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INVESTIGATIONS OF FRUIT FLIES IN HAWAII

QUARTERLY REPORT

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WORK PROJECT I-a-6. CHEMICAL CONTROL - Loren F. Steiner, Project Leader.

SUMMARY

Line Project I-a-6-1. Among 109 coded compounds screened during the quarter only pentachlorophenyl propionate and 2,4-Dinitro-6-biphenyl acetate produced mortalities of 80-85 per cent at application rates of 10 µg./fly. (The LD-50 of parathion is 0.01 µg./fly.)

Additional LD values for D. cucurbitae and D. dorsalis were obtained for several proprietary insecticides. For dorsalis, topical applications of technical methoxychlor, DDT, toxaphene, chlordane, lindane, heptachlor, and demeton in acetone yielded respective LD-50 values of 2.1, 1.1, 0.90, 0.25, 0.08, 0.05, and 0.05 µg. For cucurbitae the comparable LD-50's were 5.5, 5.0, 1.0, 0.68, 0.28, 0.09, and 0.02. Demeton was the only compound in the group more toxic to cucurbitae than to dorsalis when applied topically. Toxaphene was approximately equally toxic to the two species and so might be more useful in cucurbitae control than DDT if residual tests produce similar results.

Residual LD-50 values for deposits on glass ware established for emulsifiable and wettable powder formulations of DDD (Rothane) (19.0 and 11.5 µg./cm.^{2}), of Perthane (13.0 and 6.6 µg./cm.^{2}), and of methoxychlor (10.2 µg. for wettable powder only, the emulsifiable being of too low toxicity at the dosage used). Against cucurbitae both formulations of DDD and methoxychlor emulsifiable were comparatively non-toxic at the concentrations tested. Perthane was almost as toxic to cucurbitae as to dorsalis, and like toxaphene is worthy of field testing for cucurbitae control.

In still further residual tests of formulations the LD-50's for methoxychlor deposits from suspensions, solutions, and emulsions, were 10, 62, and 95 µg./cm.^{2} for dorsalis and 52, 260, and 280 for cucurbitae, respectively. LD-50's for DDT deposits from suspensions, solutions, and emulsions were 4, 46, and 14 µg./cm.^{2} for dorsalis and 9, 450, and 70 for cucurbitae. LD-50's for DDD deposits from suspensions, solutions and emulsions were 10, 24, and 32 µg./cm.^{2} for dorsalis and 28, 153, and 185 for cucurbitae.

Line Project I-a-6-2. The 25 acres of scrub guava above Tripler Army Hospital was surveyed by periodic fruit sampling with the intention of using it in field tests of insecticides. Its failure to produce an adequate crop prevented its use. However, the survey was completed in order to provide a record of infestation fluctuations in a guava crop in the absence of any controls other than biological. Samples of fruit were taken from 12 plots at approximately weekly intervals for the 12-week crop season from Sept. 4 to Nov. 19, inclusive. Seasonal mean infestations for the 12 separate plots ranged from 8.6 to 20.9 larvae per pound of fruit. Weekly means of the combined plots ranged from a low of 3.3 on Oct. 14 to a high of 36.3 on Sept. 9 (equivalent to from 20 to 85 per cent of the individual fruits infested. The per cent parasitism (based on emergence) ranged from 39.0 to 76.9 on the different sample dates with no correlation evident between the infestation index and percent parasitization. Neither was there any correlation between infestation density and fruit abundance.

Evidence reported previously indicated that dieldrin sprays (as well as chlordane and aldrin) reduced the percentage of emergence of oriental fruit flies from pupae and the percent of parasites in the emerged material. Further studies on guava have indicated that dieldrin sprays may give a substantial control of larvae already present in guava fruit at the time of their application and that the sprays may increase mortality in mature larvae after they leave the fruit, but before pupation. The dieldrin sprays also reduced total emergence from pupae and the percentage of parasites that were able to emerge. The dieldrin sprays had no depressive effect on the total percentage of parasitization when the sprayed trees were immediately adjacent to unsprayed areas. When dead pupae from dieldrin-sprayed fruits were dissected there was a higher percentage of parasites in them than in pupae from unsprayed fruits. This was true even in fruit picked as early as 1 hour after the spray application if the fruit is held until the eggs and larvae hatch and are allowed to mature.

