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J.B. Dimond, D.G. Mott, W.P. Kemp, and J.H. Krall

**MAINE AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF MAINE AT ORONO**

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A FIELD TEST OF MATING-SUPPRESSION
USING THE SPRUCE BUDWORM SEX PHEROMONE

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INTRODUCTION

Spruce budworm sex pheromone, formulated as Hercon^R flakes⁵ (1) was dispersed from aircraft over forest land in Maine in late June, 1980. A major goal was to sample pheromone concentrations in air, through chemical means, to determine whether the Hercon flake formulation would provide the steady, sustained release of chemical believed required for interfering with the mating process of the moths. That the flakes did have these properties has been reported elsewhere (2).

As long as pheromone was going to be applied for purposes of analyses of air, we believed that the opportunity of studying some behavioral effects on spruce budworm populations should be exploited. This report describes these studies. The principal body of data involved the ability of male budworm moths to orient to point sources of pheromone (baited traps) in pheromone treated and untreated forest blocks, but attempts were also made to monitor fertility levels among females and to measure populations of eggs.

METHODS

The pheromone used was Fulure, 97:3(E:Z)-11-tetra-decenal. This was formulated in Hercon flakes which are made up of plastic laminae, the nature of which control the release of the chemical within (1). Flakes were about 4 mm square.

Field blocks were designed to provide evaluation of several dosages of flakes, and to attempt to provide some isolation of treated populations from surrounding untreated populations. Measuring reductions in populations of fertile eggs, which is the ultimate test of the success of pheromones for male confusion (3,4), is impossible if invasion of fertile moths cannot be eliminated, or at least quantified.

Blocks were established in coastal Maine near Machias (Fig. 1). The largest block was 145 ha of spruce-fir forest in Whitneyville. This was

⁵ Hercon Division, Health-Chem Corp., New York

bordered on the east by the open marsh of the Machias River, and on the other sides by young hardwood forest occupying a recent burn. Thus, some degree of isolation from other stands of budworm hosts was provided. This block was treated with the lowest dosage of pheromone, approximately 4 flakes/m² or 50 gms of pheromone per ha. A second 130 ha block, in nearby Marshfield, was established as an untreated control for the Whitneyville block.

The northeast corner of the Whitneyville block, 9 ha., was established as a special sub-block for the air analysis studies (2). Very high dosages of pheromone, about 40 flakes/m², were applied here; the intent of the excessive dosage was to insure positive measurements with the air sampling equipment.

The last two blocks were islands, each about 30 ha in size, in Machias Bay. These, Salt Island and Bare Island (Fig. 1) were largely forested with spruce-fir, and were uninhabited. Salt Island was treated at twice the rate of pheromone applied to the Whitneyville block, 8 flakes/m² or 100 gms/ha. Bare Island was an untreated control. In the remaining text, we refer to the three dosages and blocks as low dose (Whitneyville), medium dose (Salt Island), and heavy dose (air analysis sub-block).

Pheromone flakes were applied to all treated blocks at the first sign of moth emergence on the mainland, June 29-30. Insect development was delayed on the cooler islands. Flakes were applied with a Cessna 180 aircraft, equipped with a flake-dispensing device slung under each wing (1). Flakes in a hopper were fed at a predetermined rate through a tube leading to a spinning, flanged cone, which propelled the flakes into a circular pattern in the aircraft wake. A connected system sprayed the flakes with a glue for adhesion to tree foliage. This device and the aircraft modifications were designed and furnished by Hercon and their consultants.

Guidance of the dispensing aircraft was provided by a combination of fixed flags in tree tops and moveable flag persons with helium-filled balloons, plus an observer aircraft. These procedures, plus lack of drift of the flakes, led to a precise application. This was monitored by counting flakes on m² squares of black plastic, exposed in forest openings.

The most extensive evaluation procedure was measurement of the ability of male budworm moths to orient to traps baited with synthetic pheromone lures. This is a common method of evaluation (4, 5, 6).

If the atmosphere is saturated with synthetic pheromone, male moths should contact point sources of pheromone emission less often, be they females or pheromone baited traps. Traps were Pherocon IC⁶ sticky traps, and these were baited with PVC lures emitting Fulure (7) attached to the trap tops with pins. Lures were formulated with 0.03 percent Fulure and 3 percent Fulure. The stronger lures were used on the islands where budworm populations were expected to be sparse. Trap placement was in grids of 25 traps, spaced 40 m apart to prevent cross attraction (8) between traps. Of 25 traps, 20 were baited and 5 were blanks; the latter were used to correct catches in baited traps for accidental entrapment.

