Project Number: 79-A6

Mating Disruption of the Navel Orangeworm

Annual Report

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Title: Project 79-A6 Navel Orangeworm Research
Pheromone Field Testing

Prepared by: Peter J. Landolt

Objectives: 1) To explore feasibility of using pheromone to control n.o.w. by disruption of mating; 2) to explore feasibility of using pheromone to control n.o.w. by mass trapping; and 3) to develop existing pheromone as a useful tool for monitoring n.o.w. activity in the field.

Field Attractiveness of the Synthetic NOW Pheromone, (Z,Z)-11,13-hexadecadienal

Interpretive Summary: The navel orangeworm pheromone (Z,Z)-11,13-hexadecadienal, appears to be only slightly attractive to male navel orangeworm. Trap tests this season indicate that navel orangeworm populations must be quite high before significant catches can be expected in Pherocon traps baited with optimum doses of the synthetic pheromone. This would make it unsuitable as a tool for monitoring adult emergence, since such information is needed principally during spring and early hullsplit when populations are not usually high.

Experimental Procedure: Seven separate tests were conducted during the 1979 field season evaluating the attractancy of the synthetic navel orangeworm pheromone (HDDA). All involved baiting Pherocon sticky traps with pheromone and checking subsequent numbers of males trapped. The following tests were carried out:

1. Attractancy of 1 cm pieces of PVC containing 5% pheromone. Set up in mid April and run for 2 weeks. (6 reps.)
2. Attractancy of 1 cm pieces of PVC containing .01% HDDA .1%, 1%, 5%, and 5% with an antioxidant. Set up mid April and run 3 nights. (3 reps.)
3. Attractancy of pheromone in rubber septa at 7 µg, 1.5 µg, and 30 µg doses. Set up in late April and run 4 nights.
4. Attractancy of isomer blends of pheromone in rubber septa. 1:1, 1:3, and 3:1 ratios of the ZZ and EZ isomers tested. Set up in late April and run for 4 nights. (3 reps.)
5. Attractancy of pheromone at doses of .1, and 1.0, and 5.0 mg in Zoecon rubber septa. Six reps. run in early May for 4 nights; 6 reps in late May for 4 nights.
6. Attractancy of pheromone in rubber septa at doses of .3, 3, 30, and 100 µg. Set up in early July and run for 4 nights. (3 reps.)
7. Attractancy of pheromone in rubber septa at doses of 30 µg. Set up August 9, and run for 56 nights, with 2nd application 21 days after start. (3 reps.)

Results:

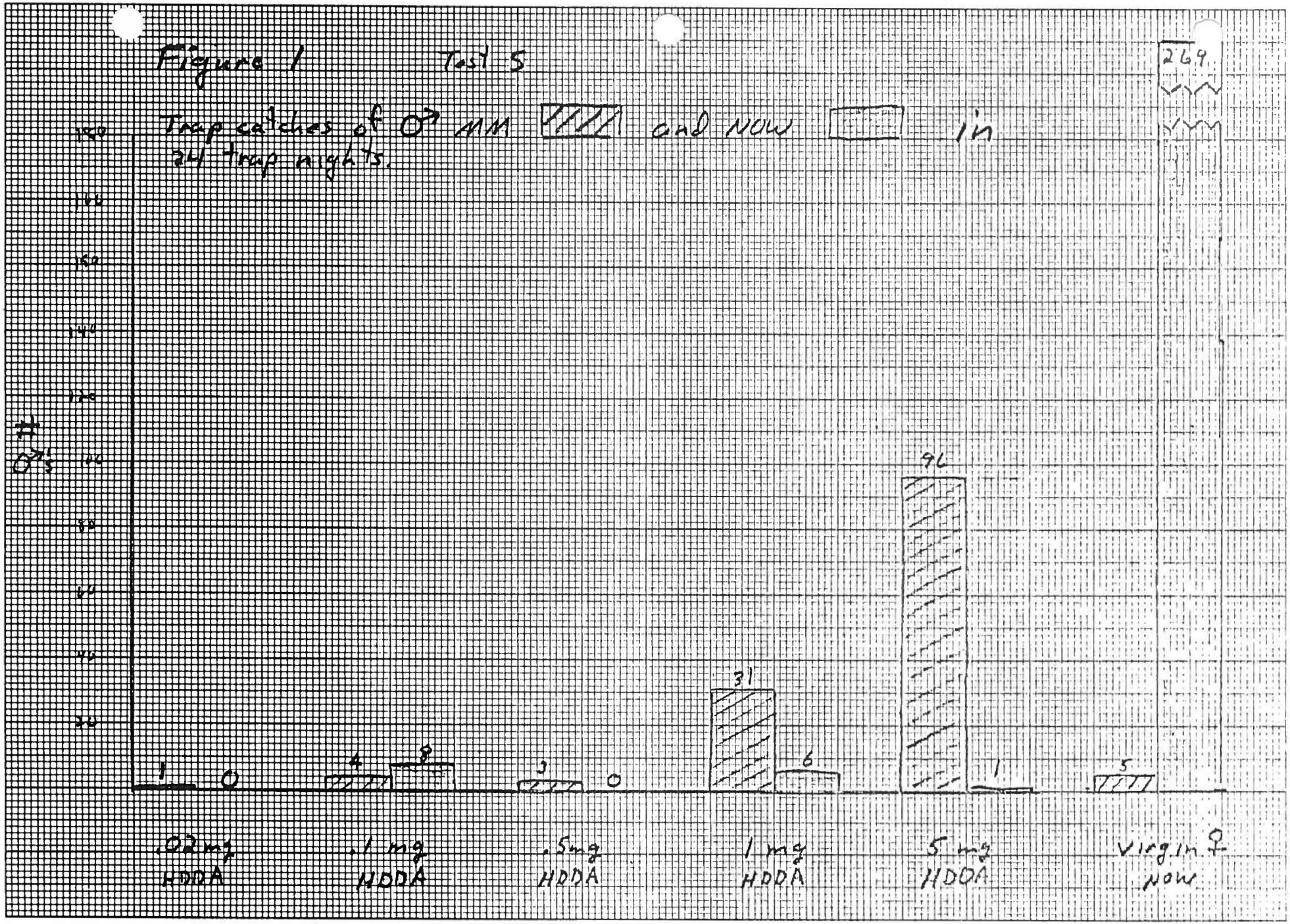
Tests 1 and 2 with PVC formulated pheromone, and tests 3 and 4 with rubber septa, did not catch male n.o.w. Results of tests 5, 6, and 7 are presented in figures 1, 2, and 3. In test 5, some n.o.w. were caught with .1 mg and 1 mg doses while many *Pyralis farinalis* males were caught with 1 mg and 5 mg doses. In test 5, a few n.o.w. males were caught with 30 mg and 100 mg doses. 30 mg doses in test 7 caught 29 n.o.w. males total with 168 trap nights, compared to 5007 in virgin female baited traps and 0 in the blanks.

