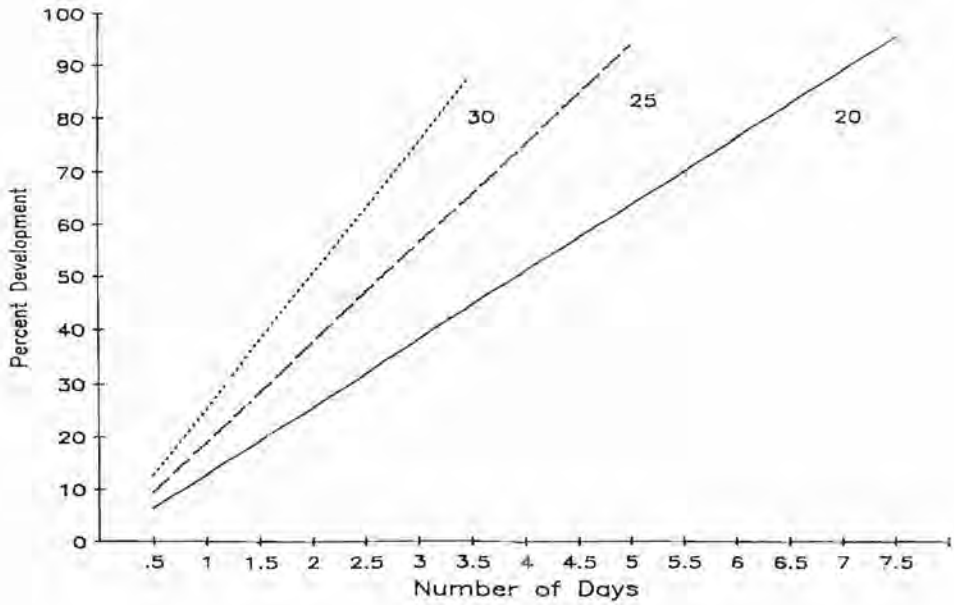
3 by the equation y = 0.0124x - 0.121:R square = 0.93 and was highly significant. The developmental threshold (y intercept) was calculated to be 9.8 degrees C and the thermal constant (1/slope) as 80.5 degree-days C.

Figure 3. Development of *Leptinotarsa decemlineata* Eggs in Relation to Temperature.



From this information the maximal age in days beyond which parasitism rates will decline significantly can be predicted for three likely rearing temperatures (Figure 4). For example, if CPB are reared at 25 degrees C, eggs could be collected every three days and still maintain reasonable parasitism.

Figure 4. Number of Days Reared at three Temperatures in Relation to Percentage of Total CPB Egg Development.



Field Studies

Edovum puttleri Release

Results from last years field test, where Edovum puttleri was released in potatoes for the control of Lepinotarsa decemlineata (CPB), indicated higher parasitism rates during the second generation of beetles, presumably because of warmer temperatures. This year we made releases into late planted potatoes to coincide with second generation egg masses, the premise being that control of these beetles might reduce the numbers of overwintering adults and make the incorporation of E. puttleri into an IPM program feasible.

Fourteen experimental plots were planted during the first week in June, each with six six-meter rows of Katadin potatoes. Each plot was separated by at least 100 meters. By planting this late we were able to avoid (to a large extent) the overwintering population of beetles. Each plot was seeded with 14 CPB adults (1:1 F:M) on 8/6. E. puttleri were released twice in seven plots; 250/plot on 8/8 and 500/plot on 8/11. Apparently our seeding rate was too low, so on 8/20 we released an additional 20 CPB into each plot. Twenty-four vines were sampled every four to five days for CPB adults, eggs and larvae. When an egg mass was found it was marked and subsequently monitored for hatch, predation or parasitism.

CPB populations never built to expected levels, probably because of cool temperatures during the month of August. E. puttleri became established in six of the seven release plots. No egg masses were found in the seventh plot. E. puttleri was also recovered in one of the control plots located behind the laboratory. Even though CPB populations were very low, there was a significant reduction in mean peak larval densities in the six release plots (Figure 5).

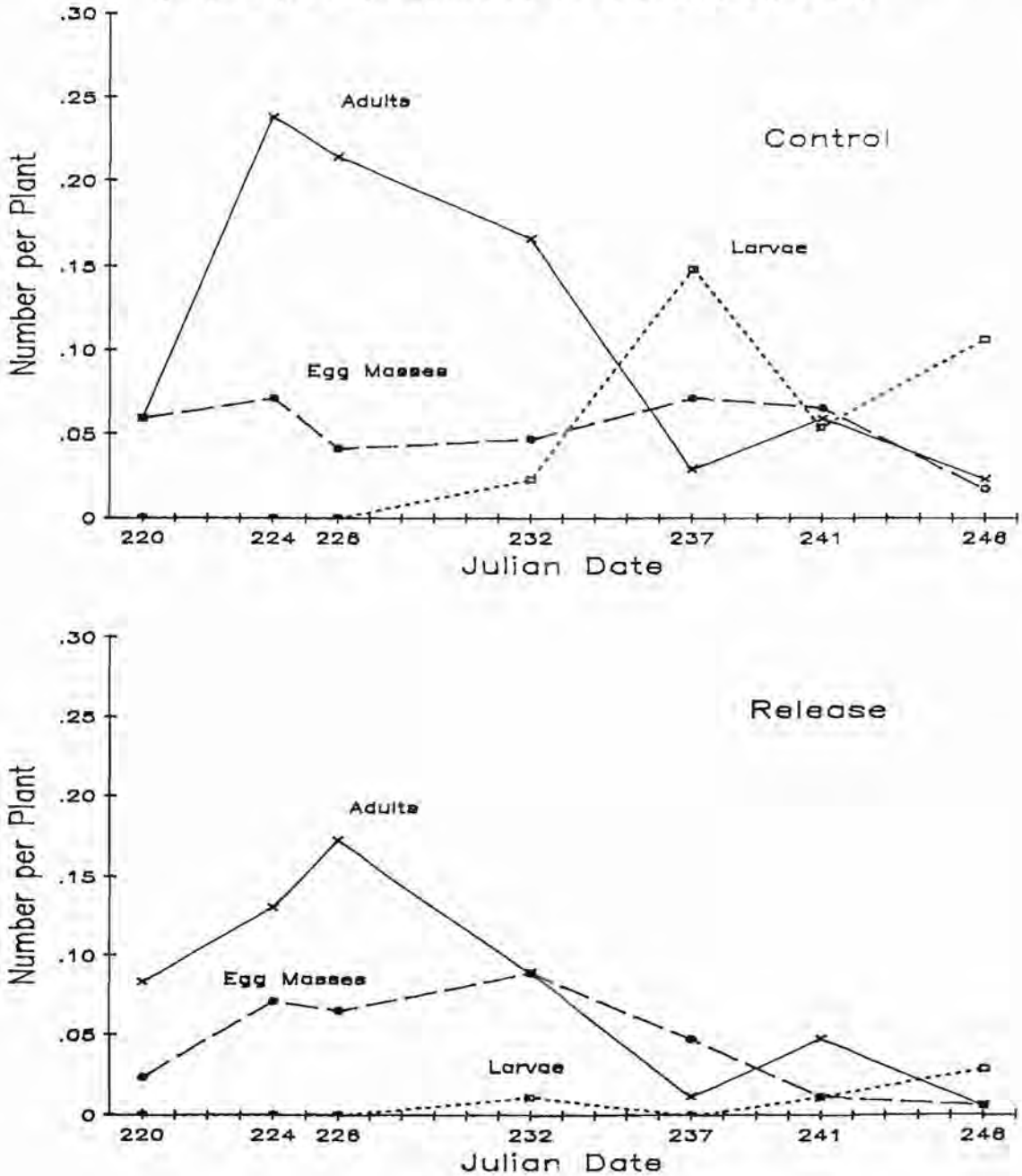
	Mean (SE) peak larval density	N	t-value	Sig.
Control	0.20 (0.079)	7	1.837	0.09
Release	0.05 (0.029)	7		

Total parasitism and predation in the plots were as follows:

	Total # of egg masses	% Parasitism	% Predation	Total Mortality
Control	52	3.8	25.0	28.8
Release	45	22.2	37.8	60.0

Rates of predation were not significantly different between the two treatments. Chrysopa sp. adults were commonly found in the plots and larvae were observed twice feeding on CPB eggs.