Traps were suspended at mid-crown heights, midway between two adjacent host-tree crowns. To accomplish this, nylon monofilament lines were placed, with a bow and arrow, over branches of the two adjacent trees. The proximal ends of the two lines were connected to angler's snap-swivel, through which was run a third line. This apparatus was raised to mid-crown height by tightening and fastening the distal ends of the first two lines. The third line, running through the snap-swivel, raised and lowered the trap for replacement of the sticky trap bottoms. Some traps were temporarily inactivated by wind damage or human error, but at least 14 baited and 4 unbaited traps were always operating on each grid. For the first 5 days of trapping, traps were exposed for 24-hour periods, but these soon began to saturate as moth numbers increased. Thereafter, traps were exposed for 2 hours per day, or in some cases 3 hours per day on the islands. There were still some problems with traps becoming saturated with moths, but 2 hours was the minimum time that a field crew could service a trap grid. Two days of trapping were missed, due to heavy rain or wind, on the mainland trap grids. Five days were missed on the islands due to fog as well.

Zoecon Corporation, Palo Alto, CA

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Two trap grids, plots 2 and 3, were established in the low pheromone Whitneyville plot. One grid, plot 1, was located in the high dose, Whitneyville air analysis area. A grid, plot 4, was located in the Marshfield control area. These four plots provided comparisons among areas of roughly equal intensities of budworm populations and of phenologies. Plot 5 was established on treated Salt Island and plot 6 on untreated Bare Island. Budworm populations on the islands were lower, and later in development than mainland blocks, and the two islands were compared only with each other.

A propane fueled light trap was operated in the vicinity of each of the two mainland blocks and on the two islands. These were placed in openings or fields outside of forests in the mainland areas, and on rocky points on the islands. Large catches in traps so located are believed to reflect moth invasions rather than localized moth activity, particularly if catches contain many females (D.O. Greenbank, Canadian Forestry Service, Fredericton, N.B. pers. comm.). Evidence of moth invasions was useful in explaining variations in catches in pheromone traps.

At intervals throughout the period of moth flight, moths were captured with Malaise traps, 3-5 per plot, suspended between host tree crowns, and also with a gasoline powered vacuum device. Females in these collections were dissected and mating status evaluated through presence or absence of spermatophores.

Attempts were made to expose tethered, virgin females on tree crowns (9), to determine rates of mating in the blocks receiving various treatments, and to determine the mating status of dead females falling out of the air onto sheets of particle board (10).

Egg populations in the blocks were sampled by two methods. In one, fir and spruce branches were pruned from upper crowns of co-dominant trees, and searched for egg masses. This was done on two occasions using single branches from 20 host trees along a transect crossing each block. Eggs were recorded as hatched or unhatched, and the latter were placed in vials for further incubation. In the second procedure, pruned branches from the same transects were boiled in water. This removes the eggs from the foliage and separates them (C. A. Miller, Maritimes Forest Research Centre,

Canadian Forestry Service, Fredericton, pers. comm.). The boiled, floating eggs can be skimmed from the surface of the boiling tank and counted. This procedure can be used only to estimate unhatched egg populations and not fertility. Boiling of collected branches was done on 5-7 occasions spread over the period of oviposition.

Finally, PVC lures, Hercon flakes with and without glue, and virgin female moths were exposed in Pherocon traps in an area not treated with pheromone flakes. The purpose of this was to estimate the relative potencies of these different pheromone sources in attracting males. Each treatment was replicated in three traps and on three days.

RESULTS

Disorientation of Male Moths

Trap catches are expressed as numbers of moths captured per trap per hour. This eliminates differences caused by different numbers of traps operational on a given day and different lengths of exposure. Baited-trap captures were corrected by subtracting the mean catch per period in unbaited traps.

The pheromone treatment produced marked reductions in the ability of males to locate pheromone-baited traps (Fig. 2), at least when moth populations were below maximum density. Figure 2 represents trap catches in the high pheromone dosage trap grid, plot 1, and the Marshfield control, plot 4. Trap catches were at or near zero for the first few and last few days of moth activity in the treated plot. In the middle period, trapped moths increased somewhat but remained well below the control. We can use numbers of moths accidentally caught in unbaited traps as an index of moth abundance. Accidental catches were zero until July 5 and after July 21. In between there was a steady rise to about 8 moths per trap on July 12, followed by a steady decline. Increased catches in baited traps in the treated plot corresponded with the period of moth abundance in the area. If the daily percent differences in catches between plots 1 and 4 are averaged over the trapping period, the mean is 94 percent. Another comparison is the mean daily catch per trap hour for the two plots. This is 0.7 moths per trap hour for plot 1 and 10.2 for plot 4. The difference here is 93 percent.