In further tests of the residual effectiveness of deposits on field-sprayed guava foliage that was brought into the laboratory and placed in a cage with flies, lindane emulsions and suspensions applied at rates of 0.5 to 1.5 lb. toxicant per 100 gal. were of little value by the third day after spraying. Dieldrin 50 WP and DDT 50 WP at rates of 1.5 and 3.0 lb. toxicant per 100 gal., respectively, performed well against both dorsalis and cucurbitae up to 3 or more weeks after the application. Emulsifiable dieldrin residues were non-toxic after 3 days. With both dieldrin and DDT wettable powders the 1.5 and 3.0 lb. application rates were far more effective than rates of 1/3 as much. For example 0.5 lb. dieldrin produced mortalities of dorsalis of 50 percent or more for only 4 days whereas 1.5 lb. remained that effective for 16 days. DDT at 1 lb. was 50 percent or more effective for 5 days whereas 3 lb. produced 50 percent mortalities for 24 days.

The effectiveness of demeton (Systox 23 Em.) against dorsalis larvae inside guava fruit was studied further at an application rate of 3 lb. per 100 gal. (6 lb. per acre). One hundred oriental fruit fly eggs were applied to the cut ends of each ripe fruit removed periodically after the spray application. The treatment reduced the mean number of larvae (alive in the fruit 6 days after the egg application) from 61 to 8 (in fruit removed 1 hour after spraying) and gave almost complete control of natural and artificial infestations during the period from 14 to 71 days after spraying. The treatment was still more than 80 percent effective at the end of 78 days.

Tests with fruits that were bagged while spraying was in progress indicated that 2 or more days were required for the toxicant to reach effective levels in fruits not receiving direct applications but the effective period in these bagged fruits was greatly shortened (to about 30 days) presumably because of exclusion of toxicant from the surface of the immature fruit and stems at time of spraying. This indicates a need for thorough coverage. When 14 grams demeton per 8-ft. guava tree was applied to the trunks only, excellent control was obtained in fruit ripening during the period from 12 to 69 days after treatment. Applications to the bark of single branches gave control only in the fruit on those branches, there being no translocation to other parts of the trees. The application of concentrates to the trunk was less effective than when the same quantity of systemic toxicant was applied in a dilute spray to the whole tree.

In further bait-spray development studies involving the tray technique and small guava trees in a well replicated set up, the highest rate of return per pound of poisoned yeast hydrolysate applied was 1.4 million dorsalis and cucurbitae in 5 days after treatment. Cucurbitae outnumbered dorsalis 2 to 1 in these tests.

Further tests with a dry-mix parathion-partially hydrolyzed yeast protein formulation indicated that it was somewhat less effective than the fresh tank mix. When compared on an equal solids basis a liquid yeast hydrolysate (42.8% solids) from Nutritional Bio Chemicals was not significantly more effective than their finished dry yeast hydrolysate. Additional tests with sugar continued to indicate that its inclusion in the protein hydrolysate-parathion (or malathion) formulas was deleterious to the attraction of cucurbitae. Although caramelization of raw and granulated sugars improved their performance in liquid baits it had an adverse effect on the attraction of sugar-protein-insecticide deposits on foliage. Diazinon and chlorthion were much less effective in yeast hydrolysate sprays than was parathion.