Figure 5. Phenology of CPB Life Stages on Potatoes in Control and Edovum puttleri Release Plots
(7 plots/date/treatment; 24 plants/plot/date)



Field Insectary

One experimental plot was set up to be used as a field insectary for *E. puttleri*. Several hundred field collected CPB adults were released into this plot during the second week in August. The field was then monitored for egg production. When adequate numbers were found, *E. puttleri* from Mission Biological Control Laboratory were released into the plot. Approximately 100 parasitized egg masses were collected and shipped to the Mission Laboratory for reintroduction into their laboratory culture.

Project Number: CPB 6.1.1
Project Title: Development of an Artificial Diet for Colorado Potato Beetle, Leptinotarsa decemlineata
Report Period: March 1, 1985 - September 30, 1986
Report Type: Interim
Project Leader: O. T. Forrester

These laboratory studies were directed toward the development of an artificial diet for Colorado potato beetle, Leptinotarsa decemlineata (CPB). Several diets were screened for CPB. All diets tested supported growth from neonate to adult. The best results were obtained with diets containing silver leaf nightshade (SLNS), Solanum elagnifolium, potato leaf powder or potato plant homogenate, Solanum tuberosum.

Plant leaf powders were prepared by oven-drying at 50°C or by air drying in brown paper bags. Dry plant materials were pulverized in a Wiley mill to 60 mesh. Plant homogenate was prepared by cutting fresh potato leaves and stems into 1-2 cm pieces and blending 100 gm of plant with 100 ml of cold water for 2 min. The diets were prepared by placing 550 ml of boiling water and the agar into a liter blender and blending at low speed for 30 sec. The remaining ingredients were then added and blended at high speed for 1 min. The diet was dispensed into 1/2 oz plastic cups at 5 ml per cup.

Neonate larvae were removed from hatched egg clusters with a 15/128 artists brush and placed in a 1/2 oz plastic cup, 5 larvae per cup and closed with a paper tab lid. Cups were held at 23-25°C, 50-60% RH and a 14 hour photoperiod. Larvae were transferred to cups of fresh diet every 4-5 days. When 4th instar larvae burrowed into the diet, they were removed and placed in a 1 oz plastic cup 3/4 full of pro-mix potting soil for pupation. Enclosed adults were held on diet, 2 pair per 6 oz cup or in screen cages on potato plants.

CPB reared from neonate to adult on artificial diet and then transferred to potato plant were fecund. Preoviposition period was 6-7 days, the same as CPB reared on potato plants. Diet-reared CPB fecundity was not reliable when kept on artificial diet. Mean preoviposition period was 35 days. Diet-reared adults were smaller than those reared on potato plants. Mean weight was 72 mg and 112 mg, respectively.

Four protein sources were substituted in the basal diet (Table 1). Developmental rate and survival were best when potato leaf powder was substituted for SLNS powder (Figure 1). The slower developmental rate and lower survival of CPB on the wheat germ diet are due in part to bacterial contamination.

Substitution of lipids in the basal diet did not affect rate of development however, it did affect survival (Figure 2). Wheat germ oil gave better results than linseed oil. Eggs produced by CPB reared on diet with olive oil did not hatch. Hatch was normal on diets with the other lipids tested.

Bacterial contamination and oxidation of this diet are problems that are difficult to address due to the lack of information on development and survival of CPB reared on artificial diet. We now have enough information to test antibacterials and preservatives to solve these problems.

Table 1. Ingredients for Colorado potato beetle basal diet.

Ingredient	Basal diet amount (ml)
Deionized water	550
Agar	10
Wheat germ	
Rolled oats	40
Plant powder*	20
Potato homogenate	
Casein	18
Micropulverized Casein	
Sucrose	16
Brewers yeast hydrolysate	8
Lactalbumin hydrolysate	
Vitamin pre-mix	2.5
Vanderzant's vitamin pre-mix	
Wesson's salt mix	4
Salt mix # II	
B sitosterol	.5
Cholesterol	.5
Methylparaben	.5
Sorbic acid	.5
Raw linseed oil	1

Figure 1. The effect of host plant powder and protein source on rate of development and survivial of Colorado potato beetle on basal diet.