The light trap catches at Whitneyville and Marshfield showed peak catches and many females suggesting moth invasions, on July 4, July 8 and 9, and July 14-19 (Appendix Table 1). These are probably related to the peak catches on plot 4 (Fig. 2) on July 11-12, and July 16-19.

Trap grids on the low dose block at Whitneyville, plots 2 and 3, indicated less disorientation of males than with the high dose of pheromone (Fig. 3), and plot 2 characteristically showed less disorientation than plot 3. We believe the latter difference reflects moth abundance. Plot 2 was an upland, mesic site with much balsam fir and red spruce; plot 3 was wet with much black spruce and cedar. Budworm larval populations were more abundant at plot 2 than at plot 3.

The average daily percent difference from the control plot was 74 and 86 percent respectively for plots 2 and 3. The two plots averaged 71 and 85 percent, respectively, lower than untreated plot 4 in catch per trap hour.

The medium dosage of pheromone applied to Salt Island (plot 5) appeared to be as effective as the high dosage applied at Whitneyville (Fig. 4). But a direct comparison should not be made since moth densities were lower on the islands. As an index of this, the mean catch per trap hour in blank traps at Marshfield (plot 4) was 1.59, while on untreated Salt Island (plot 6) this value was 0.22 or 86 percent less. Another indication was that defoliation of host trees was very heavy on the mainland, but light on the islands. Baited traps on the islands caught as many or more moths than on the mainland, but the PVC lures were stronger.

The mean daily percent reduction in trap catches on Salt Island was 93 percent. The difference in mean catch per trap hour between the two islands was 92 percent (Fig. 4).

The July 20-25 period was the period of increased captures on the treated island. This appears clearly related to a large moth invasion detected at this time by light trap. On a single night (July 21), 75 percent of all male moths and 57 percent of all female moths captured over the trapping season, were captured at the Salt Island trap. Light trap counts are given in Appendix Table 1.

Mating Status of Captured Females

Malaise traps showed no difference between treated and untreated blocks in proportions of females mated (Fig. 5). Fifty seven to 73 percent of females were unmated regardless of treatment. Examination of moths collected with the vacuum device showed a similar result (Table 1). Proportion of mated females ranged from 55 to 100 percent, with no evidence of effects due to pheromone treatment.

Deposition and Fertility of Eggs

There were no differences in egg numbers that could be ascribed to treatment. On untreated Marshfield block, numbers of unhatched eggs per 20 fir branches rose from about 100 on 7/9 to 3900 on 7/18, declining to 500 on 7/28. Numbers on spruce followed the same trend exactly, but at slightly lower numbers. On the high-dose air-analysis sub-block, there were somewhat fewer eggs, but the differences were not significant. On the same dates, unhatched-egg numbers on fir rose from 200 to 3400, declining to 200. The low-dose Whitneyville block had numbers of unhatched eggs almost exactly the same as Marshfield, 100, 3800, and 400 respectively. The island situation was similar. Here only spruce was sampled, fir being uncommon. On treated Salt Island, unhatched egg numbers rose from 100 on 7/15 steadily to 1000 on 7/29, when sampling was terminated. On Bare Island, comparable numbers were 50 rising to 1000.

We also detected no unusual numbers of infertile eggs on any blocks. When parasitized and damaged eggs were discounted, fertility rates exceeded 90 percent in all collections.

Mating Status of Tethered Females and Spent Females

Numbers of female moths tethered and exposed in the blocks, and numbers of spent females collected from drop-boards placed on the ground, were too few to produce meaningful data. Among tethered females, a first exposure (7/5) produced suggestive results. After 48 hours, dissection of 4-6 females recovered per treatment indicated that none had mated in the high pheromone dosage block, 25 percent had mated in the low dosage block, and 100 percent had mated in the untreated control. However, when the trial was repeated (7/12), the percentage of exposed moths that were mated

was a uniform 25-30 percent in all three circumstances. And in a third trial, nearly all the females died, apparently from high, mid-day temperature. A much larger effort would probably be required to use tethered females to evaluate success of pheromone applications, but the procedure is tedious and may be impractical (6).