Twelve spray applications at weekly intervals of partially hydrolyzed yeast protein 1.5 lb. and malathion 25 WP, 3 lb. in 120 gallons of water per acre were made in a 1/3 acre planting of passion fruit. Sixty-seven percent of the immature fruit and 68 percent of maturing fruit had been stung by dorsalis and cucurbitae prior to the first spray application. Stung immature fruit declined to zero within 4 weeks and never exceeded 1.5 percent thereafter. The percentage of stung maturing fruit diminished more slowly as the injured immature fruit ripened but after a steady decline, reached a low of 0.8 percent after 10 weeks and never exceeded 3 percent during the remainder of the fruiting period more than 50 days after the last spray. The area was subject to a constant heavy influx of flies from adjacent areas. Estimates of total fly kill based on dead flies in 20 sample boxes indicated that the per-acre rate during the week after each spray started at 39,000 dorsalis and 21,000 cucurbitae, ranged as low as 900 and 450 respectively and totalled slightly more than 200,000 during the 12-week period. Malathion deposits on fruit averaged 3.0 PPM on the day of spraying, 1.1 PPM 24 hours later, 0.6 PPM on the second day, and 0.1 PPM on the sixth. Larvae rarely mature in passion fruit but the ovipunctures often cause deformed or shriveled fruit and reduced set.

A post-treatment survey of infestations in the Hamakua Coast gulches used in the 1952-1953 methyl eugenol-G-22008 control experiment indicated average infestations at the 5 elevations from 300 to 1900 feet of 15.1 larvae per pound in the former treated area and 15.6 in the controls. One year earlier while the experiment was in operation infestations at 700, 1100, 1500, and 1900 feet were 65, 75, 91, and 100 percent less in the treated than in the control areas. The current survey was made in November near the peak of fruit production for the fall crop. The results indicate that the area used for the experiment was neither more nor less favorably situated with respect to oriental fruit fly attack than those areas used as controls.

A comparison of fly catches made by bait stations in the Kilauea area strongly suggests that as few as 3 poison-bait stations treated monthly with 35 ml. methyl eugenol-G-22008 solution would be as effective in removing male flies from a 120-acre area as would 19 such stations. The 3 stations in 120 acres approximate the station density used in the 6-square mile Hamakua Coast experiment.

Line Project I-a-6-4. After 18 generations of selection by exposure to parathion in the adult or larval stages no evidence of any acquired tolerance to this toxicant was obtained. Dorsalis exposed to DDT in the larval medium for 15 generations acquired a tolerance to topical applications of DDT of 30 times that of the laboratory strain. Dorsalis exposed to DDT residues in the adult stage only for 31 generations showed no appreciable increase in the 5-fold tolerance to topical applications of DDT observed as early as the 10th generation.

Topical tests with DDT and parathion on flies reared from guava on Oahu, and from 3 widely separate guava areas on Hawaii indicated that the native and the untreated laboratory flies did not differ in their tolerance to these insecticides.

Line Project I-a-6-5. Field tests with solutions of white and raw sugar protected from fermentation with salicylic acid failed to show significant fruit fly catches for either sugar. SM-14 lure cultured with strains of bacterium No. 14 which had been preconditioned to soy meal culture were significantly poorer in the field than was SM-14 cultured with bacterium No. 14 which had not been preconditioned.

Of 21 materials screened in quantitative olfactometer tests, 9 were found to be attractants and 4 enhancers for D. dorsalis while 4 were attractants and 6 enhancers for D. cucurbitae. No repellents appeared for either species.

In olfactometer concentration tests two ranges of concentrations were compared as possible bases for concentration tests of materials previously shown to be attractive in screening tests. Seven such materials were subjected to concentration tests. For D. cucurbitae allyl phthalate, civet, diethoxybenzene and diethyl phthalate all appeared to have optimum concentrations in excess of 1.0% while diethyl malonate appears to have an optimum concentration between 0.1% and 1.0%. For D. cucurbitae an optimum concentration in excess of 1.0% was indicated for diethyl phthalate, and less than 0.01% for 2,4-dihydroxybenzaldehyde.

Olfactometer tests with sugars showed that the attraction of sucrose in the olfactometer is apparently not due to an impurity but appears to be definitely olfactory in nature. The most attractive concentration of sucrose solutions was found to be in the neighborhood of 20%. Other sugar found to be as attractive as sucrose was levulose and maltose, while dextrose, galactose and arabinose were less attractive. Mannose, xylose and sorbose were not attractive at all. There was no correlation between size of sugar molecule or the chemical nature of the sugar and attractiveness. Caramelization increased attractiveness of both raw and white sugar.