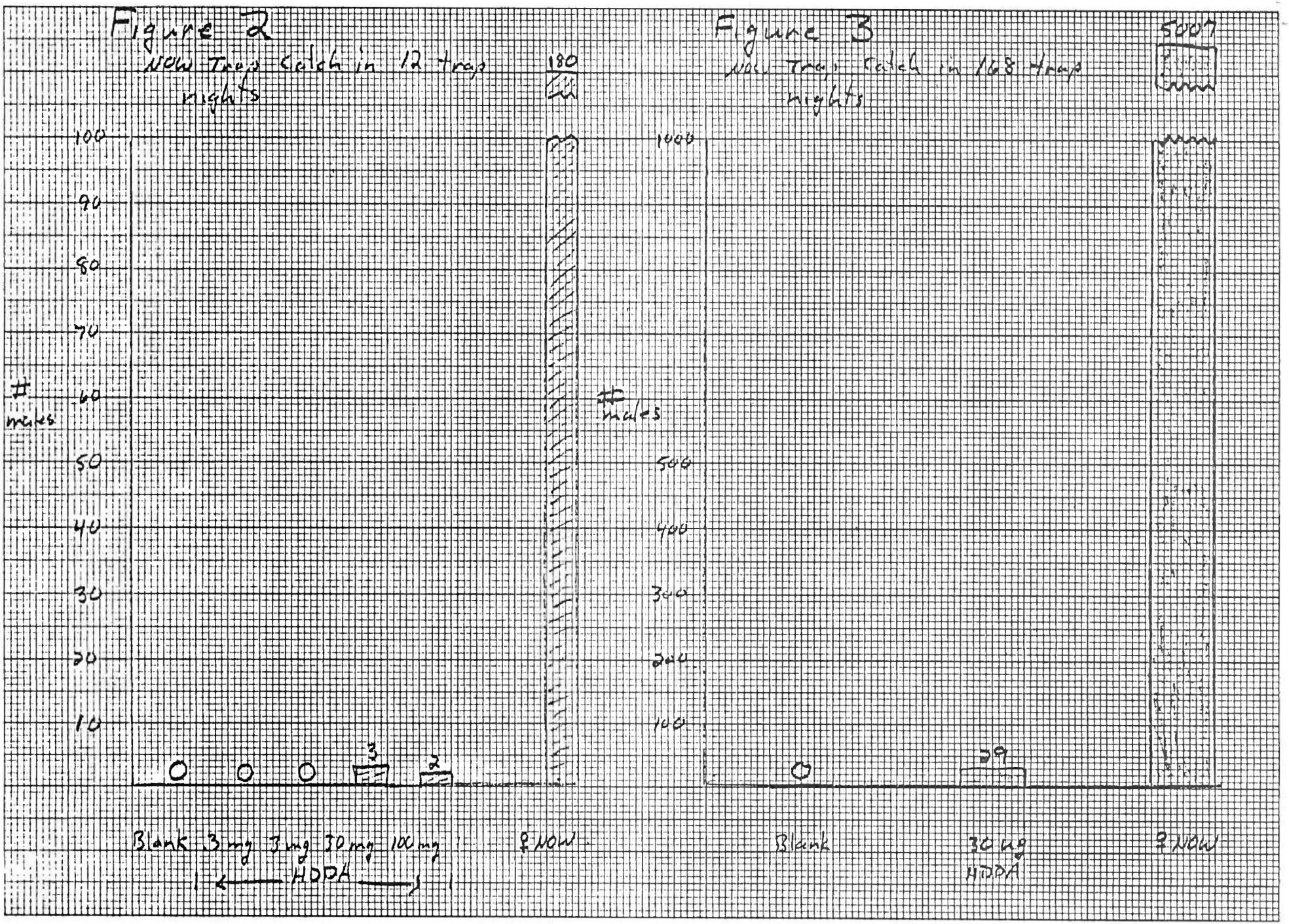
Discussion:

Previous studies indicated field attractancy of the pheromone (ZZ) -11,13-hexadecadienal for n.o.w. males and the possibility of its use as an orchard monitoring tool (Curtis, et al, 1980.) However, it now appears that its performance as a bait is related to the n.o.w. population levels. Previous tests were conducted with much higher n.o.w. populations. At lower populations, it is not a satisfactory attractant for monitoring purposes. Possibly it is one component of a pheromone system used by the moths.

Publications:

Curtis, C. E., J. A. Coffelt, K. W. Vick, P. E. Sonnet, and R. E. Doolittle. 1980. Sex pheromone of the navel orangeworm, *Amyelois transitella:* Field attraction of male to (ZZ)-11,13-hexadecadienal. Environmental Entomol. (in prep.).





Effects of Within Tree Placement on N. O. W. Trap Catch

Interpretive Summary:

Pherocon traps baited with virgin females caught significantly more males at 4 meters (mid canopy) than at 2 meters (bottom of canopy). Traps on the ground caught significantly fewer males than any of the others.

These results suggest that male activity is primarily within the canopy. Monitoring traps would best be placed in mid canopy for optimum catches.

Experimental Procedure:

Pherocon IC sticky traps, each baited with 3 virgin female n.o.w., were positioned at ground level, 2 meters, 4 meters, and 6 meters in mature almond trees. Each trap height was replicated three times and only one trap was used per tree. Trap trees were 4 trees apart in 3 rows; also 4 trees apart. Trap heights were randomized within rows.

Trap catches were monitored daily and females replaced every 3-4 days.

Results:

The results are summarized in Table I which includes average daily trap catch for each trap height tested and total trap catches. Trap catches at ground level were significantly less than catches at all other levels. Catches at 2 meters were significantly less than catches at four meters. Maximum trap catches were obtained at 4 meters, which corresponds to mid-canopy in the almond trees used in this study.

Table I N. O. W. Trap Catches at Different Heights

	Ground	2 meters	4 meters	6 meters
Daily Means $\bar{x}$/Trap Night	5.6^{a}	11.4^{b}	18.5^{c}	15.8^{bc}
Total	135	274	444	380

Average nightly trap catch and totals for 24 trap nights. Means followed by different letters are significantly different ($\alpha = .01$)

Navel Orangeworm Mating Distruption

I. Initial Tests

Interpretive Summary:

Pheromone placed in traps baited with virgin female n,o.w. reduced subsequent catches of males. The effect was maintained when pheromone sources were moved 1 ft. away from traps. Females still called in the presence of the synthetic pheromone.

Experimental Procedure:

A number of small scale tests were conducted attempting to reduce trap catches by putting synthetic pheromone on or near traps baited with virgin females. The four reported on here involved the use of a PVC formulation for pheromone release. In three of the tests, a one cm piece of PVC was pinned to each trap top. The PVC contained 5% synthetic (Z,Z)-11,13-hexadecadienal. The fourth test involved putting 4 pieces of PVC ca one foot from the trap. All traps were baited with 3 virgin female n.o.w. in a screen cage. Each test was run with 3 replications and an equal number of untreated controls. The following is a brief description of each:

1). (Figure 4) Run from 14 April to 1 May, 3 reps., each treated trap with a 1 cm piece of PVC containing 5% pheromone by weight. Traps were 6 trees apart in a single row.

2). (Figure 5) Run from 21 April to 3 May. Set up like #1, but in a different orchard. Traps were 5 trees apart in a single row.

3). (Figure 6) Run from 3 to 14 May. Treatment was a 1 cm piece of PVC with 5% pheromone pinned to trap. Treatment and check with 3 reps.

4). (Figure 7) Run from 3-14 May. Treatment was 4 one cm pieces of PVC with 5% pheromone placed about 1 ft. from trap. 3 treatment and 3 control reps. were run.

Results and Discussion:

In all 4 tests, numbers of males caught in virgin female baited pherocon traps were kept near zero for eight days. During this period, it is assumed the male attraction to our females was disrupted by the synthetic pheromone released from the PVC. After eight days males were again being trapped, presumably after the expenditure or breakdown of the pheromone.

It is possible that the males were repelled by the pheromone and not disrupted by an inability to locate the females. However, later experiments more or less resolved this point.

FIGURE 4 Cumulative trap catch; treatment was ten pieces of PVC with 5% pheromone in trap. Controls had blank pieces of PVC.