Collections of dead moths on drop boards produced very small numbers of insects, less than one female per board per collection period. Males were more numerous, but still not common. Removal of specimens by ants and vertebrates was suspected as contributing to the small numbers. Numbers were too small for any meaningful analysis.

Attractive Power of Baits, Flakes, and Females

Over three exposure periods, between July 9 and 15, the relative attractiveness of PVC baits, Hercon flakes, and captive virgin females did not change (Table 2). The PVC bait attracted the greatest number of male moths ($p < 0.05$). Single and double Hercon flakes, with or without the adhesive, showed lesser attractiveness, but with no difference between them. The attractiveness of a single virgin female could not be distinguished from that of the unbaited traps.

DISCUSSION

Trap captures showed that the Hercon flake formulation of budworm pheromone interfered with the ability of males to locate pheromone traps in the treated plots. Sanders and Silk (5) reported very similar results with the same pheromone system. The difference in catches between treated and untreated plots usually exceeded 95 percent and was often 100 percent when moth densities were low. When moth densities were high, the disorientation effect of the pheromone treatments weakened. Results showed a dosage response of the pheromone at high moth densities, which interacted with the different population densities of mainland and the islands. All doses were equally effective at low moth densities.

These trapping results may be an underestimate of the true disorientation effect of the treatments because of problems with trap saturation. Once trap catches exceed about 50 moths, trap efficiency decreases (8). Obviously, trap saturation would occur more often in

untreated plots 4 and 6, than in treated plots; traps would still be operating efficiently in treated plots, while not in untreated plots. To attempt to reduce problems with trap saturation, we reduced daily exposures from the original 24 hours to 2 hours. There was still some saturation in the 2-hour periods, however. Twenty-five to 30 percent of traps exceeded 50 moths, the saturation level, in both plots 4 and 6 during the periods of heavy moth activity.

Should a similar project be undertaken again, some steps would be needed to reduce saturation of traps. More staff or smaller trap grids would reduce time to service a grid. However, more significant might be placement of traps. We had them suspended at mid-crown level in an attempt to maximize catches. It appears that we could have made them somewhat more difficult for moths to locate by suspending them near ground level, probably reducing saturation, and avoiding considerable effort and expense of placing and tending traps.

In spite of the evidence of male disorientation displayed with trap results, other measures of female fertility and egg hatch suggest no regulation of budworm numbers provided by the treatments. Two factors prevent drawing firm conclusions from this, however. One of these was the heavy incidence of moth invasions. In spite of our efforts to obtain isolation by choice of islands and inland sites surrounded by non-host forest, moth invasions occurred on 7 to 9 nights at each site, as indicated by light trap captures. Moths were trapped in large numbers on the islands before pupae there had matured. A Maine Forest Service light trap, operated in nearby Edmunds Twp., showed the highest total captures in 19 years in the region (Fig. 6). The second factor involves lack of extensive knowledge of oviposition and activity behaviors of unmated compared to mated females. Outram (11) showed that unmated females lay fewer eggs and smaller egg masses. Unmated females may also deposit eggs in different locations and be less vulnerable to capture with Malaise traps or suction devices. We are not sure that we were looking in the right places for sterile eggs. Nevertheless, our results suggest that no significant level of population reduction is achieved with pheromone applications at budworm outbreak densities, agreeing with the test of Sanders and Silk (5). This

is in spite of the fact that several studies have shown significant reduction of mating in caged budworm moths (5,9). Wind tunnel experiments show that some males can locate females in high concentrations of pheromone, and field applications may, at most, only delay oviposition (5).

Attempting to locate isolated locations for tests such as these may be futile with insects as mobile as budworm moths. Our islands were within 0.5-1 km of the mainland. But, more remote islands of small size were exposed and lacked forest. Remote, forested islands were too large to treat in entirety with the resources available. More recently, cages have been used to address the problems of moth invasion (5).

The Hercon flake formulation worked well in providing a sustained release of pheromone, as indicated by the air sample studies of Weisner and Silk (2), and some observations of our own. Where baits, flakes, and virgin females were compared in traps, the flakes were nearly as attractive as baits, and much more attractive than virgin females. We also checked, periodically, five traps that were baited with single flakes and exposed on untreated Bare Island. We were concerned that flake effectiveness might not persist through the delayed period of moth emergence on the islands. Flake-baited traps continued to attract males through the end of the experiment. Sanders and Silk (5) report that, using the same pheromone system, excessive numbers of flakes failed to adhere to foliage in the tree crowns. We did not investigate this problem.

Table 1. Data for vacuumed moth collections, 1980 pheromone trials, Machias, Maine.