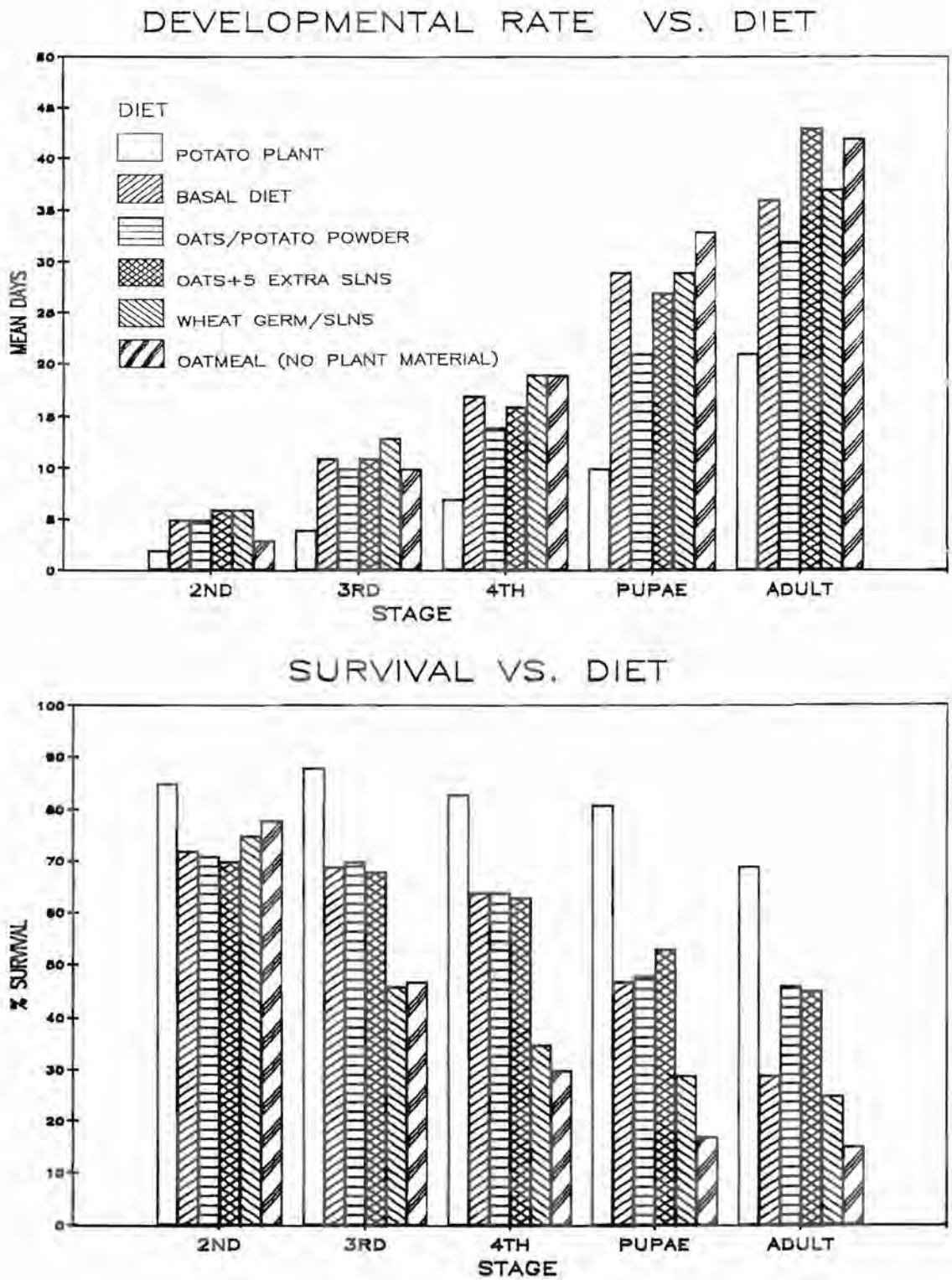
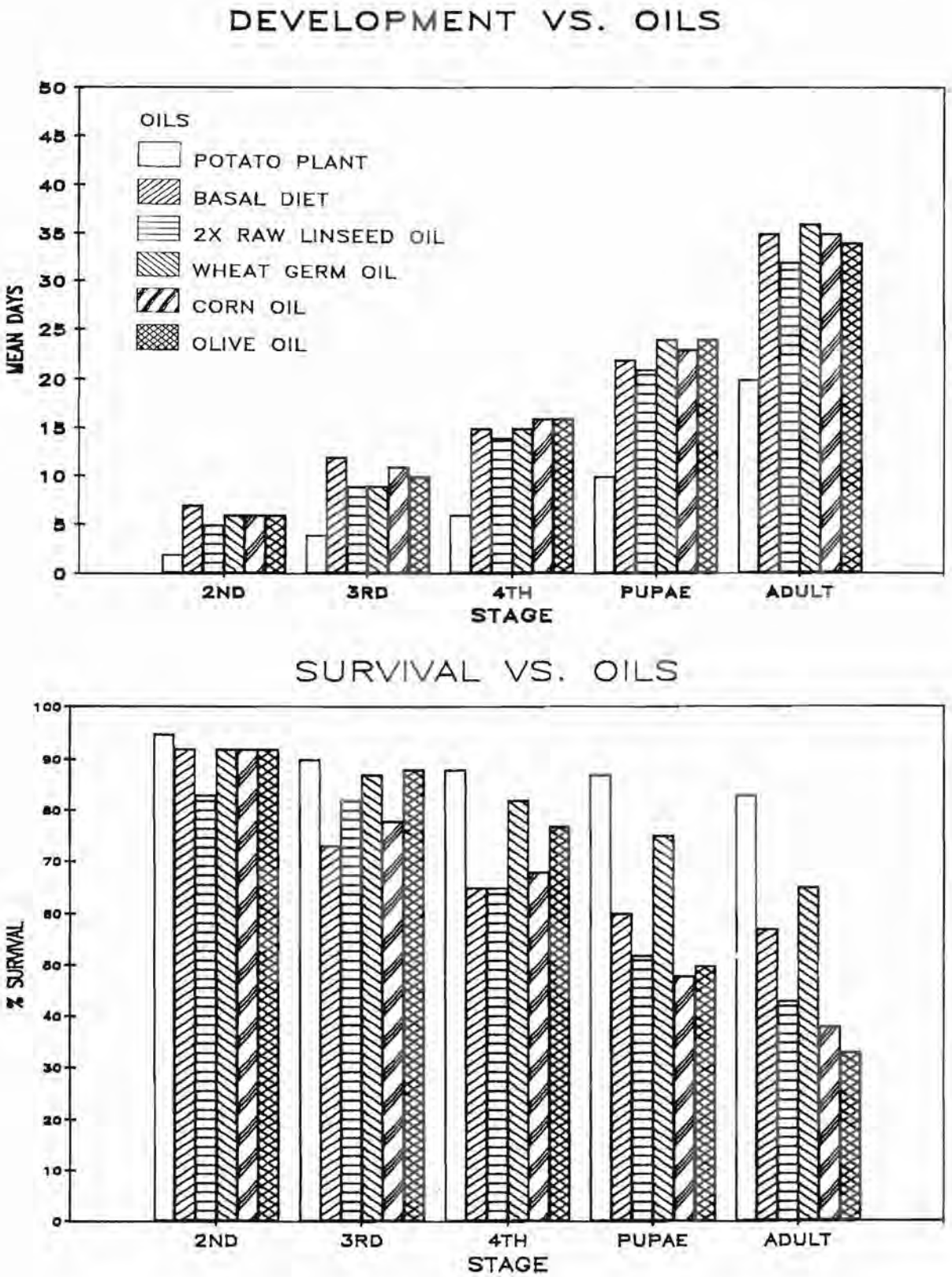


Figure 2. The effect of lipid substitution on rate of development and survival of Colorado potato beetle on basal diet.



Project Number: AW 1.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasite Redistribution Program
Report Period: October 1, 1985 to September 30, 1986
Report Type: Interim
Project Leader: P. C. Kingsley

Introduction

This project was initiated in 1981 to evaluate the effects of alfalfa weevil (AW) parasites on AW populations and subsequent changes in the local economics of growing alfalfa. The movement of principally two species, Bathyplectes anurus and Microctonus aethiopoides, from eastern areas where these species have been well established to areas further west, has been coordinated from the APHIS Biological Control Laboratory in Niles, Michigan. Recovery surveys, three years post-release, indicate widespread success of the redistribution methods.

The movement of these two species has also been documented in our intensive sampling of five areas, each with six sites and five fields per site (Figure 1). Areas I-IV have been sampled for six years, whereas, the Nebraska area was added to the survey in 1983.

Methods

Survey methods, as described in the 1985 Evaluation Handbook, remained the same as the previous year.

Results

Alfalfa weevil populations in the three western areas (NE, IA, MO, IL) showed a general decline this year, ending a climbing trend of previous years. In areas III (OH) and IV (PA, NJ) however, populations continued to increase (Figure 2, Table 1A). It is interesting to note what appears to be a five year cycle of weevil populations in all areas.

The number of parasite species recovered in each of the three western areas has doubled since 1981 (Table 1B, Figure 3). The two parasite species mentioned above are primarily responsible for this increase. At the initiation of our program M. aethiopoides was well established east of the Mississippi River, and B. anurus was firmly established only in area IV (PA, NJ)(Figure 4). Over the six survey years both species have shown steady upward trends in establishment. For example, in 1981 B. anurus was recovered from only 30% of the 30 area II (IL, IA) fields, but by 1986 over 70% of these fields had this species established. In Ohio this expansion of B. anurus range has apparently been at the expense of its congener, B. curculionis (see Appendix 1). Similarly, in area I (IA, MO) M. aethiopoides was recovered from 40% of the fields in 1981 vs. 100% in 1984. Parasitism by B. anurus has correspondingly increased with establishment in areas II (OH) (from 3% in 1981 to 19% in 1986) and area II (IL, IA) (from 0% to 8.7%). More dramaticaly, M. aethiopoides has gone from parasitizing 2% to 57% of the adult weevils over the six survey years (Figure 5). All this evidence attests to the effectiveness of the parasite redistribution program.

Overall trends in disease rates by area and year are shown in figure 6 and a generalized seasonal phenology of alfalfa weevil adults and larvae in figure 7. Seven new county records were recovered this year from evaluation samples.

Additional and more detailed information on alfalfa weevil densities and parasitism rates by species summarized on a site and area basis can be found in six annual archival data reports.

Figure 1. 1986 AW Evaluation Areas and Sites
(5 Areas, 6 Sites/Area, 5 Fields/Site).

Table 1A. Six-year area summary for peak alfalfa weevil larval densities.

Area	1981	1982	1983	1984	1985	1986
	Mean (SE) Peak Larval Density Number per 100 Sweeps					
O NB			262.3ab[1/] (26.5)	706.7a (164.93)	731.3b (101.25)	443.3ab (90.35)
I MO, IA	619.5b (105.0)	193.3ab (36.2)	351.7b (56.1)	253.0b (28.56)	367.7a (83.20)	181.7a (32.20)
II IA, IL	846.3b (199.3)	251.5ab (59.0)	207.0a (33.8)	345.4b (77.43)	694.2b (72.01)	281.1a (72.01)
III OH	92.1a (22.3)	97.5a (22.9)	161.8a (8.4)	245.8b (31.12)	686.2b (112.16)	817.9c (92.82)
IV NJ, PA	218.1a (39.9)	381.7b (87.9)	171.1a (34.8)	52.8b (13.67)	268.7a (46.34)	727.9bc (189.41)

Table 1B. Six-year area summary for peak alfalfa weevil parasite species diversity.