In a test of various concentrations of Clorox to treat SM-14 lure to remove objectionable odors there was some loss of attractiveness with concentrations of Clorox as low as 2.0%.

WORK PROJECT I-a-6. Chemical Control - Loren F. Steiner, Project Leader.

Line Project I-a-6-1. Preliminary Laboratory Testing of Insecticides for Fruit Fly Control. (I. Keiser, J. R. Holloway, M. Fujimoto, L. F. Steiner)

Screening Tests of Coded Compounds (Keiser, Holloway)
By I. Keiser

During this quarter 109 coded compounds from the Division of Insecticide Investigations were tested topically, and the results are listed in table 1.1. Two compounds, 2,4-Dinitro-6-biphenylyl acetate and pentachlorophenyl propionate, gave mortalities of 85 and 80 percent, respectively. However, these were tested at the usual screening concentration of 10 micrograms of compound per fly. DDT gave a mortality of 60 percent at 1 microgram toxicant per fly and parathion (previous studies) gave 50 percent average mortality from 1/100th microgram toxicant per fly. Dosage mortality curves will be run with the above two coded compounds.

Tests of Proprietary Compounds (Keiser, Holloway, Steiner)
by I. Keiser

An extensive series of tests was made during this quarter to determine, under laboratory conditions, the comparative toxicities of different proprietary compounds to adult D. dorsalis and D. cucurbitae fed a controlled laboratory diet. These studies will be continued until accurate LD values have been established for all promising proprietary compounds in all available formulations. Most were initially compared at 1 or 2 standard dosage levels against D. dorsalis only. Table 1.2 lists the results with chlordane and heptachlor tested topically. In the first test, it was not possible to arrive at an adequate curve for D. dorsalis with chlordane, and the entire test was repeated. Heptachlor, at the 50 per cent level is approximately 5 times as toxic against D. dorsalis as is chlordane, about one-fifth as toxic as parathion, and twenty times as toxic as DDT. Chlordane is about 3 times as toxic against D. dorsalis as compared with D. cucurbitae, and heptachlor at least twice as toxic at the 50 percent level. These two experiments were conducted using cucurbitae that differed greatly in weight. The smaller flies (90 per gram) in test 2 had substantially lower LD-values than the larger flies (49 per gram) in test 1 on a per-fly basis but the reverse was true with one exception when calculated on a weight basis.

Table 1.3 presents the data from topical tests where lindane and demeton (Systox) were compared with DDT. At the 50 percent level, lindane is approximately 10 times as toxic against D. dorsalis as is DDT. Systox is about 15 times as toxic as DDT against D. dorsalis. Against D. cucurbitae, however, the results show more contrast. Lindane is again 10 times as toxic as DDT at the 50 percent level, but Systox is about 100 times as toxic as DDT against D. cucurbitae. This is due to the fact that both DDT and lindane are only one-third as toxic against D. cucurbitae as against D. dorsalis, while Systox is twice as toxic. To date, Systox is the only insecticide tested which is appreciably more toxic against D. cucurbitae than against D. dorsalis. However, parathion and malathion are at least as toxic to D. cucurbitae as to D. dorsalis.

Table 1.1.—Comparative toxicity of DDT and coded compounds from the Division of Insecticide Investigations against adult D. dorsalis, when applied as a topical treatment.1/