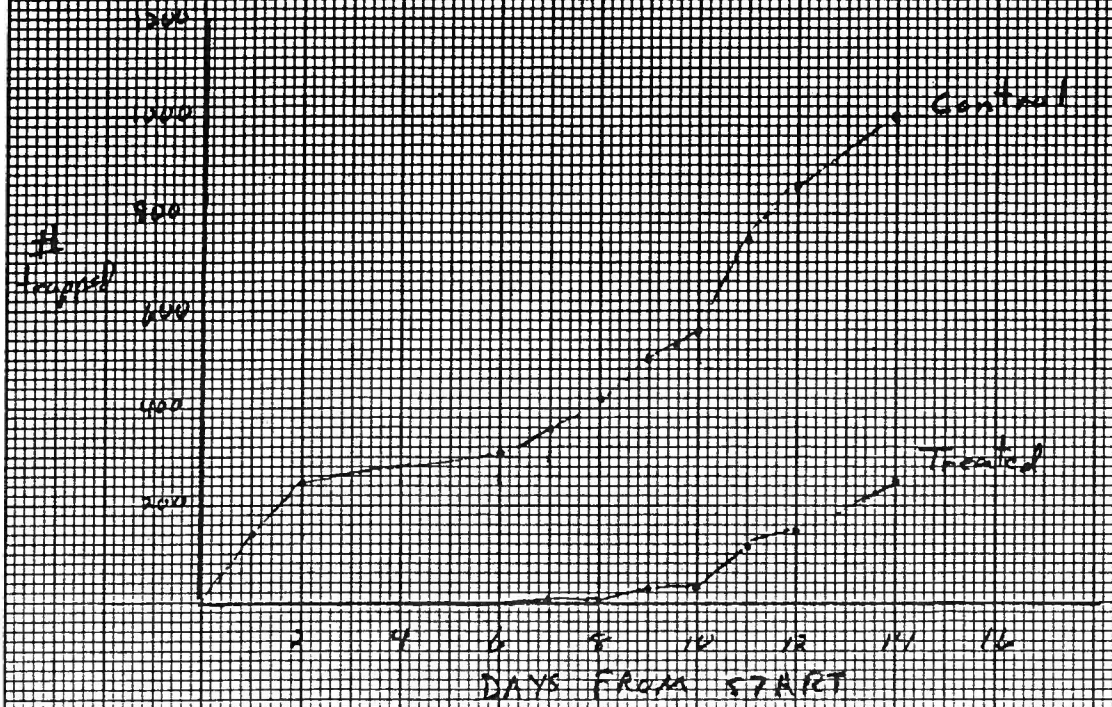


FIGURE 5 Same as above

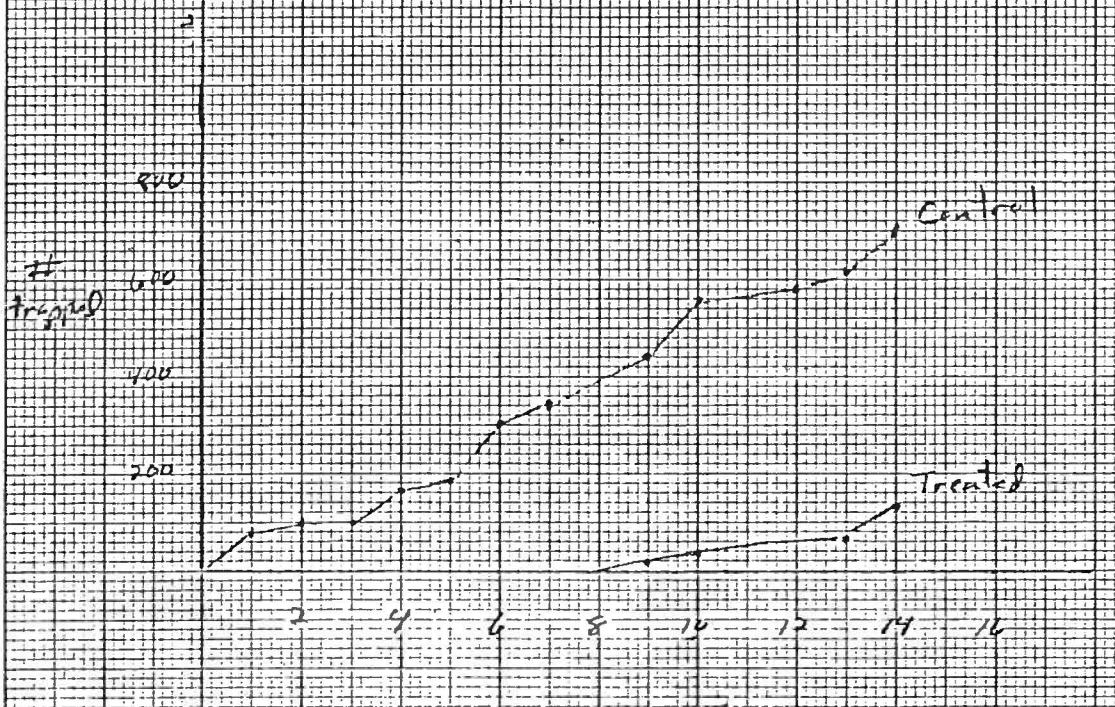


FIGURE 6 Cumulative trap catches

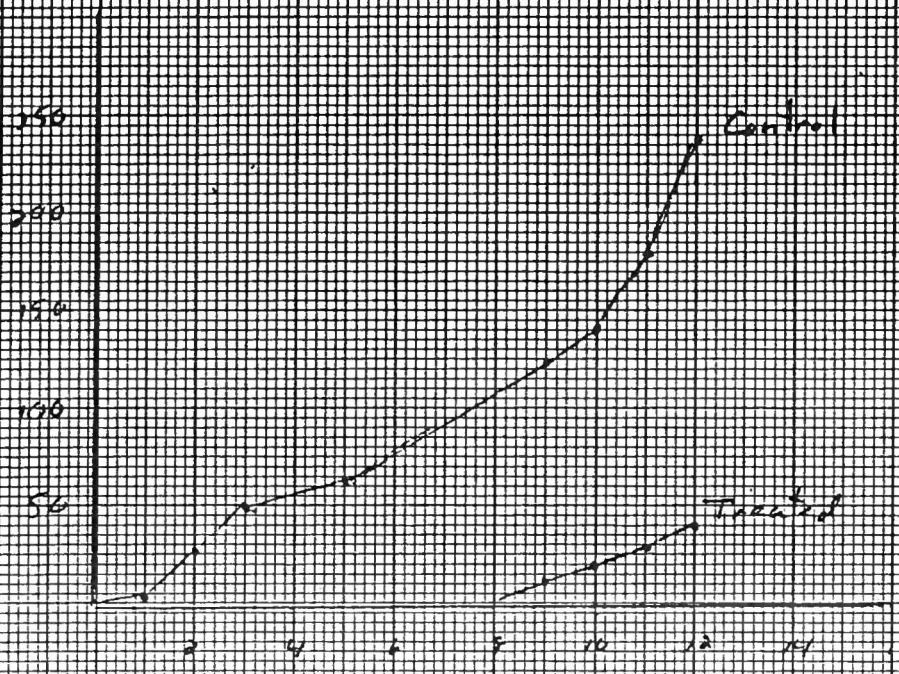


FIGURE 7 Cumulative trap catches. Treatment at 5% PVC 14 from trap

Navel Orangeworm Mating Disruption

II Screening for Possible Disruptant Materials

Interpretive Summary:

The (Z,Z), (Z,E) and (E,E) isomers of 9,11-tetradecaformate, Virelure, and (Z,Z)-11,13-hexadecadienal, reduced catches of virgin female baited Pherocon traps to near zero for 18 days when placed in the trap at 4 mg doses. All were in a PVC formulation except Virelure, which was in Hercon laminates. The olefinic tetradecaformates (Z9,Z11, and a combination of both) disrupted less efficiently.

In a comparison of the disruptive effects of the isomers of the n.o.w. pheromone 11,13-hexadecadienal, the (Z,Z) (E,Z) and (Z,E) isomers gave very similar results, showing a significant reduction in trap catch. The reductions were not as complete as in the previous test probably because of the lower initial dose (.4 mg vs 4 mg). The (E,E) isomer showed no disruption effects.

These studies indicated possible disruptant materials to be tested further; the 9,11-tetradecaformates, Viralure and the (Z,Z), (Z,E) and E,Z) isomers of 11,13-hexadecadienal.

Experimental Procedure:

Two studies were conducted comparing possible disruptant materials. Both involved using Pherocon IC sticky traps baited with virgin female navel orangeworm adults (3 in a screen cage per trap). Traps were positioned 2 meters high in almond trees; only one trap per trap tree. Trap trees were 4 trees apart, in trap rows 4 rows apart. Treatments were randomized within rows and replicated 3 times; each replicate in a different row.

In Test A, treatment consisted of pinning the formulated materials to the inside of the trap top. The following materials were tested:

1). Z-9-tetradecaformate in a 5% PVC formulation. Each treated trap received 4 mg in a 1 cm PVC cylinder.

2). Z-9-tetradecaformate in a 3% PVC formulation. Each treated trap received 4 mg in a 1.7 cm PVC cylinder.

3). Z-9-tetradecaformate; 2 mg in a 5 cm PVC cylinder, and Z-11 tetradecaformate; 1.9 mg in a 8 cm PVC cylinder.

4). (Z,Z)-9,11-tetradecaformate in a 5% PVC formulation. Each treated trap received 4 mg in a 1 cm PVC cylinder.