Area	1981	1982	1983	1984	1985	1986
	Mean (SE) Number of Parasite Species/Site					
O NB			1.5a[1/] (0.22)	1.5a (0.22)	2.3a (0.33)	3.0a (0.26)
I MO, IA	1.7a (0.21)	2.3a (0.21)	2.3a (0.33)	2.0a (0.00)	3.2ab (0.31)	3.0a (0.26)
II IA, IL	2.2a (0.17)	2.5a (0.33)	2.5a (0.34)	3.0b (0.45)	3.8b (0.31)	4.8b (0.17)
III OH	3.5b (0.22)	3.8b (0.31)	3.5b (0.34)	3.3b (0.24)	4.2b (0.31)	4.7b (0.21)
IV NJ, PA	4.5c (0.29)	4.0b (0.26)	3.8b (0.17)	3.0b (0.00)	4.2b (0.17)	4.3b (0.21)

1/ Values in columns followed by the same letter are not significantly different at the 95 percent level of confidence [Newman-Keuls multiple comparison test].

Table 2A. Six-year summary of overall percent parasitism of alfalfa weevil adults.

	% Adult Parasitism (Total Parasitized/Total Dissected)					
Area	1981	1982	1983	1984	1985	1986
O NB			0.04a[1/] (1/2630)	0.07a (2/3074)	0.05a (20/4000)	0.48a (14/2901)
I MO, IA	02.3a (126/5378)	13.2a (313/2374)	16.5b (724/4392)	22.7d (467/2054)	34.3d (1411/4118)	56.5d (813/1439)
II IA, IL	39.1b (1849/4724)	35.9c (598/1666)	35.1c (478/1361)	17.2bc (293/1707)	27.4c (1635/5960)	38.8c (602/1552)
III OH	32.0b (562/1759)	26.6b (287/1081)	18.3b (421/2295)	20.2dc (424/2095)	26.3c (1425/5422)	35.5c (2265/6381)
IV NJ, PA	26.2b (107/408)	32.6bc (587/1799)	19.1b (230/1026)	14.0b (103/734)	20.7b (694/3361)	29.3b (992/3384)

Table 2B. Six-year summary of overall percent parasitism of alfalfa weevil larvae.

	% Larval Parasitism (Total Parasitized/Total Dissected)					
Area	1981	1982	1983	1984	1985	1986
O NB			24.8c[1/] (1209/4871)	22.2b (1102/4957)	22.3c (1495/6720)	34.0b (1960/5759)
I MO, IA	14.4c (976/6760)	18.1a (358/1983)	13.8a (932/6773)	15.2a (720/4725)	20.1b (1329/6600)	37.0c (2370/6409)
II IA, IL	08.1a (586/7266)	40.0c (1096/2739)	29.0d (1672/5771)	25.5cc (1295/5078)	17.2a (1051/6124)	39.6d (2837/7158)
III OH	11.6b (452/3900)	26.0b (903/3609)	15.7b (1065/6778)	22.6b (1204/5337)	21.0b (1769/8410)	23.6a (1243/5278)
IV NJ, PA	30.9d (889/2881)	26.3b (1290/4904)	27.1cd (1443/5331)	14.1a (461/3271)	23.3c (1645/7058)	43.1e (2479/5748)

1/ Values in columns followed by the same letter are not significantly different at the 95 percent level of confidence [Chi-square].

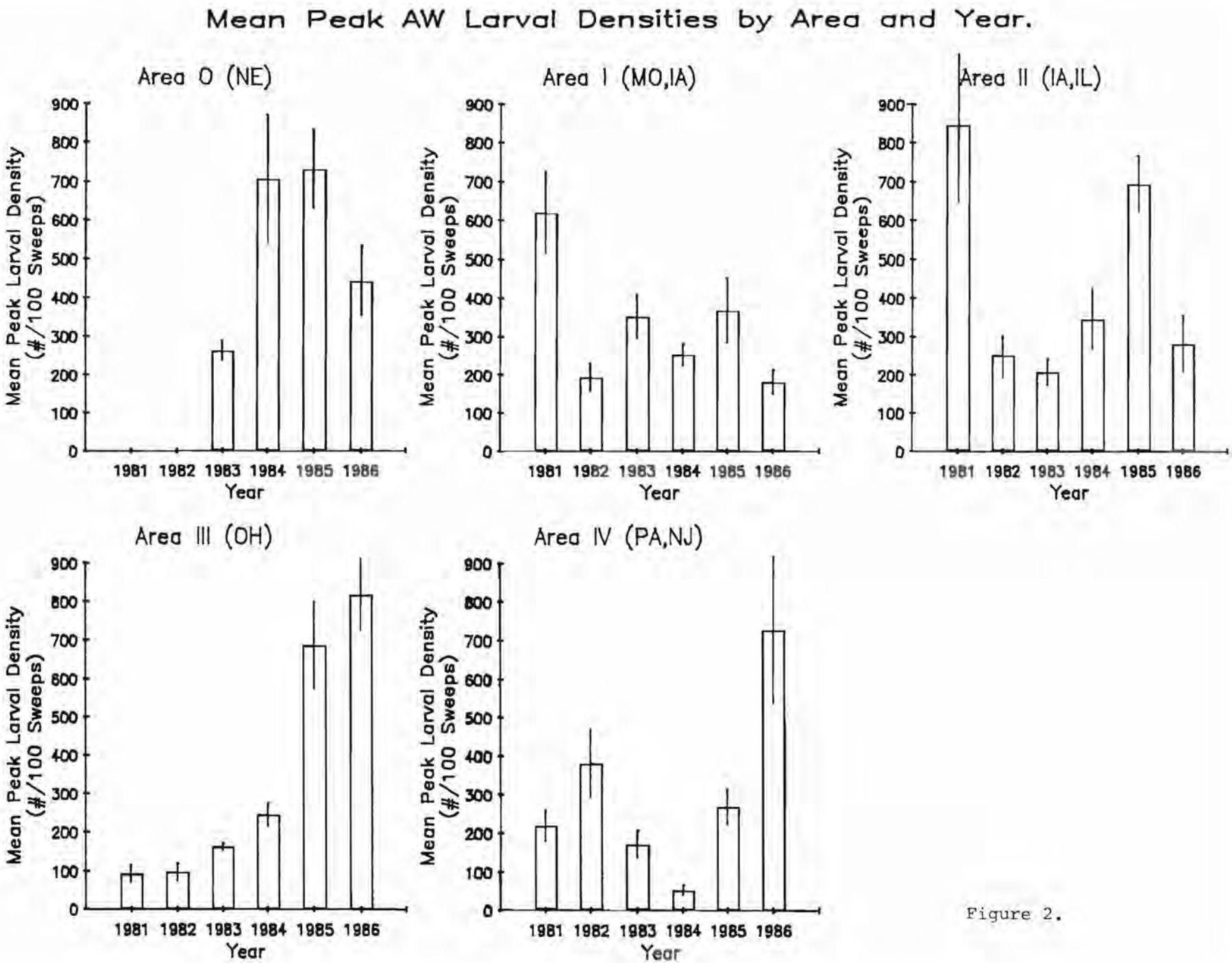

Mean Peak AW Larval Densities by Area and Year.
Area 0 (NE)
Area I (MO,IA)
Area II (IA,IL)
Area III (OH)
Area IV (PA,NJ)
Mean Peak Larval Density (#/100 Sweeps)
0
100
200
300
400
500
600
700
800
900
1981 1982 1983 1984 1985 1986
Year

Figure 2.