E Compound	Per cent mortality after 24 hours2/	E Compound	Per cent mortality after 24 hours2/	E Compound	Per cent mortality after 24 hours2/	E Compound	Per cent mortality after 24 hours2/
3628	10	3684	0	3862	8	4255	3
3629	15	3688	0	3865	85	4256	5
3630	18	3690	0	3887	80	4257	20
3631	10	3691	5	3892	18	4258	0
3632	15	3702	0	3913	5	4259	10
3634	10	3703	0	3915	0	4260	8
3638	23	3704	0	3920	33	4261	10
3643	13	3768	0	3922	0	4263	5
3644	5	3770	0	4144	0	4264	15
3645	10	3771	5	4145	5	4266	0
3646	20	3772	8	4146	0	4267	0
3647	15	3773	5	4147	3	4268	0
3648	15	3774	0	4149	5	4298	0
3667	10	3775	0	4150	0	4299	5
3668	8	3776	3	4205	0	4300	0
3669	38	3777	3	4206	5	4301	0
3670	23	3778	0	4240	5	4302	5
3671	10	3780	3	4241	0	4304	0
3672	15	3781	0	4242	3	4306	0
3674	13	3784	13	4243	0	4307	3
3675	18	3785	10	4244	13	4309	3
3677	20	3786	5	4245	10	4310	3
3678	30	3788	3	4246	10	4311	0
3679	28	3789	8	4249	15	4312	5
3680	5	3791	13	4250	5	4313	3
3681	3	3795	0	4252	13		
3682	3	3796	3	4253	10		
3683	0	3845	3	4254	8	DDT	60

1/ Each mortality listed is average of 2 cages, 20 flies per cage, or 40 D. dorsalis mixed sex, tested with each material. Coded compounds were tested at 10 micrograms compound per fly and DDT at 1 microgram toxicant per fly in 1 microliter acetone solution. Insects laboratory-reared, fed yeast protein hydrolysate since emergence.

2/ No food or water during 24-hour holding period—usual procedure in topical tests.

Table 1.2--Comparative toxicity of chlordane and heptachlor against adult fruit flies when tested as a topical treatment.1/

Method of evaluation	Test	Percent mortality after 24 hours2/							
		D. dorsalis				D. cucurbitae			
		LD-50		LD-95		LD-50		LD-95	
		Chlordane	Heptachlor	Chlordane	Heptachlor	Chlordane	Heptachlor	Chlordane	Heptachlor
Microgram insecticide per fly.	1	-	0.042	-	0.15	1.00	0.25	2.75	0.62
	2	0.25	0.05	0.86	0.11	0.68	0.09	1.95	0.40
Microgram insecticide per gram of fly.	1	-	2.8	-	9.9	49.0	12.3	134.8	30.4
	2	15.2	3.0	52.1	6.7	61.3	8.1	175.7	36.0

1/ For test number 1 each LD is listed based on mortality curves of 6 dosages, 20 flies of each species (2 cages--20 flies per cage) at each dosage level, or a total of 240 flies of each species treated topically with each insecticide. For test number 2, each LD listed is based on mortality curves of 6 dosages for D. dorsalis, and 4 dosages for D. cucurbitae, duplicated (2 cages--20 flies per cage) as in test 1. The 1,760 flies (treated individually with 1 microliter proper acetone solution per fly thorax) were laboratory-reared, fed yeast protein hydrolysate since adult emergence.

2/ Technical 100 percent materials tested.

Table 1.3--Comparative toxicity of DDT, lindane, and demeton (Systox) against adult fruit flies when tested as a topical treatment.1/

Method of evaluation	Test	Percent mortality after 24 hours											
		D. dorsalis						D. cucurbitae					
		LD-50			LD-95			LD-50			LD-95		
		DDT	Lindane	Demeton	DDT	Lindane	Demeton	DDT	Lindane	Demeton	DDT	Lindane	Demeton
Micrograms insecticide per fly	1	-	0.140	0.057	-	0.50	0.22	-	0.37	0.040	-	1.20	0.140
	2	0.75	0.076	0.046	2.1	0.32	0.15	2.8	0.28	0.022	12.5	1.10	0.073
Micrograms insecticide per gram of fly	1	-	9.3	3.8	-	33.1	14.60	-	24.3	2.6	-	79.8	9.2
	2	54.3	5.7	3.3	152.2	23.2	10.9	224.0	22.4	1.8	1000.0	88.0	5.8

1/ Each LD listed based on mortality curves of 6 dosage levels, 20 flies of each species (2 cages--20 flies per cage) at each dosage level, or a total of 240 flies of each species treated topically with each insecticide. The 2400 flies (treated individually with 1 microliter proper acetone solution per fly thorax) were laboratory-reared, fed yeast protein hydrolysate since adult emergence. Different species kept in separate cages. All insecticides 100 per cent technical except Systox 23 Em.