5). (Z,E)-9,11 tetradecaformate in a 2% PVC formulation. Each treated trap received 4 mg in a 2.5 cm PVC cylinder.

6). (E,E)-9,11-tetradecaformate in a 2% PVC formulation. Each treated trap received 4 mg in a 2.5 cm PVC cylinder.

7). (Z,Z)-11,13-hexadecadienal and antioxidant in a 3.62% PVC formulation. Each treated trap received 4.05 mg in a 1.4 cm PVC cylinder.

8). Virelure in Hercon laminates. Each treated trap received 3 in^2 containing 19.45 mg Z-11-hexadecenal and 1.25 mg Z-9-tetradecenal.

9). Control, with no treatment or formulation.

In Test B, treatment consisted of pinning four pieces of formulated material to foliage ca one foot from the trap. The following materials were tested:

1). (Z,Z)-11,13-hexadecadienal, 4 rubber septa containing 100 mg each.

2). (E,Z)-11,13-hexadecadienal, 4 rubber septa containing 100 mg each.

3). (Z,E)-11,13-hexadecadienal, 4 rubber septa containing 100 mg each.

4). (E,E)-11,13-hexadecadienal, 4 rubber septa containing 100 mg each.

5). Controls with no treatment on formulation.

In both A and B, each trap contained 3 virgin female n.o.w. in a screen cage. Females were replaced every 3-4 days.

Results:

Cumulative trap catches for all treatments and controls are shown in Fig. 8 for Test A, and Fig. 9 for Test B.

In Test A, close to a 100% reduction in trap catch was obtained with Virelur the isomers of 9,11-tetradecaformate, and the navel orangeworm pheromone (Z,Z)-11 13-hexadecadienal, for 18 days. Cumulative trap reductions were 85% for Z-9-tetradecaformate, 87% for Z9 + Z11 tetradecaformates and 95% for Z11 tetradecaformate alone.

In Test B, trap reductions were 70, 68, and 66% for the (E,Z), (Z,Z), and (Z,E) isomers of 11,13-hexadecadienal after 8 days. The (E,E) isomers had no effect.

Discussion:

These results, obtained with disruptant materials placed close to the females, must be interpreted with caution. Trap reductions following such treatments could be from effects other than disruption of short or long range anemotaxis. Repellency and inhibition of females should also be considered. Comparisons are also somewhat arbitrary, since some formulations and release rates were not the same. However, since these tests were conducted primarily to screen materials for further disruption work, some conclusions may be made about their relative performance.

In Test A (Fig. 8) Viralure, (Z,Z)-11,13-hexadecadienal, and the isomers of 9,11-tetradecaformate reduced trap catches close to 100%. Since it might be a disruptant effect, these materials deserve further investigation.

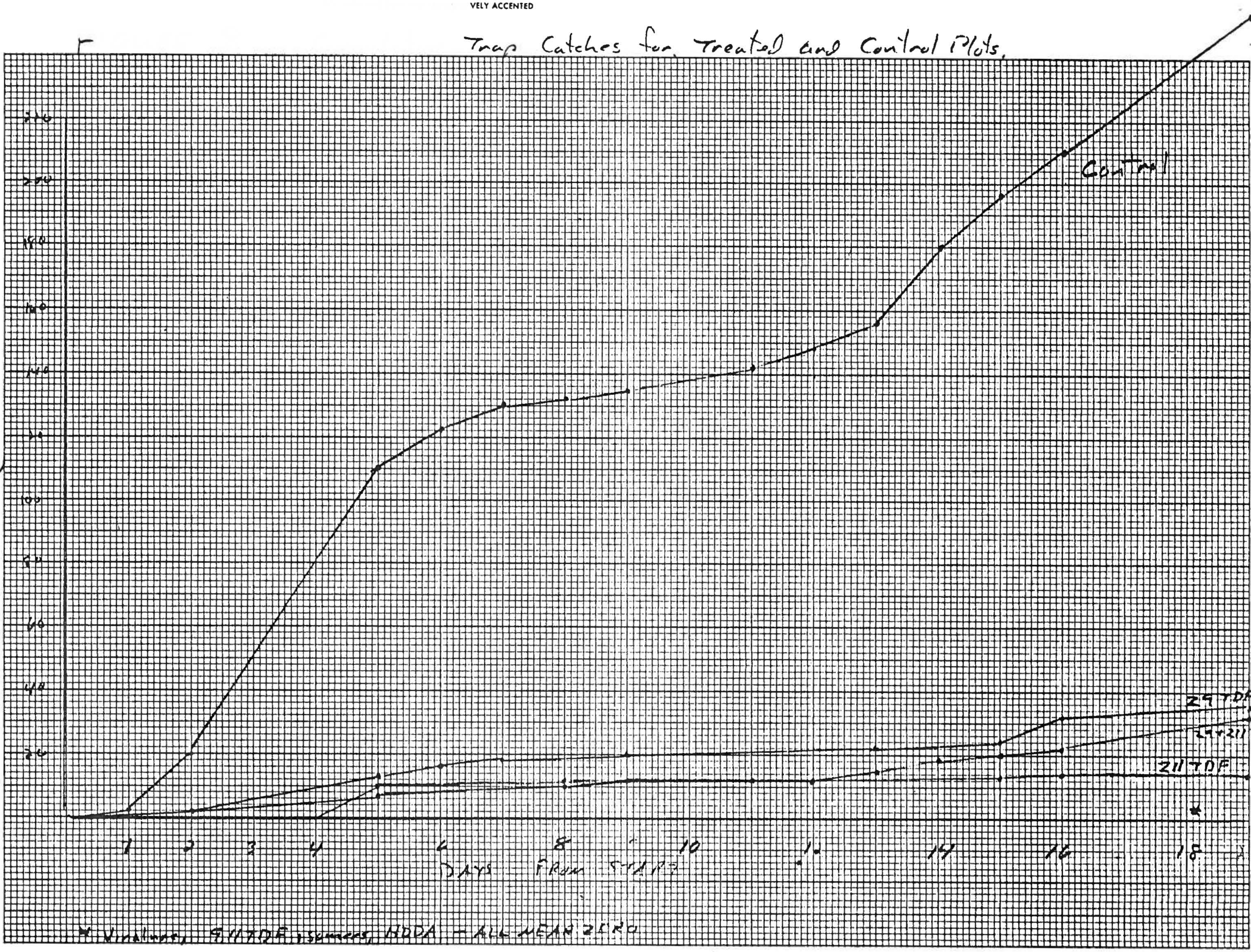
The olefinic tetradecaformates also reduced trap catches, but not as well. If a 100% reduction cannot be accomplished with material in the trap, it seems unlikely widespread disruption could be obtained.