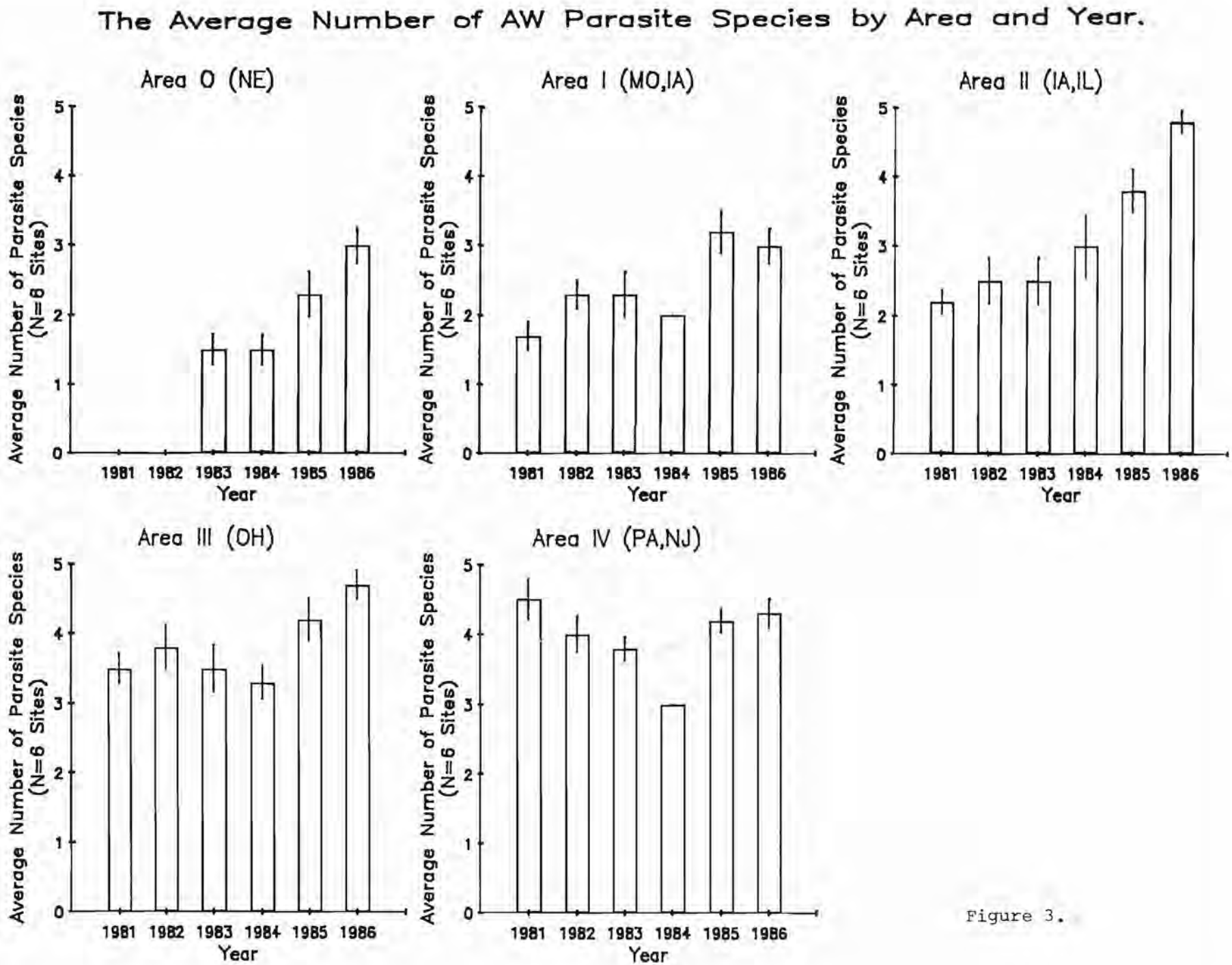

The Average Number of AW Parasite Species by Area and Year.
Area 0 (NE)
Area I (MO,IA)
Area II (IA,IL)
Area III (OH)
Area IV (PA,NJ)
Average Number of Parasite Species (N=6 Sites)
0
1
2
3
4
5
1981
1982
1983
1984
1985
1986
Year

Figure 3.

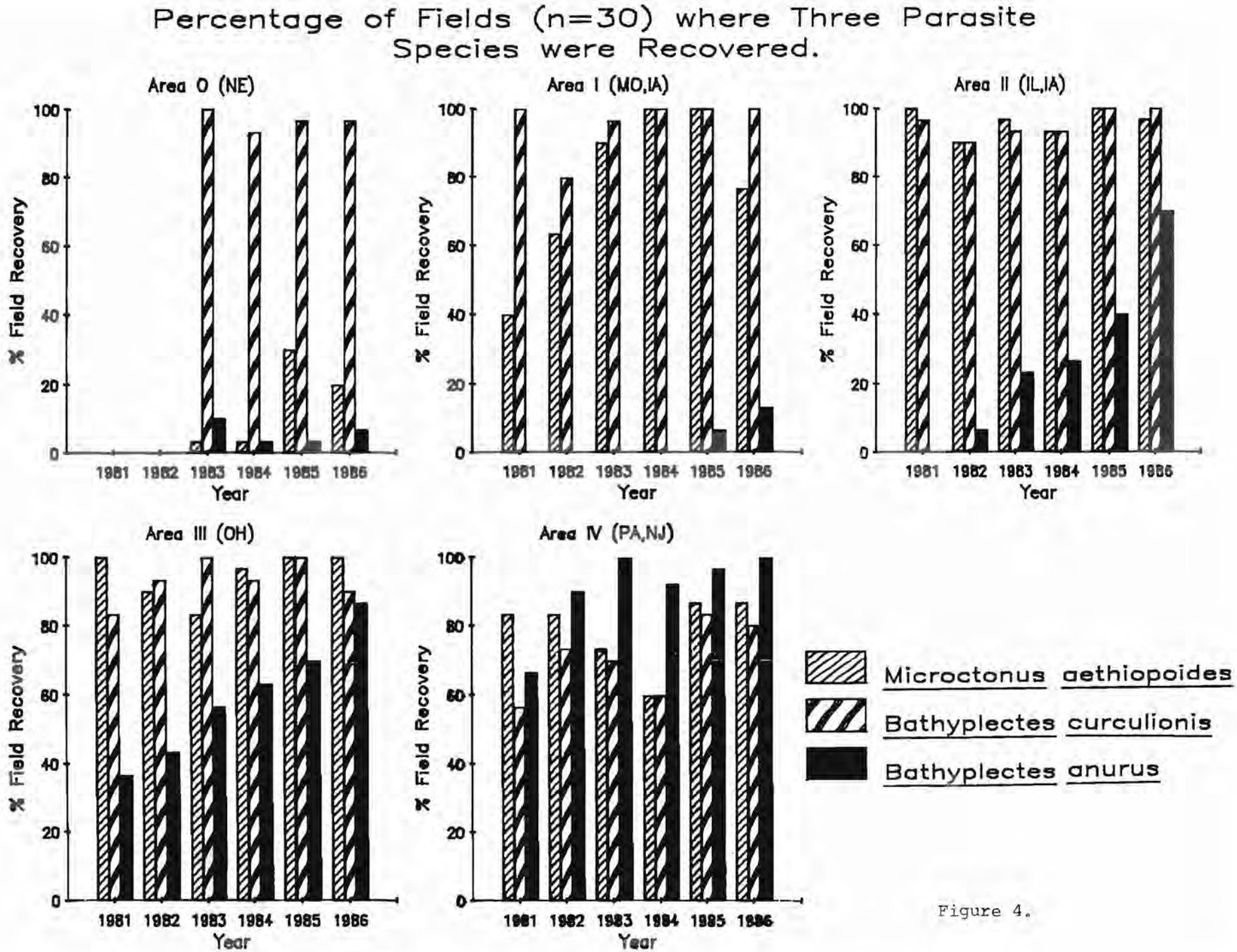

Percentage of Fields (n=30) where Three Parasite Species were Recovered.
Area 0 (NE)
Area I (MO,IA)
Area II (IL,IA)
Area III (OH)
Area IV (PA,NJ)
% Field Recovery
Year
0
20
40
60
80
100
1981
1982
1983
1984
1985
1986
Microctonus aethiopoides
Bathyplectes curculionis
Bathyplectes anurus

Figure 4.