Another series of topical tests were conducted in which toxaphene and methoxychlor were compared with DDT. The results are summarized in table 1.4. Against D. dorsalis, DDT and toxaphene have about the same toxicity, while methoxychlor is 1/2 as toxic. Against D. cucurbitae, however, both DDT and methoxychlor are about 1/5 as toxic as toxaphene at the 50 percent level. At the 95 percent level, DDT is 1/7 and methoxychlor 1/12 as toxic as toxaphene. Toxaphene may, therefore, be far more useful than DDT in cucurbitae control than hitherto suspected if residual tests perform similarly. Toxaphene has not been given much consideration in our work on dorsalis because early in these investigations it showed no advantage over DDT.

Topical tests, comparing Perthane and Rothane, were completed for D. dorsalis and already reported. During this quarter these compounds were tested residually in the laboratory and the results are listed in table 1.5. The wettable powders were more toxic than their corresponding emulsions, against D. dorsalis, and Perthane was more toxic than Rothane against this species. Against D. cucurbitae Perthane wettable powder showed approximately the same toxicity as against D. dorsalis.

As compared to the LD-50 for DDT wettable powder (3.0 and 7.5 μg./cm.^{2} for dorsalis and cucurbitae), the residual toxicity of Perthane wettable powder was only half as great against dorsalis but equal to DDT against cucurbitae. Perthane emulsifiable, however, with LD-50s of 13.0 and 18.0 for the two species may be compared with previously established values for DDT-Em. of 14.0 and 1000.0. Perthane emulsifiable as well as the wettable powder should, therefore, be tested in melon fly control experiments. No curves were possible with methoxychlor emulsion against D. dorsalis, although the wettable powder at the same concentration of toxicant showed mortalities approximately the same as those caused by Rothane. Also, no curves were possible with Rothane emulsion or suspension against D. cucurbitae, although the same concentrations were adequate against D. dorsalis. Therefore, detailed tests were set up to study the effect of formulation on toxicity, and the differential effectiveness against the two species tested. In the first such test (table 1.6) Rothane solution, emulsion and suspension were studied. The results show that Rothane emulsion and solution are 5 to 7 times as toxic against D. dorsalis as D. cucurbitae at the 50 percent level. At the 95 percent level, they are 12 to 20 times as toxic. Deposits from the Rothane suspension are 2 to 3 times as toxic against D. dorsalis at the 50 percent level, as compared with those from solutions and emulsions, and 6 to 7 times as toxic at the 95 percent level. No curve was possible with D. cucurbitae as the heavy deposits from the higher suspension deposits necessary with this species caused repellent action, and mortalities actually decreased. See table 1.6 footnotes 3 and 4.

Table 1.7 shows the results of a similar, but independent test with methoxychlor. The suspension deposits from the wettable powder were 6 to 9 times as toxic against D. dorsalis as were the emulsion or solution. Approximately the same relationship held with D. cucurbitae, but methoxychlor was only 1/2 to 1/3 as toxic against this species as it was to D. dorsalis.

Table 1.4.--Comparative toxicity of DDT, toxaphene, and methoxychlor against adult fruit flies when tested as a topical treatment.1/

Method of evaluation	Per cent mortality after 24 hours											
	D. dorsalis						D. cucurbitae					
	LD-50			LD-95			LD-50			LD-95		
	DDT	Toxa-phene	Methoxy-chlor	DDT	Toxa-phene	Methoxy-chlor	DDT	Toxa-phene	Methoxy-chlor	DDT	Toxa-phene	Methoxy-chlor
Micrograms insecticide per fly	1.1	0.9	2.1	4.3	4.0	8.5	5.0	1.0	5.5	21.0	3.0	37.5
Micrograms insecticide per gram of fly	73.0	60.0	150.0	300.0	280.0	570.0	335.0	72.0	360.0	1650.0	210.0	2600.0

1/ Each LD listed based on mortality curves of 6 dosage levels, 20 flies of each species (2 cages--20 flies per cage) at each dosage level, or a total of 240 flies of each species treated topically with each insecticide. The 1440 flies (treated individually with one microliter proper acetone solution per fly thorax) were laboratory reared, fed yeast protein hydrolysate since emergence. All insecticides 100 per cent (technical). Different species kept in separate cages.