Date	1			2			3			4			5			6		
	♀ (♀ _m)	♂	0 ^x	♀ (♀ _m)	♂	0 ^x	♀ (♀ _m)	♂	0 ^x	♀ (♀ _m)	♂	0 ^x	♀ (♀ _m)	♂	0 ^x	♀ (♀ _m)	♂	0 ^x
7/06/80	1 (1)	24		-- (--)	---		-- (--)	---		0 (0)	16		-- (--)	--		-- (--)	--	
7/07/80	1 (0)	39		-- (--)	---		1 (1)	36		1 (1)	22		-- (--)	--		-- (--)	--	
7/08/80	3 (3)	4		-- (--)	---		0 (0)	0		5 (2)	40		-- (--)	--		-- (--)	--	
7/09/80	2 (1)	49		0 (0)	33		0 (0)	20		-- (--)	---		-- (--)	--		-- (--)	--	
7/10/80	2 (1)	4		20 (17)	104		-- (--)	---		13 (10)	120		-- (--)	--		-- (--)	--	
7/11/80	7 (6)	50		-- (--)	---		-- (--)	---		-- (--)	---		-- (--)	--		-- (--)	--	
7/15/80	3 (1)	41		9 (6)	88		11 (7)	120		25 (20)	137		-- (--)	--		-- (--)	--	
7/17/80	3 (2)	44		15 (12)	57		18 (7)	142		19 (17)	85		-- (--)	--		-- (--)	--	
7/22/80	16 (15)	8		2 (0)	13		3 (3)	18		5 (3)	7		-- (--)	--		-- (--)	--	
7/24/80	-- (--)	---		-- (--)	---		-- (--)	---		-- (--)	---		3 (3)	36		9 (9)	44	
Total	38 (30)	263		46 (35)	295		33 (18)	336		68 (53)	427		3 (3)	36		9 (9)	44	
Percent ♀ _m	79			76			55			78			100			100		

Table 2. Counts of moths per 100 minutes attracted to sticky traps baited various ways.

		Time 1	Time 2	Time 3	Mean
PVC Bait	1	--	--	43.8	
	2	32.3	40.9	25.6	35.3
	3	34.7	36.1	33.9	
Single Flake	1	16.7	14.4	6.6	
	2	12.4	9.4	14.9	
	3	9.0	21.5	24.4	
Double Flake	1	5.8	3.5	5.8	6.9
	2	10.2	12.3	9.1	
	3	5.7	5.6	3.9	
Single, Glue	1	29.5	24.4	11.5	12.6
	2	11.0	10.0	4.2	
	3	6.7	5.9	9.8	
Double, Glue	1	15.5	8.0	18.1	10.6
	2	8.7	6.3	3.3	
	3	7.1	5.3	23.1	
Female	1	2.5	2.3	0.9	1.2
	2	2.4	1.2	0.0	
	3	1.5	0.0	0.0	
Unbaited	1	1.6	2.7	0.0	3.0
	2	5.7	4.8	5.6	
	3	3.5	1.1	1.9	

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Appendix Table 1. Numbers of total moths and female moths captured in four light traps.

Date	Whitneyville	Marshfield	Salt Island	Bare Island
7/2	0	0	-- ^{1.}	--
7/3	0	0	--	--
7/4	14(3) ^{2.}	1	21(10)	52(23)
7/5	0	6	0	6(5)
7/6	0	0	--	--
7/7	0	0	--	--
7/8	6(1)	1	--	0
7/9	14(4)	1	7	21(13)
7/10	1	2	--	0
7/11	8	28	--	--
7/12	2	4	0	3(1)
7/13	--	--	--	--
7/14	7(2)	42(8)	0	0
7/15	17(6)	20(3)	--	0
7/16	9(2)	12	1(1)	4(1)
7/17	85(27)	--	6(1)	2
7/18	28(6)	37(12)	0	40(19)
7/19	20(7)	87(34)	0	--
7/20	--	--	--	--
7/21	7(4)	31(13)	90(20)	0
7/22	0	--	0	5
7/23	0	1	--	--
7/24	0	3(2)	2(2)	11(5)
7/25	4(1)	1	1(1)	8(4)
7/26	--	4(3)	--	0
7/27	--	--	--	--
7/28	0	--	--	--
7/29	0	--	--	0
7/30	--	--	0	0
7/31	--	0	--	4(3)
8/1	--	--	--	--
8/2	--	--	--	--
8/3	--	0	--	2(2)
8/4	--	--	0	3(2)