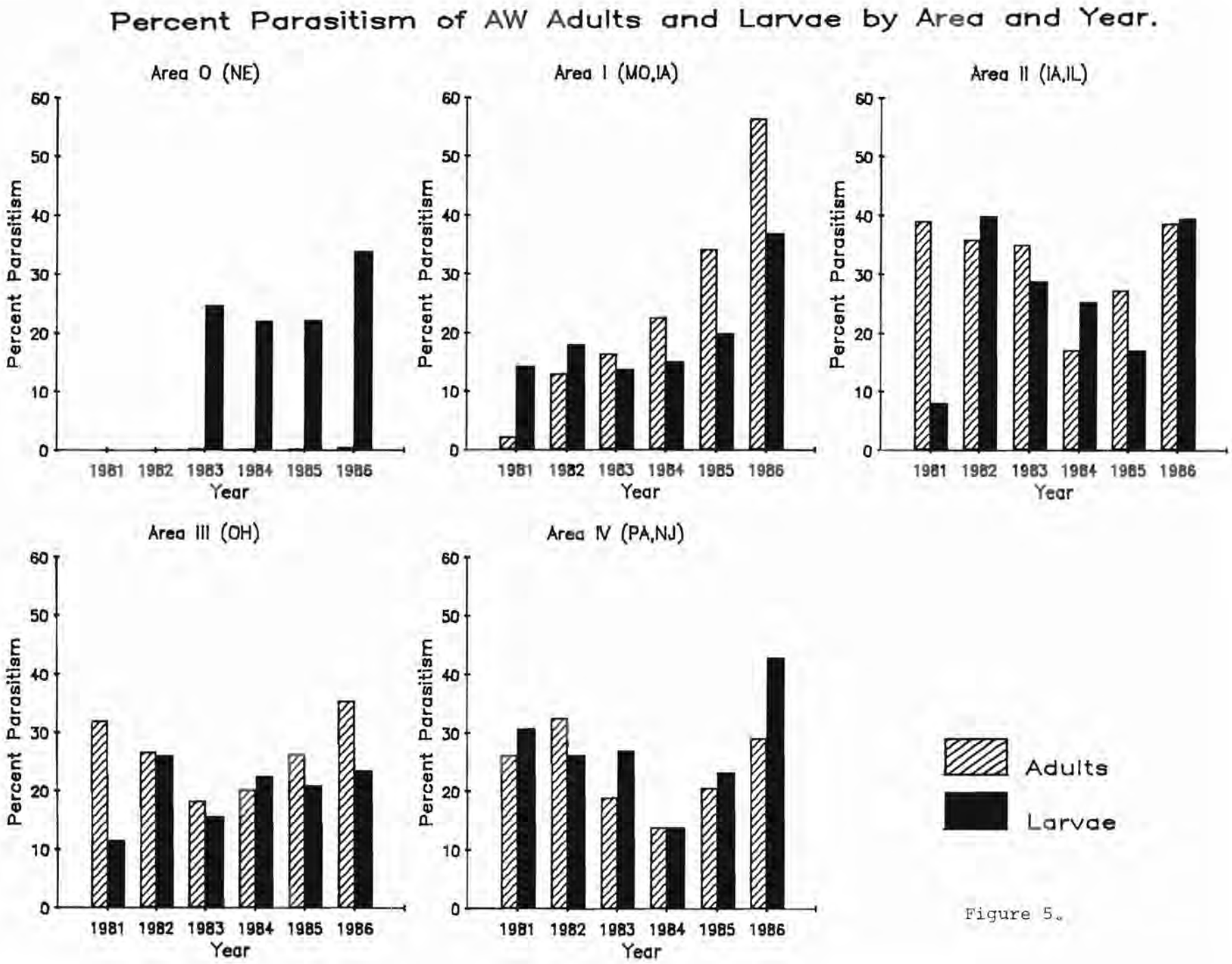

Percent Parasitism of AW Adults and Larvae by Area and Year.
Area 0 (NE)
Area I (MO,IA)
Area II (IA,IL)
Area III (OH)
Area IV (PA,NJ)
Percent Parasitism
Year
0
10
20
30
40
50
60
1981
1982
1983
1984
1985
1986
Adults
Larvae

Figure 5.

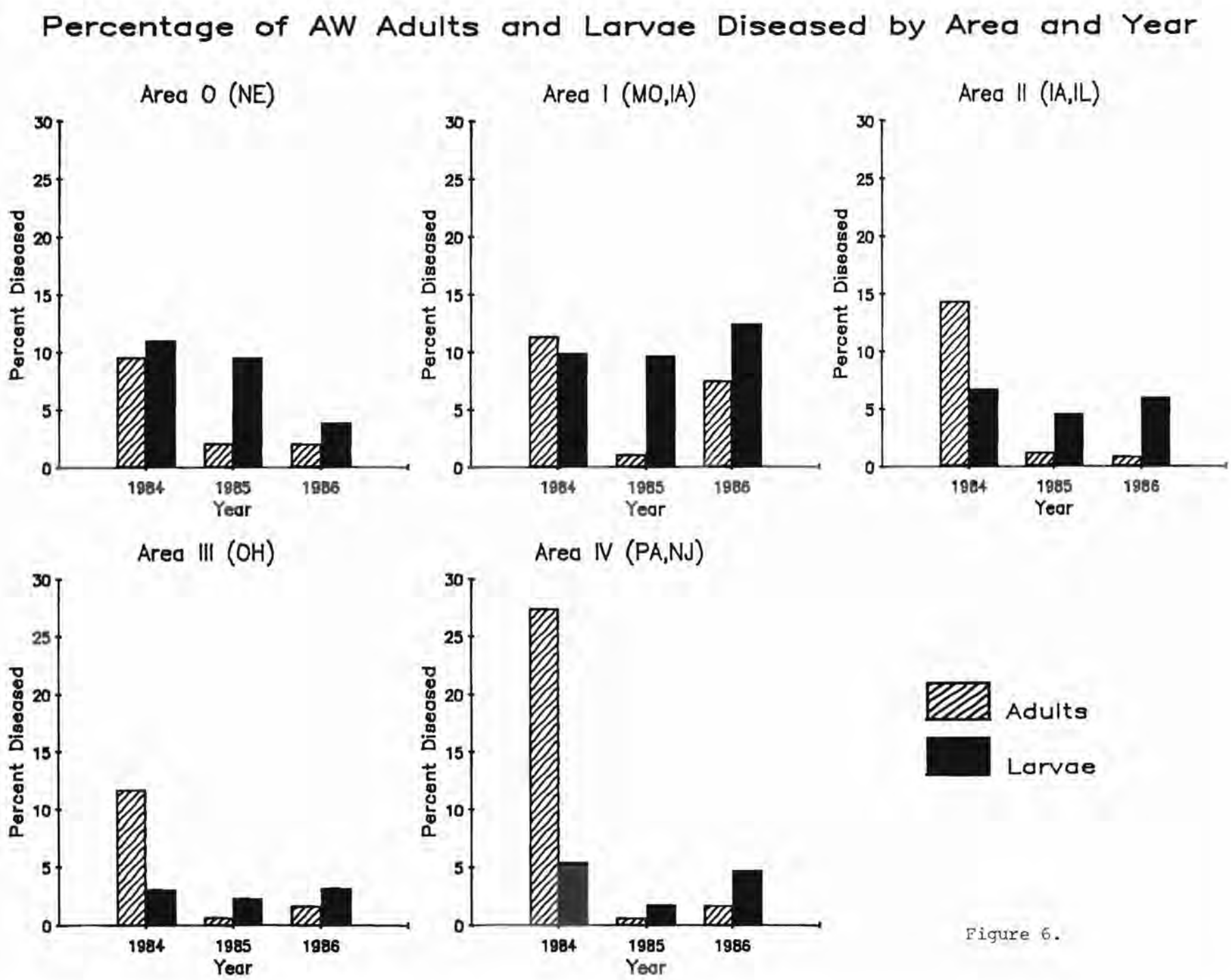

Percentage of AW Adults and Larvae Diseased by Area and Year
Area 0 (NE)
Area I (MO,IA)
Area II (IA,IL)
Area III (OH)
Area IV (PA,NJ)
Percent Diseased
Year
1984
1985
1986
0
5
10
15
20
25
30
Adults
Larvae

Figure 6.