Table 1.5.--Comparative toxicity of Rothane, Perthane, and methoxychlor against adult fruit flies when tested as a laboratory residual treatment on glass. 1/

Formulation 3/	Micrograms insecticide per square centimeter of glass surface 2/											
	D. dorsalis						D. cucurbitae					
	LD-50			LD-95			LD-50			LD-95		
	Rothane	Perthane	Methoxychlor	Rothane	Perthane	Methoxychlor	Rothane	Perthane	Methoxychlor	Rothane	Perthane	Methoxychlor
Emulsifiable	19.0	13.0	- 4/	125.0	65.0	- 4/	- 4/	18.0	- 4/	- 4/	85.0	- 4/
Wettable powder	11.5	6.6	10.2	48.0	11.5	32.0	- 4/	6.7	22.0	- 4/	16.2	72.0

1/ Each LD listed based on mortality curves of 5 dosage levels, 100 flies of each species (2 petri dish cages--50 flies per cage) at each dosage level, or a total of 500 flies of each species for each insecticide formulation tested. The 6000 flies tested were laboratory reared, fed yeast protein hydrolysate since adult emergence.

2/ Sugar water on dental roll available to flies for entire 24-hour holding period. Different species kept in separate cages. Two ml. proper concentration water emulsion or suspension per 100 mm. petri dish. Deposits 24 hours old before introduction of flies.

3/ All wettable powders 50 per cent. Rothane and methoxychlor 25 per cent emulsifiable; Perthane--50 per cent emulsifiable.

4/ Ineffectual at dosages tested (0.9-25.5 µg. toxicant/square centimeter glass surface).

Table 1.6—Comparative toxicity of Rothane in different formulations against adult fruit flies when tested as a laboratory residual treatment.[1]

Formulation[2]	Micrograms insecticide per square centimeter of glass surface			
	LD-50		LD-95	
	D. dorsalis	D. cucurbitae	D. dorsalis	D. cucurbitae
Emulsion	32.0	200.0	185.0	2300.0
Solution	24.0	128.0	153.0	3000.0
Suspension	10.0[3]	—[4]	27.5[3]	—[4]

1/ LD values each determined from 7 dosage levels, 3 replicate cages, each species, 30 cucurbitae or 50 dorsalis per replicate, flies laboratory-reared, held on standard protein hydrolysate-sugar-water diet after emergence, on water alone during the 24-hour exposure period.

2/ Solution—25 per cent emulsifiable commercial stock solution diluted with xylene; emulsion—25 per cent emulsifiable commercial stock solution diluted with water; suspension—50 per cent wettable powder suspended in water.

3/ D. dorsalis mortalities averaged 35, 89, 90, 87, 91, 68, and 70 percents, respectively for 8, 16, 32, 63, 127, 256, and 509 micrograms toxicant per square centimeter of glass surface. One hundred percent mortality was never achieved, and the comparatively heavy deposits at the highest levels tested were repellent to some extent.

4/ D. cucurbitae mortalities averaged 9, 27, 35, 41, 29, 36, and 30 percents, respectively, for 8, 16, 32, 63, 127, 256, and 509 micrograms toxicant per square centimeter of glass surface. Fifty percent mortality was never achieved and the comparatively heavy deposits at the highest levels tested were repellent to some extent.