1. The symbol -- indicates no data.

2. The first number is total moths; the number of females is in parenthesis.

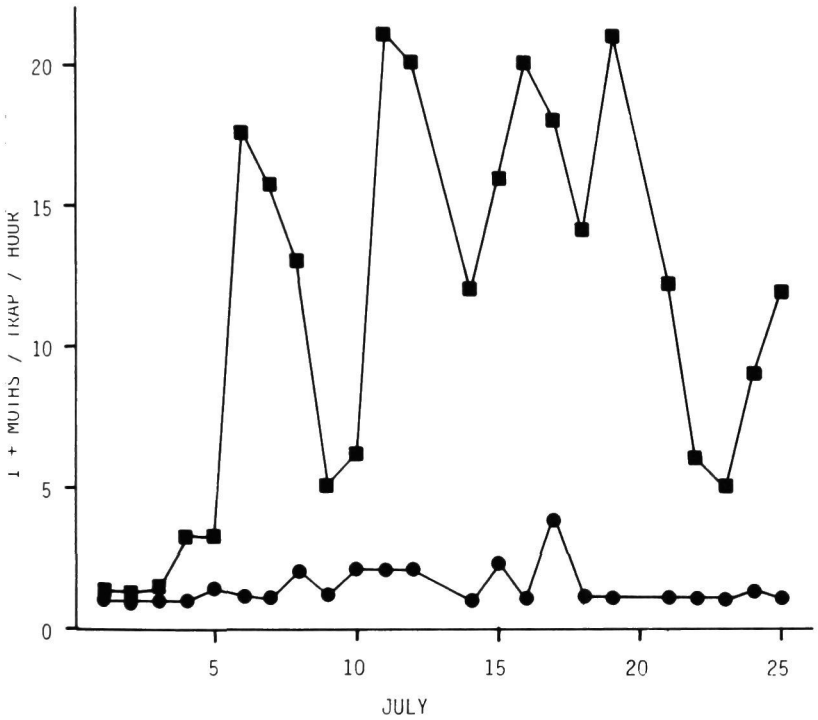


Figure 2. Spruce budworm moths captured in pheromone baited traps in high-dose plot 1 (lower curve) and untreated plot 4 (upper curve).

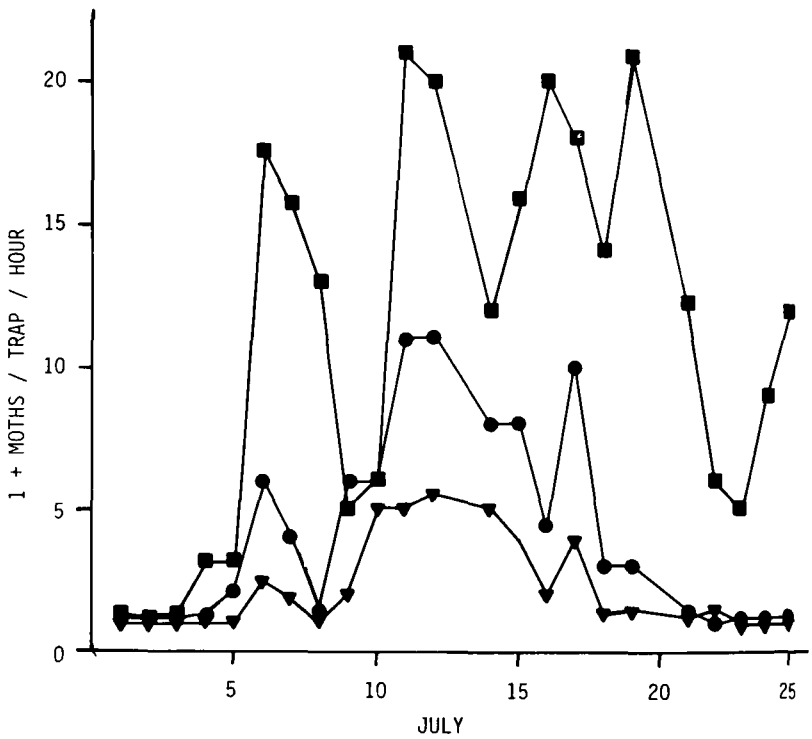


Figure 3. Spruce budworm moths captured in pheromone baited traps in low dose plot 2 (middle curve) and plot 3 (lower curve) and in untreated plot 4 (upper curve).