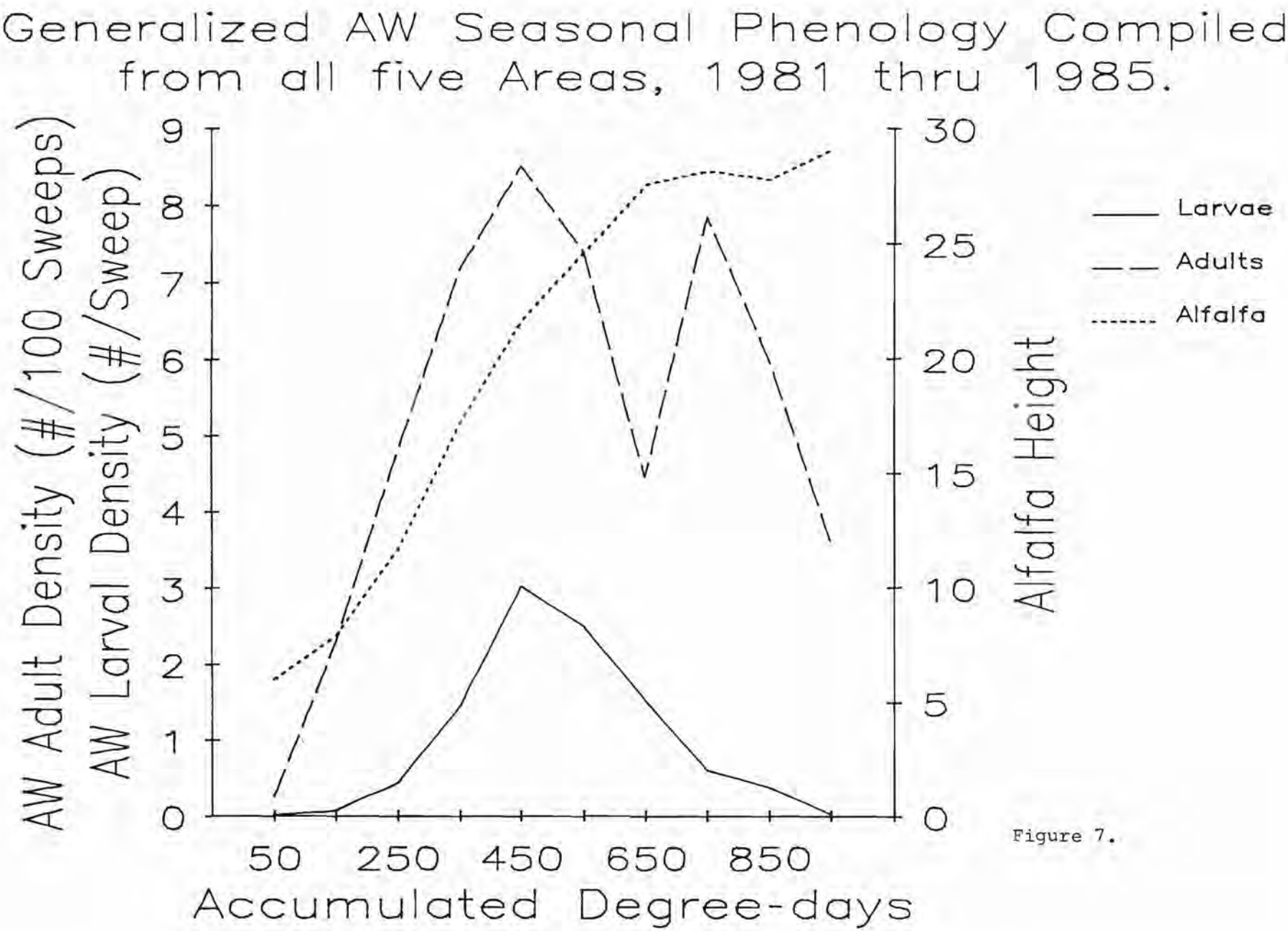

Generalized AW Seasonal Phenology Compiled
from all five Areas, 1981 thru 1985.
AW Adult Density (#/100 Sweeps)
AW Larval Density (#/Sweep)
0
1
2
3
4
5
6
7
8
9
Alfalfa Height
0
5
10
15
20
25
30
50
250
450
650
850
Accumulated Degree-days
Larvae
Adults
Alfalfa

Figure 7.

Appendix 1.

Presented as a poster at the
1986 National Entomological
Society of America meeting
in Reno, Nevada.

The displacement of an established alfalfa weevil parasite by its recently colonized congener in Ohio.

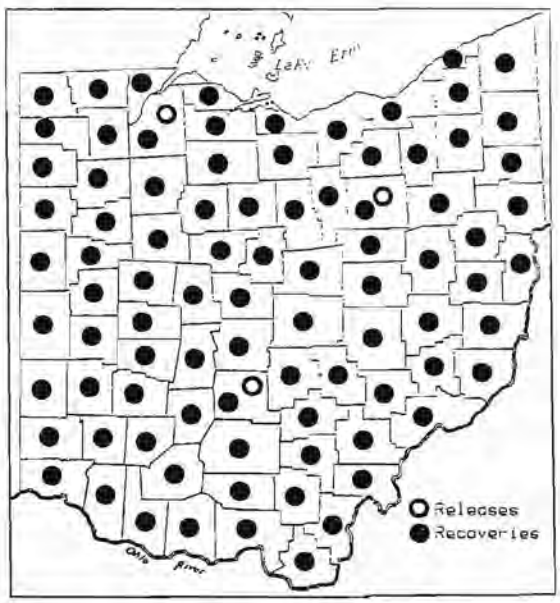
P. C. Kingsley and C. P. Schwalbe

USDA-APHIS-PPQ
Otis Methods Development Center
Otis ANGB, MA

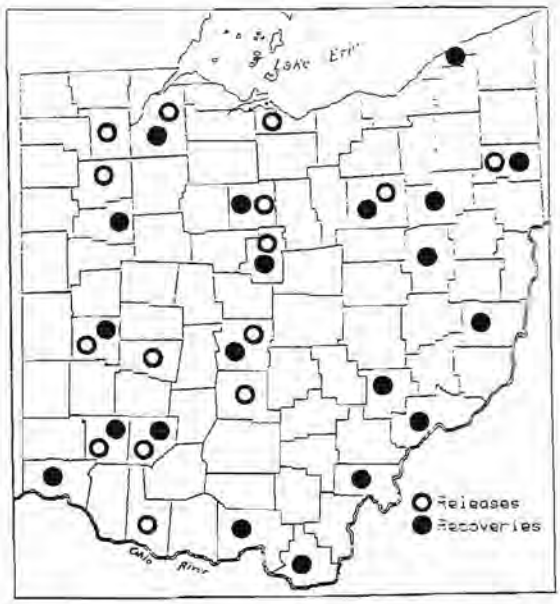
As part of a long term national evaluation of the USDA Cooperative Alfalfa Weevil Biological Control Program, 30 alfalfa fields have been intensively sampled in Ohio for six years. Five fields in each of six counties were sampled nine times during the first and early second cuttings for weevil and parasite populations. Parasitism was assessed by dissections and rearing.

Two introduced species, Bathyplectes anurus (Thomson) and B. curculionis (Thomson) (Hymenoptera: Ichneumonidae), are the major larval parasites of the alfalfa weevil Hypera postica (Gullenhal) (Coleoptera: Curclionidae) in Ohio. B. curculionis was first recovered from Ohio in 1963 and has been established throughout the state since the early 1970's. B. anurus, although well established in the northeast, is a slow diperser and was not recovered in Ohio until 1972. Over 100,000 wasps were released in Ohio between 1970 and 1981.

Releases and New County Records for Bathyplectes curculionis in Ohio.

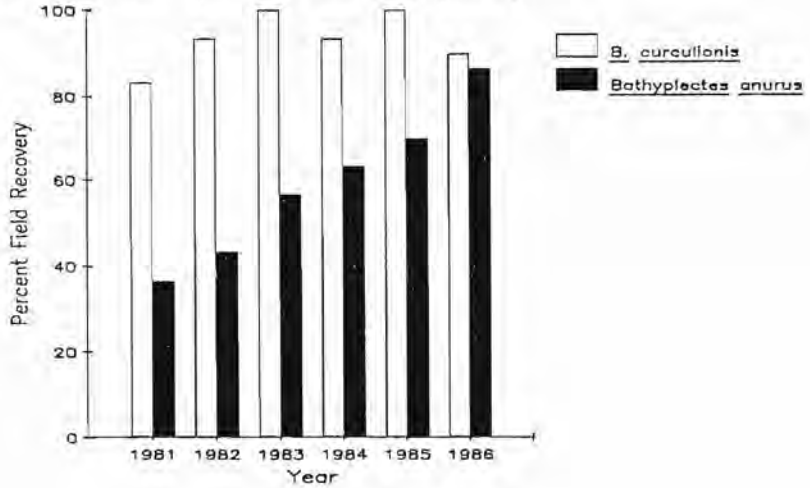


Releases and New County Records for Bathyplectes anurus in Ohio.