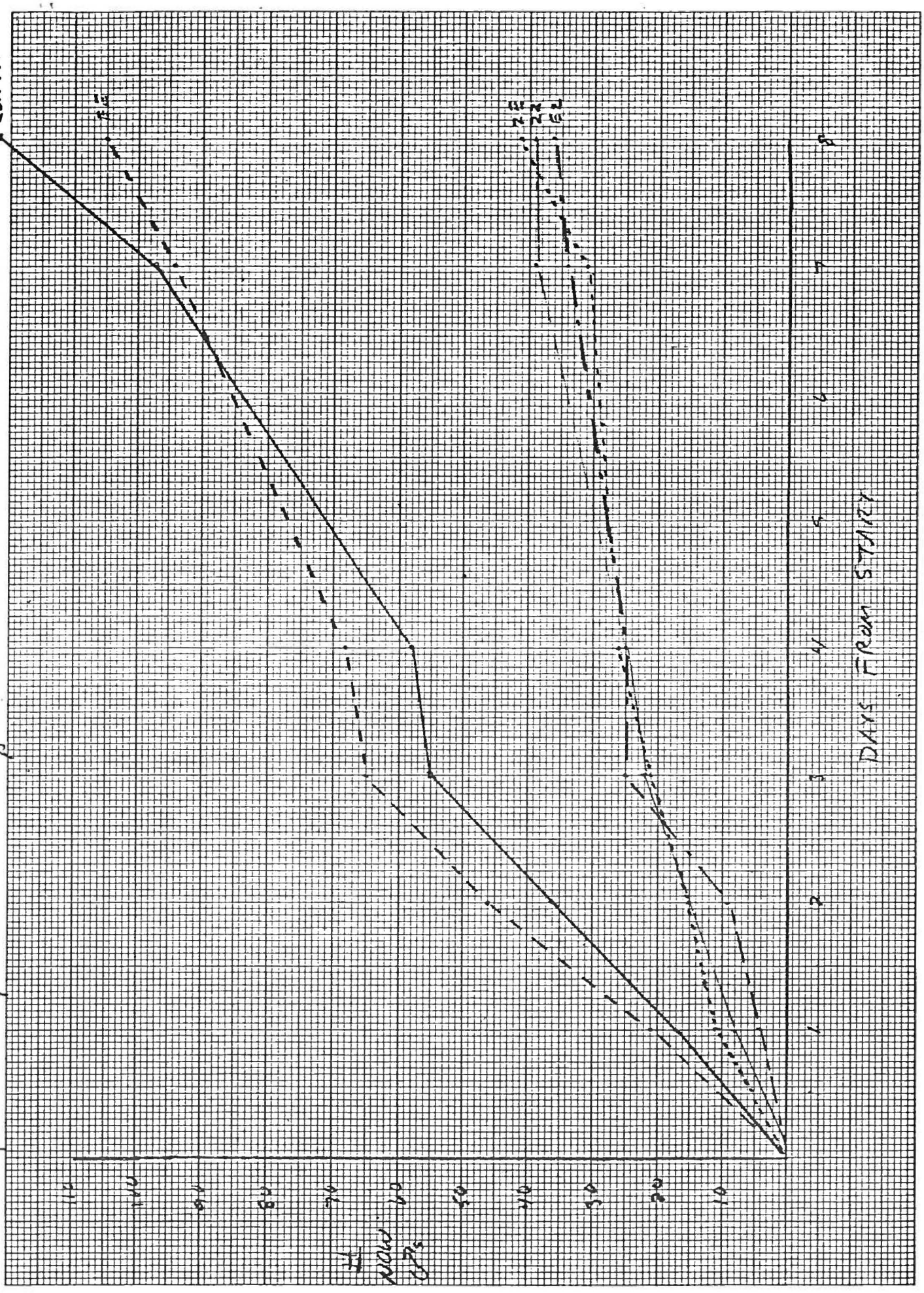
It is interesting that the pheromone used in this test lasted throughout the 19 day test period when all previous PVC formulated pheromone tests faltered after 8 days (see previous section). Possibly this is a result of the antioxidant incorporated in the formulation, preventing the breakdown of the pheromone upon exposure to air.

In Test B, (Fig. 9) trap reductions were considerably less than those in Test A. The (Z,Z), (Z,E), and (E,Z) isomers of the n.o.w. pheromone 11,13-hexadecadienal gave final reductions, after 8 days, of 68,70, and 66%. This could be because of the treatment difference (sources one ft. from trap), the formulation difference (rubber septa instead of PVC), or the dose difference (400 mg total vs. 4 mg).

More important however, is the sharp contrast in results between those isomers and (E,E)-11,13-hexadecadienal, which appeared to have no effect (see Fig. 9). Care must be taken in future tests to be certain of isomeric purities of samples.

Trap Catches for Treated and Control Plots.




control
DAYS FROM START
Head Size

Navel Orangeworm Mating Disruption

III Comparison of Disruptant Materials and Formulations in 9-tree Plots

Interpretive Summary

Tests comparing the mating disruptant potential of Virelure, the navel orangeworm pheromone (Z,Z)-11,13-hexadecadienal, and (Z,Z)-9,11-tetradecaformate, released in 9-tree plots, showed the pheromone to be the most promising.

Similar tests comparing formulations showed best results with Hercon laminates, over Conrel fibers or the PVC formulations.

(Z,Z)-11,13-hexadecadienal, released from Hercon laminates, reduced daily catches of males in female baited Pherocon traps over 95% for 39 days.

Experimental Procedures:

These materials and formulations were tested in a completely randomized design of 9-tree plots. Varietal arrangements and inter-plot distances were standardized within blocks. All treatments were replicated three times along with three control plots receiving no treatment.

The following treatments were tested in this study for disruption of n.o.w. mating

1). Viralure in Hercon laminates. Dose was 932 mg Z-11-hexadecenal and 39.6 mg Z-11-tetradecenal per plot. Test was conducted from 6 June to 11 June.

2). The navel orangeworm pheromone (Z,Z)-11,13-hexadecadienal, formulated in Conrel fibers, Hercon laminates, and a PVC formulation. Dose was 432 mg per plot. The test was conducted from 14 August to 30 September. However, the PVC and Conrel plots were monitored only through 3 September.

3). (Z,Z)-9,11-tetradecaformate formulated in Conrel hollow fibers, Hercon laminates, and PVC. Dose was 864 mg/plot. The test was conducted from 28 August until 12 September.

Plots were monitored for male navel orangeworm with Pherocon IC sticky traps baited with virgin female navel orangeworm. Each plot had one trap in the center tree at 2 meters height, baited with 3 females in a screen cage. Traps were checked on most days and females were replaced every 3-4 days.

Treatment involved placing dispensers of formulated disruptant on fishing line, which was then placed in the almond trees. Each plot tree, except the center trap tree, received a length of fishing line with 3 dispensers positioned near the top, middle, and bottom of the canopy. Test 3, however, received a double treatment with 2 lines and 6 dispensers per tree.

Results:

Test 1: (See Fig. 10). Cumulative and daily catches of male navel orangeworm in female baited Pherocon traps were consistently less in the Viralure plots than in the control plots. However, the difference was not significant at the .05% level in a paired t-test.

Test 2: (See Fig. 11). Initially, trap catches in all three treated plots were at zero, a 100% reduction from the check plots. The disruptive effects waned just in the plots treated with pheromone in Conrel fibers. Males began to locate calling females in the traps again, after four days. Trap catches in these plots were comparable to those in the control plots after 10 days. Traps in the PVC plots began catching males, indicating loss of the disruptive effects, after 6 days, and were comparable to those of the control plots after 13 days. Daily trap catches in the Hercon plots, however, were <1% of control plot trap catches for the first 5 days, and <5% of those for 39 days.

Test 3: (See Fig. 12). In this test, using (Z,Z)-9,11,tetradecaformate as the mating disruptant formulation results were similar to Test 2, but the disruption was less complete and broke down sooner. A 99% reduction in trap catches relative to the control plots was never achieved in the Conrel or PVC plots and for only 2 days in the Hercon plots. In the Conrel plots, a 95% reduction in trap catches occured only for the first days; in the PVC plot, for 2 days; and in the Hercon plots for 8 days.

Discussion:

Of the three formulations tested, Hercon laminates appear to be the most promising for future mating disruption work with the navel orangeworm pheromone or with (Z,Z)-9,11-tetradecaformate. In both of the comparative tests conducted, the disruptive effects were greater and lasted much longer with the disruptant material formulated in Hercon laminates. As was suggested previously, the pheromone may break down in the PVC formulation by oxidation upon exposure to air. The same problem may occur with the Conrel hollow fibers. However, the formate is thought to be more stable, but showed similar results in formulation comparisons.

Virelure performed poorly, even though the dose was more than twice that used in Test 2. Trap catches in Virelure plots were not significantly different from those in control plots. This was not expected since Virelure reduced trap catches to zero for 18 days when place directly in the trap (See Fig. 8). It may be repellent, or have an effect on other precopulatory behaviors or short range attraction. Although the formate disrupted, as indicated by reductions in males caught at virgin female traps, it was inferior to the pheromone. Results obtained with the pheromone (Z,Z)-11,13-hexadecadienal indicate it disrupts longer and more effectively with less material.

FIGURE 10 - Cumulative trap catches for control and [illegible]-treated 9-tree plots. Not significantly different at the .05 level in a paired t test.