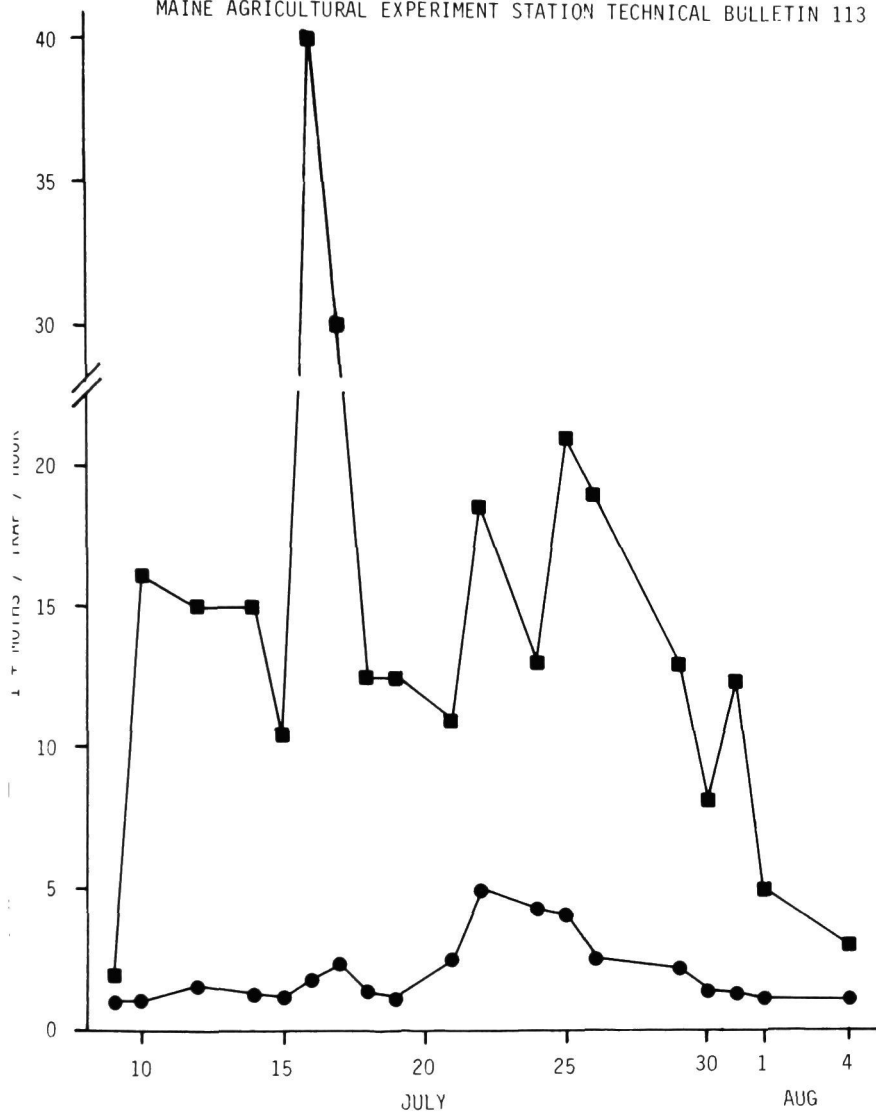


Figure 4. Spruce budworm moths captured in pheromone baited traps moderate dose plot 5 (lower curve) and untreated plot 6 (upper curve).