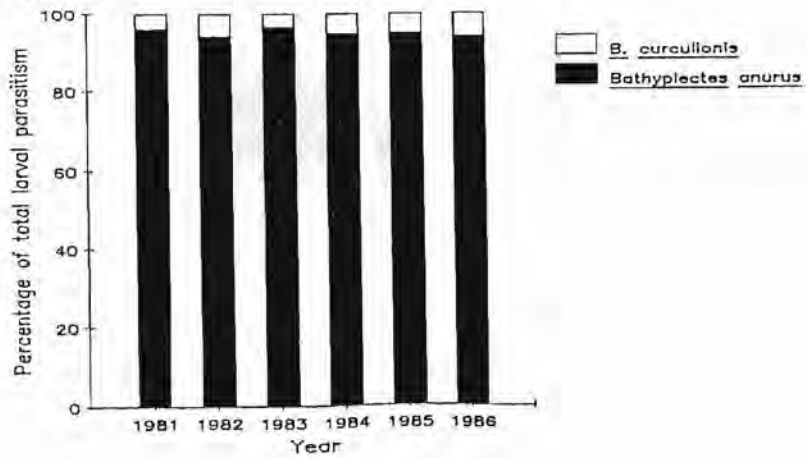
During the six years of our survey, this species has clearly increased its range as indicated by the percentage of the 30 fields sampled where B. anurus was recovered; from less than 40% in 1981 to 85% of the fields in 1986.

Percentage of fields in Ohio where two alfalfa weevil parasites were recovered (N=30 fields/year).



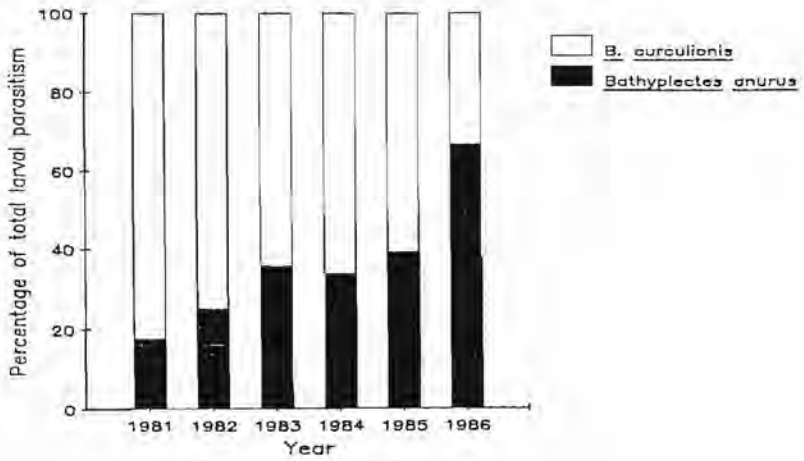
As predicted by Dowell (1977), B. anurus has become the dominant member of the larval parasite guild when sympatric with its congener B. curculionis. Data from 30 fields located in Pennsylvania and New Jersey, where B. anurus has been established since the early 1970's show the culmination of this trend for B. anurus dominance. Here, B. anurus has accounted for over 93% of the total larval parasitism since 1981.

Relative percent parasitism of two alfalfa weevil larval parasites in 30 Pennsylvania and New Jersey alfalfa fields.

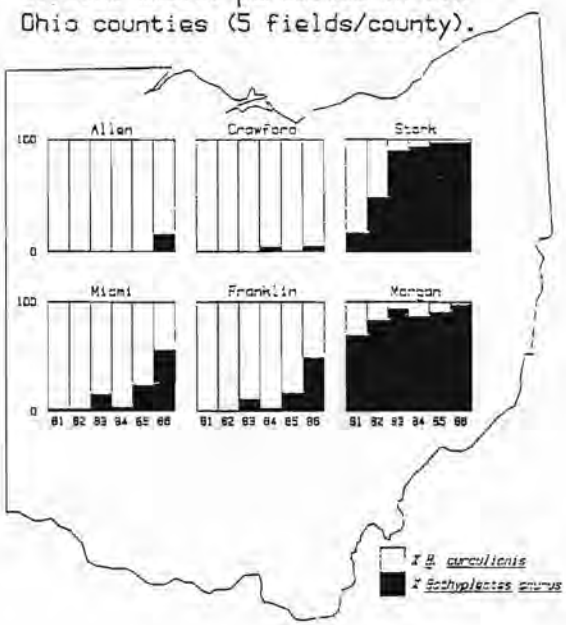


In Ohio, this struggle for dominance is taking place now. Since 1981, B. anurus has increased its portion of the parasitism by both species from 17% to 66% in 1986.

Relative percent parasitism of two alfalfa weevil larval parasites in 30 Ohio alfalfa fields.

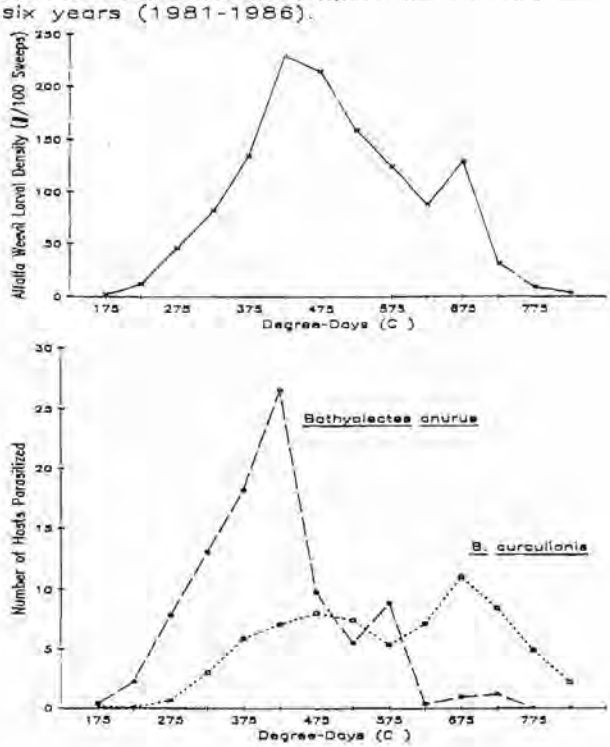


Relative percent parasitism of two alfalfa weevil parasites in six Ohio counties (5 fields/county).



Several reasons were sited by Dowell for the competitive advantage of B. anurus over B. curculionis. These include: better synchronization with its host, elimination of supernumeraries earlier, and the encapsulation of B. curculionis eggs.

Generalized phenology of alfalfa weevil larvae and the number parasitized, based on 30 alfalfa fields sampled in Ohio for six years (1981-1986).



In addition to being more competitive, B. anurus may be more effective. That is, the overall larval parasitism is significantly higher where B. anurus accounts for more than 50% of the parasitism by both species.

Overall parasitism of alfalfa weevil (Hypera postica) larvae in Ohio alfalfa fields where each of the two congeners, Bathyplectes anurus and B. curculionis, are dominant in terms of relative parasitism.

Greater than 50% Parasitized by	Overall Larval Parasitism	Number Dissected	Number * of Fields
B. anurus	26.3%	13,145	63
B. curculionis	14.7%	20,627	117

P < .001 Chi-Square

* 30 fields/year x 6 years

Dowell, R. V. and D. J. Horn 1977. Adaptive strategies of larval parasatoids of the alfalfa weevil. Can. Entomol 110:507-12.

☆ U. S. GOVERNMENT PRITNING OFFICE: 1987--500-296--60132