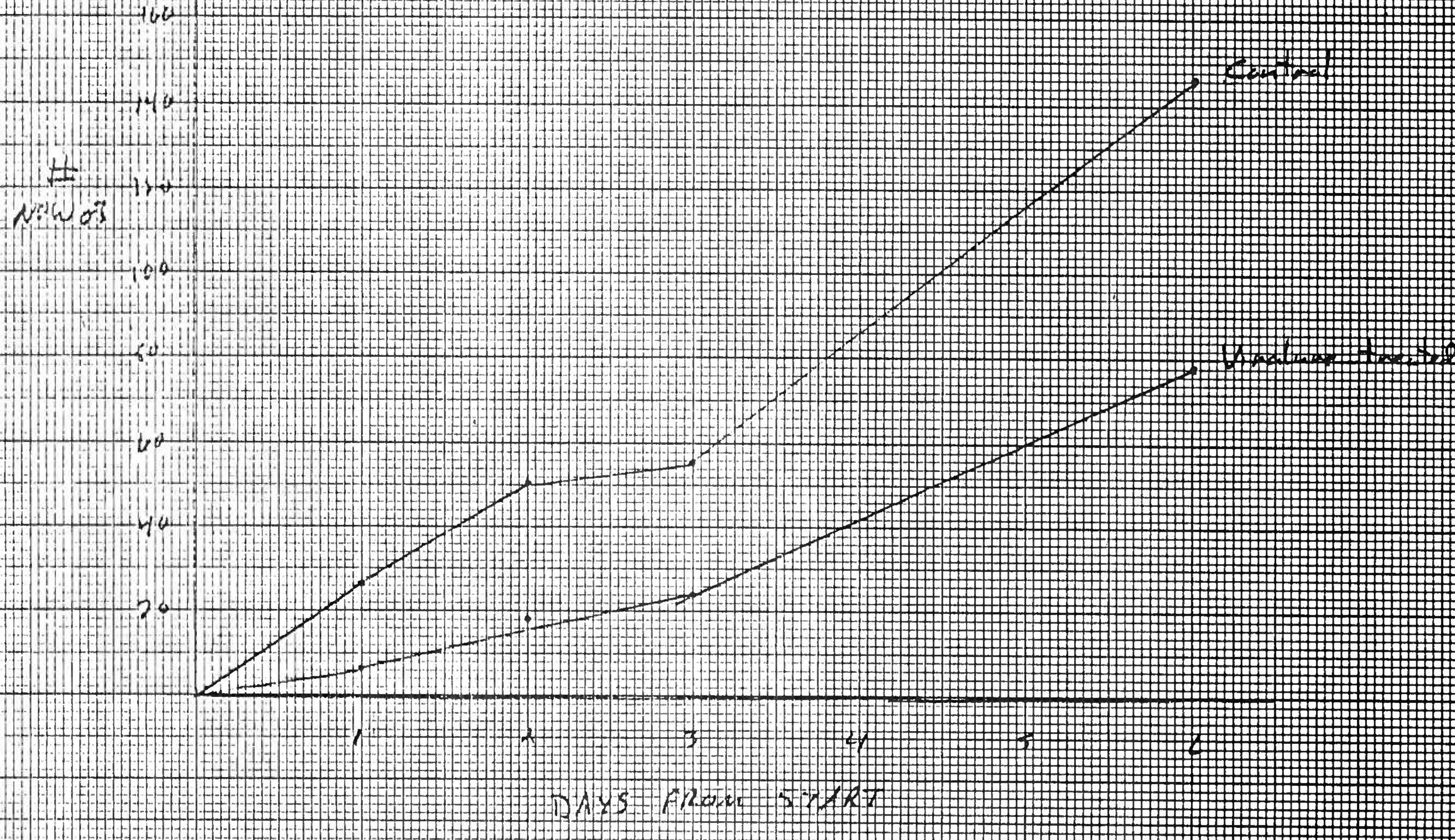
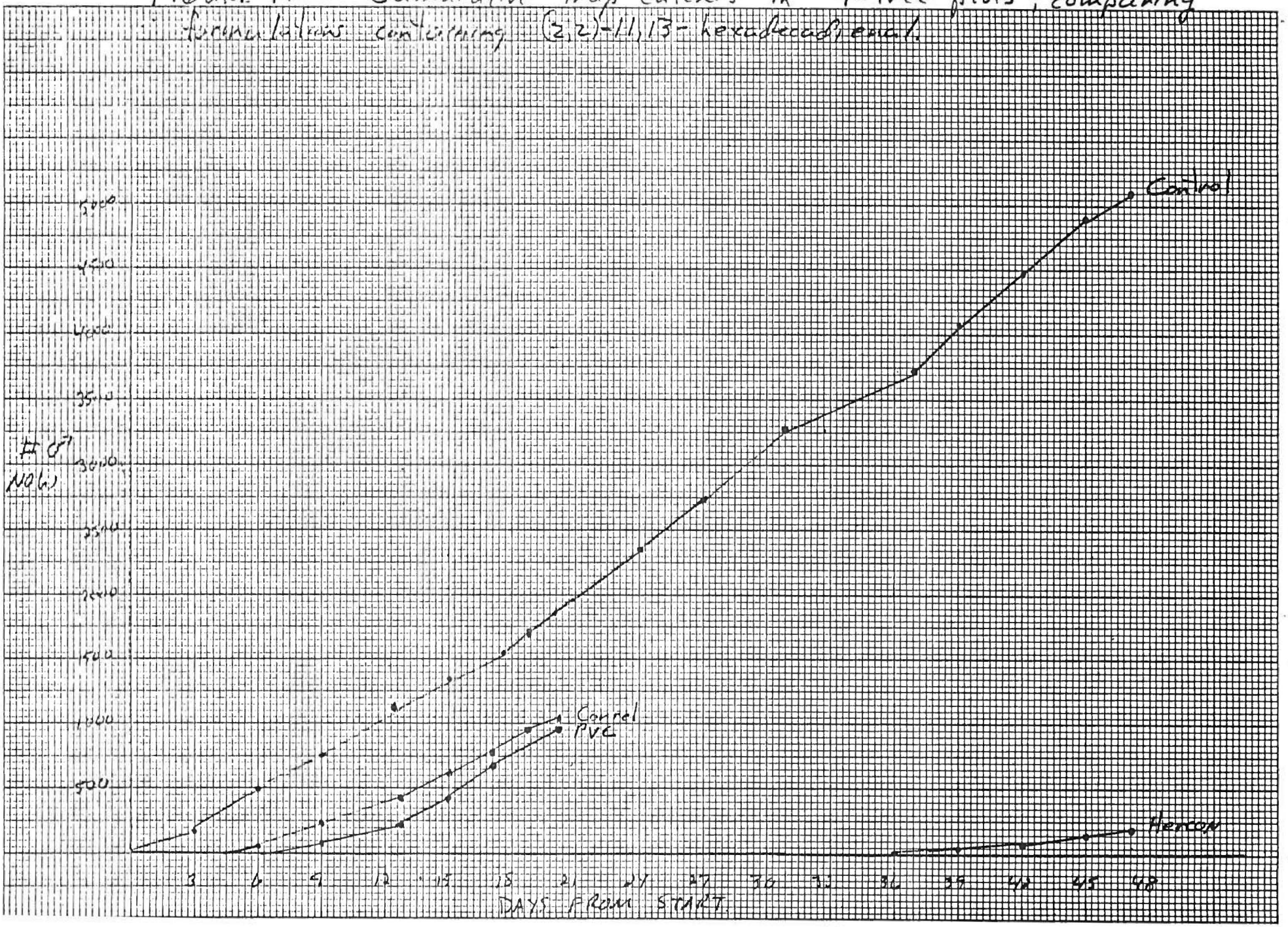