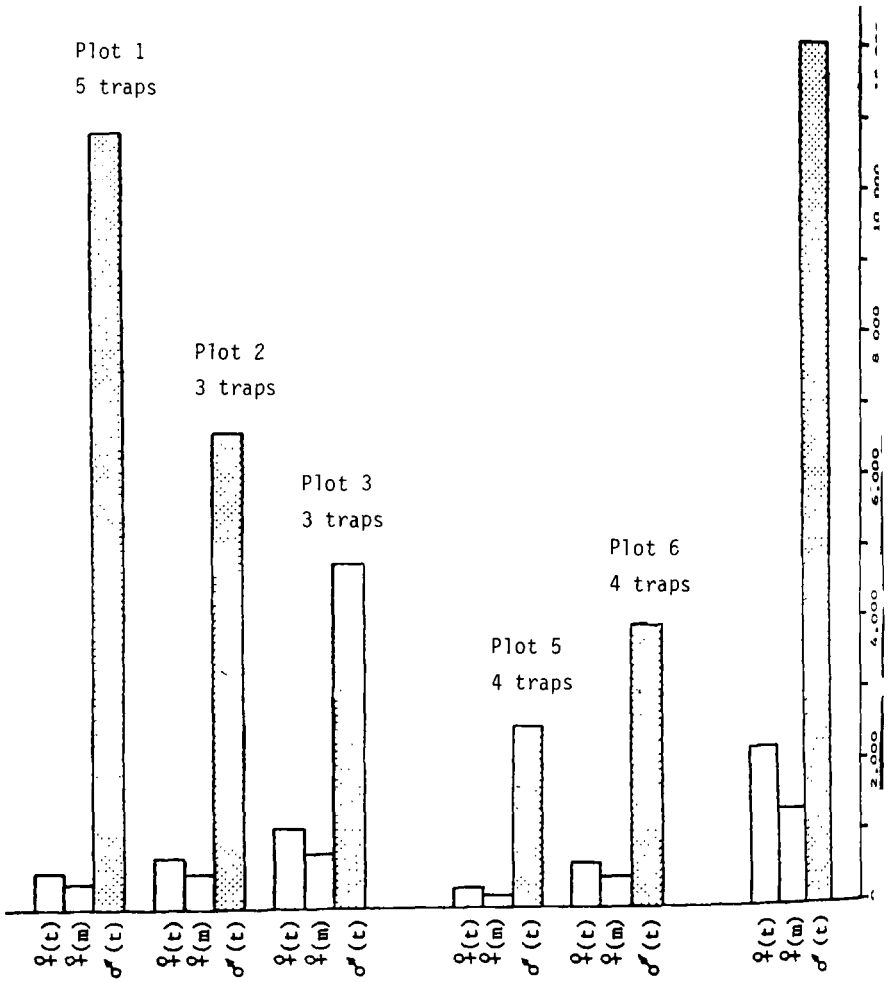


Figure 5. Numbers of total female moths, mated female moths, and total male moths collected in Malaise traps in pheromone-treated and in control plots.

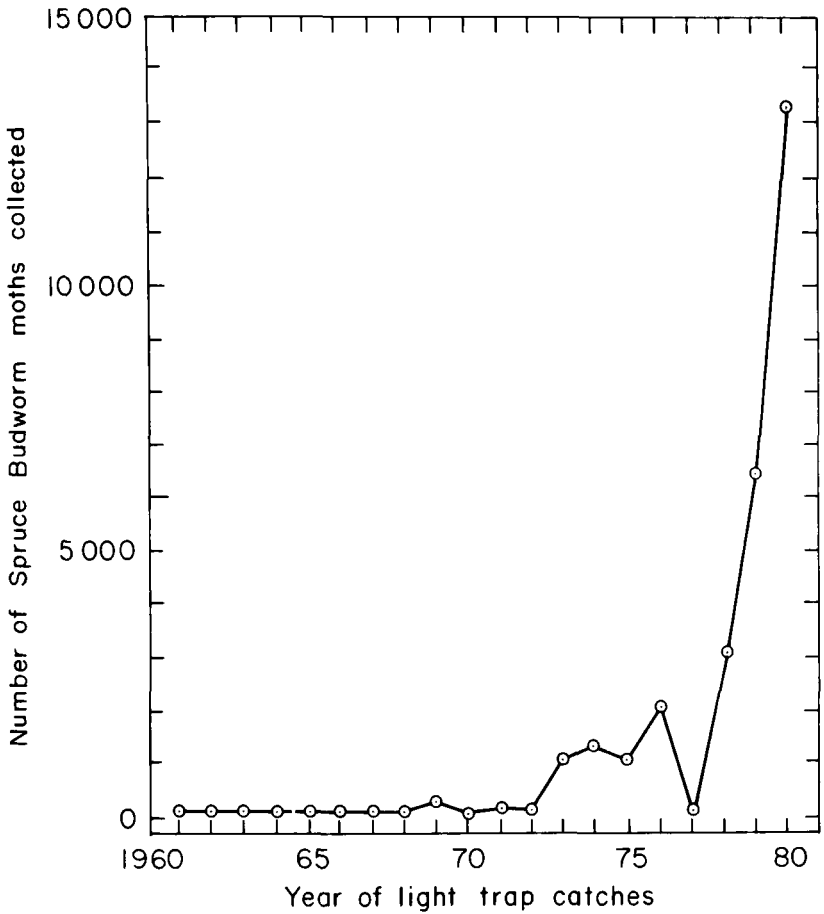


Fig. 6. NUMBER OF SPRUCE BUDWORM MOTHS COLLECTED IN A LIGHT TRAP AT EDMUNDS TWP., MAINE, IN 1960-1980. (Redrawn from Devine and Connor, 1981)