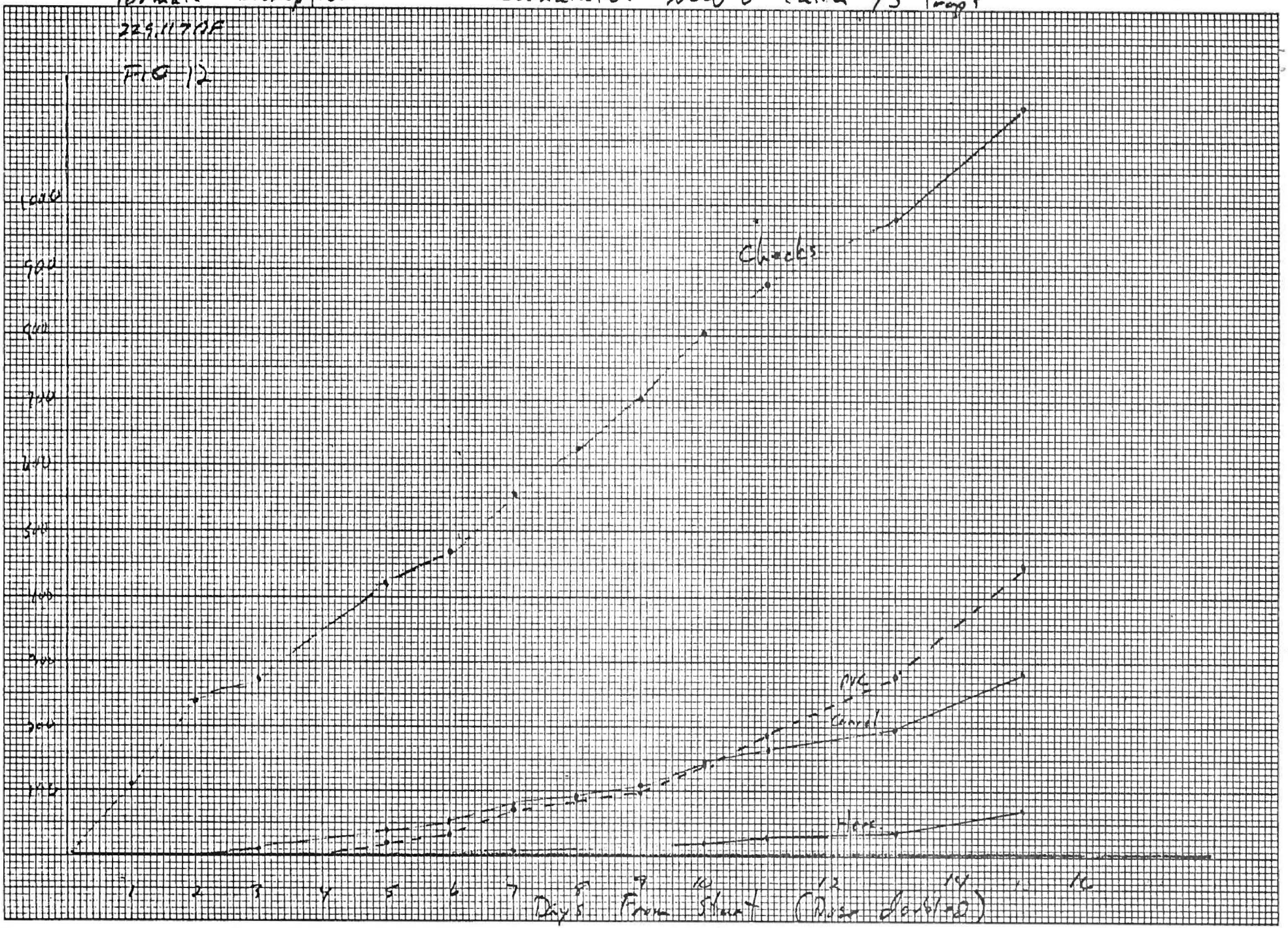
FIGURE 11 Cumulative trap catches in 9-tree plots, comparing formulations containing (Z,Z)-11,13-hexadecadienal.



Formate Disruption cumulative NOW of catch /3 traps

229.1705F

FIG 12



Navel Orangeworm Mating Disruption

IV Disruption with (Z,Z)-11,13-hexadecadienal Formulated in Hercon Laminates

Interpretive Summary:

Results of these tests indicate that navel orangeworm mating can be reduced by > 95% per night, in the lower canopy. This is after the application of the pheromone (Z,Z)-11,13-hexadecadienal in Hercon laminate dispensers, at an initial dose of 3.6 g/acre.

The effectiveness of the disruptant was reduced in the upper part of the canopy and fell off sharply when the dose was reduced from 3.6 to 2.28 g/acre.

The results are very promising but reductions in moth populations or nut damage have yet to be demonstrated.

Experimental Procedure:

Three separate tests are reported here, attempting to demonstrate the efficacy of the pheromone as a mating disruptant.

The first, described previously, utilized a completely randomized design, with 9-tree plots of mature almond trees. The treatment involved placing three pheromone impregnated Hercon laminate dispensers on fishing lines which were hung in the almond trees. Dose was 432 mg/plot, and each tree except the center trap tree received such a length of fishing line. The treatment was replicated three times. These, and three control plots, were monitored with a virgin female baited Pherocon trap at 2 meters in the center tree of each plot. This test was carried out concomitant with the test comparing formulations reported on in the previous section. It was conducted from 15 August to 30 September.

The second test was set up like the first, but with monitoring traps at 2 meters and at 5 meters. It was conducted from 7 September to 28 September.

The third test involved a one acre treated plot and a one acre control plot at either end of a single uniform orchard block. Treatment was the same as the previous tests, with dispensers on fishing line hung in all but five trees in which the traps were hung. Dose was 2.28 grams pheromone/acre, compared to 3.6 g/acre in previous smaller tests. The treated plot was monitored with 10 female baited Pherocon traps at 2 meters and 5 meters in the five trap trees. The control plot was monitored only at 2 meters in 5 trees. This test was conducted from 21 September to 19 October.

Mating success was also checked with dealated lab-reared females placed overnight in test plots. Dealated females were placed, with a pile of twigs, in white enameled pans positioned in almond trees. Ten females were used per pan. These were left overnight and collected the following morning. Successful mating was indicated by subsequent oviposition of viable eggs, and was a useful indicator of disruptive effects when treated and control plots were compared. Mating success was measured in this way in all three tests reported here.

Results:

The treated plots in Test 1 (Fig. 13) had trap catches that were <5% of the control plot trap catches for 39 days. This was with Pherocon traps baited with virgin females at 2 meters in the center tree of each plot and pheromone released from Hercon laminate dispensers.

In Test 2 (Fig. 14), Pherocon traps were placed at 2 meters and at 5 meters. The high traps in the treated plots had trap catches >95% below those of the high control plot traps for only 16 days. Traps at 2 meters were >98% below the corresponding low traps in control plots throughout the 22 day study.

The disruptive effects of the treatment in Test 3 (Fig. 15) began to break down even sooner. Traps at 5 meters in treated plots began catching substantial members of males after 4 days, and after 16 days in traps at 2 meters.

Results of the mating success trials with dealated females are summarized in Table 2. Two were run in Test 1 with 3 of 62 mated in the treated plots and 27 of 62 mated in the control plots. In Test 2, none of 29 females in the treated plots and 19 of 29 in the control plots were mated. In Test 3, 0 of 50 in the treated plot, and 15 of 54 in the control plot were mated. This gave a total in the three plots of 2% mated, vs 42% in the central plots, a 95% reduction.

Discussion:

The results of these experiments indicate the mating disruption effect of the pheromone released is less effective in the upper part of the canopy. This might be because of dilution of the pheromone released by wind, or possibly because of greater male moth activity higher in the canopy. A higher level of pheromone is needed at that level. The reduction in effectiveness that occurred in Test 3 with 2.28 g/acre instead of 3.6 g/acre suggests that 3.6 g is a minimum amount even at 2 meters.

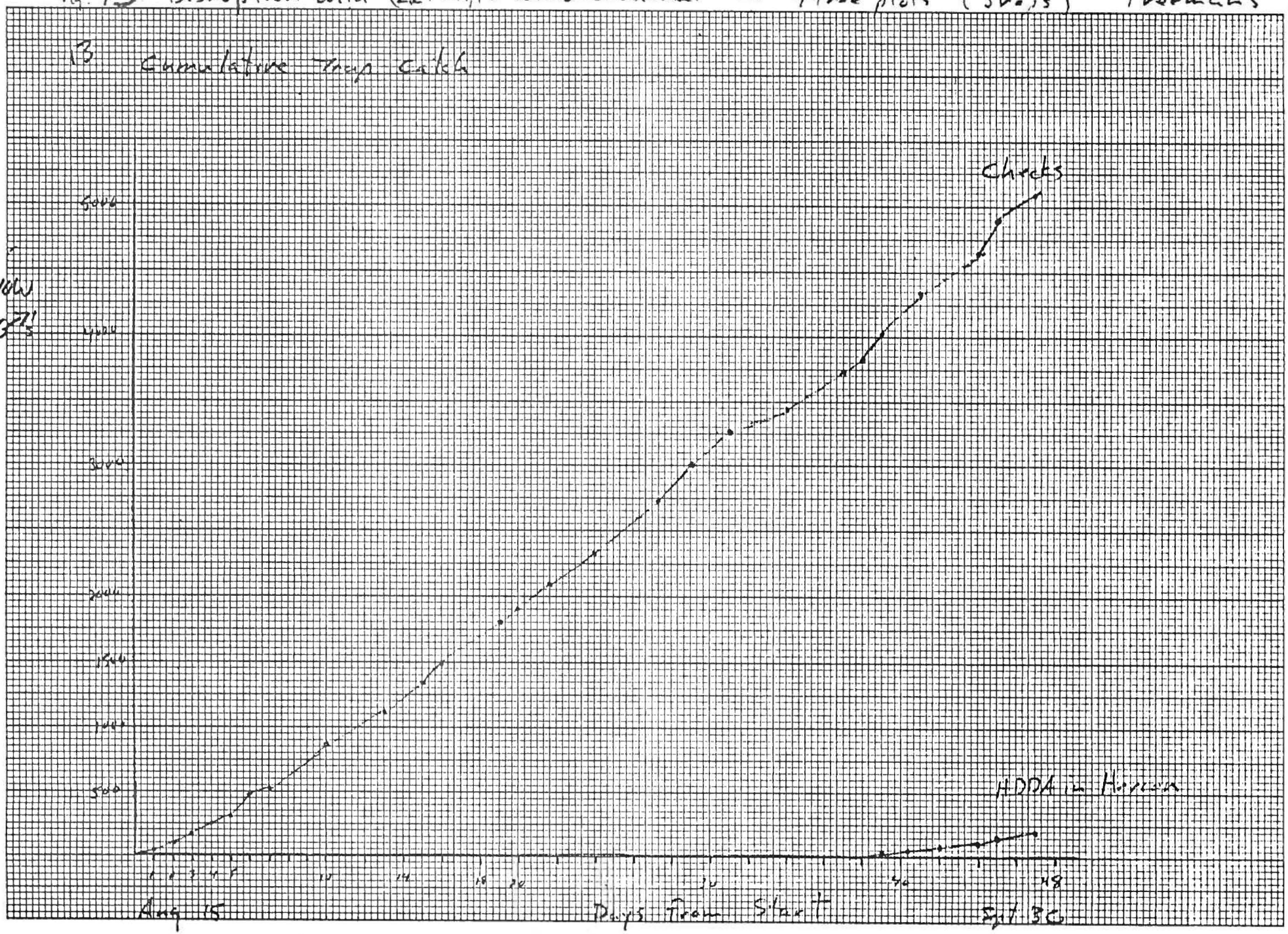
The results of the mating success trials add support to the hypothesis that the pheromone released in the test plots was disrupting mating of navel orangeworm. The >95% reduction in mating success, however, was with lab-reared females and was for a single night's exposure only. It is not known how the lab-reared females' attractiveness compare with wild females'. Also, since females will call and attract males on consecutive nights, a 95% reduction in mating on the first night becomes only a 90.25% reduction on the 2nd, and an 81.5% reduction by the 4th night, assuming constant attractancy. Also it isn't known how much mating must be reduced to control an expanding moth population and, ultimately, reduce nut damage. Experiments will have to be run on a larger scale, investigating the relationship between disruptant, population, and damage levels.

Table 2

		Treated			Check		
Test	Date	In Copula	c viable eggs	Total	In Copula	c viable eggs	Total
I	21 Aug	1	1	32	18	13	33
	27 Aug	0	2	30	0	14	29
II	6 Sept	0	0	29	0	19	29
III	2 Oct	0	0	31	11	12	25
	8 Oct	0	0	29	8	3	29

Fig. 13 Disruption with (2,2)-11,13-hexadecadienal in 9-tree plots (3 reps) Freeman's

13 Cumulative Trap Catch



HADA Disruption. Herron Sandwiches Semantic + Shapovalov

Fig. II

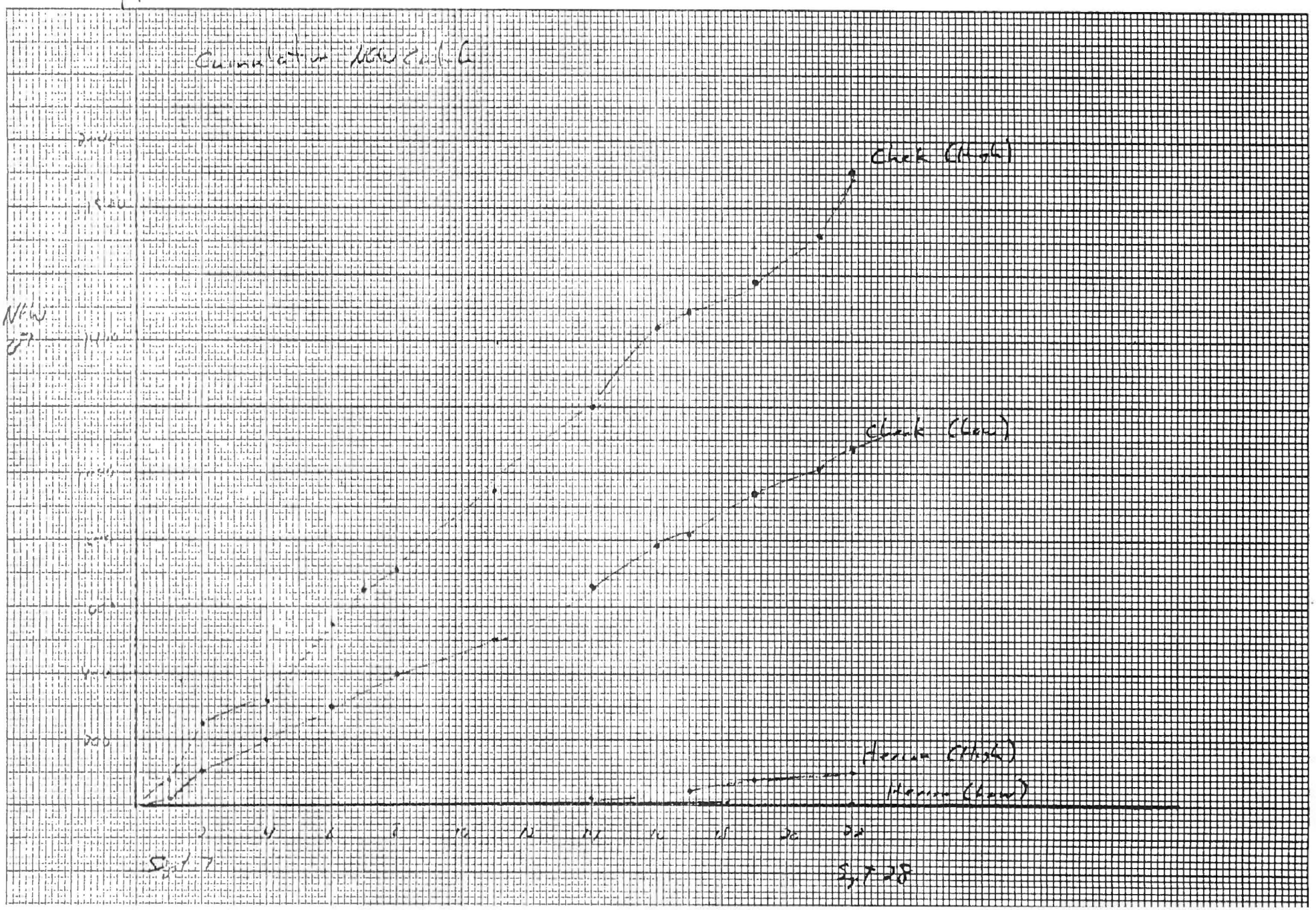


Fig.

FPI-MI 20 X 20 TO 1 INCH
5TH, 10TH AND 20TH LINE PROGRESSIVELY ACCENTED

15 9x9 tree (1 acre) plots (Freeman's) 228/acre