Table 1.7.--Comparative toxicity of methoxychlor in different formulations against adult fruit flies when tested as a laboratory residual treatment.1/

Formulation 3/	Micrograms insecticide per sq. cm. of glass surface 2/			
	LD-50		LD-95	
	D. dorsalis	D. cucurbitae	D. dorsalis	D. cucurbitae
Emulsifiable 25%	95.0	250.0	280.0	725.0
Solution	62.5	230.0	260.0	1350.0
Wettable Powder, 50%	10.2	24.0	52.0	120.0

1/ Each LD listed based on mortality curves of 5 dosage levels; 90-150 flies of each species (3 petri dish cages--30 to 50 flies per cage) tested with each methoxychlor formulation at each dosage level. The 5,760 flies tested were laboratory-reared, fed yeast protein hydrolysats since adult emergence.

2/ Sugar water on dental roll available to flies for entire 24-hour holding period. Different species kept in separate cages.

3/ Emulsifiable diluted with water. Wettable powder suspended in water. Solution was of technical methoxychlor dissolved in xylene. Two milliliters proper concentration emulsion, suspension, or solution pipetted into 100 millimeter petri dish. Deposits 24 hours old before introduction of flies.

Table 1.8 lists the results of a similar, but independent, test with DDT. Against D. dorsalis and at the 50 percent level, the wettable powder is approximately 4 times as effective as the emulsion and 12 times as effective as the solution. At the 95 percent level, the wettable powder is 8 times as effective as the emulsion and 50 times as effective as the solution. While DDT suspension from wettable powder is only twice as toxic against D. dorsalis as compared with D. cucurbitae at the 50 percent level, it is 10 times as toxic at the 95 percent level. DDT emulsion is 70 times as toxic against D. dorsalis as compared with D. cucurbitae and the solution only 6 times as toxic at the 50 percent level. As mentioned above, these tests will be continued to include most of the promising proprietary compounds. The results to date, however, suggest graphically the marked differences in toxicity of different formulations of the same insecticide and against closely related species treated identically.

Table 1.8. Comparative toxicity of DDT in different formulations against adult fruit flies when tested as a laboratory residual treatment. 1/

Formulation 3/	Micrograms insecticide per square centimeter of glass surface 2/			
	LD-50		LD-95	
	D. dorsalis	D. cucurbitae	D. dorsalis	D. cucurbitae
Solution	46.0	300.0	450.0	—
Emulsion	14.0	1000.0	70.0	—
Suspension	3.75	7.50	9.0	100.0

1/ Each LD listed based on mortality curves of 7 dosage levels, 90-150 flies of each species (3 petri dish cages--30 to 50 flies per cage) tested with each DDT formulation at each dosage level. The 1,920 flies tested were laboratory reared, fed yeast protein hydrolysate since adult emergence.

2/ Sugar water on dental roll available to flies for entire 24-hour holding period. Different species kept in separate cages.

3/ Solution--technical material dissolved in xylene; emulsion--commercial 25 percent product diluted with water; suspension--50 percent wettable powder suspended in water. Two milliliters proper concentration emulsion, suspension or solution pipetted into 100 millimeter petri dish. Deposits 24 hours old before introduction of flies.

Line Project I-a-6-2. Field Testing of Insecticides for Fruit Fly Control
(L. F. Steiner, K. Ohinata, I. Keiser, R. K. S. Lee, R. N. Kinoshita, J. R. Holloway, M. Fujimoto)

Dorsalis Infestations In Unsprayed Guava
(Steiner, Lee, Kinoshita)

The wild guava area above Tripler Army Hospital comprising about 25 acres of scrub trees made accessible by bulldozed trails has not borne a guava crop suitable for field tests since 1952. A straggling crop throughout early 1953 kept a low fly population in the area. A good mid-season bloom led us to plan for late season field tests but the drought which affected guava throughout leeward Oahu suppressed fruit production to such an extent that no tests were set up. However, since we had initiated the usual periodic collection of guava samples to establish the pretreatment infestation level, sampling was continued in order to obtain needed information on infestation indices in the absence of any insecticide treatment in an area where comprehensive field experiments with insecticides have been and will be conducted.

The 25 acres of guava are distributed over a total area of about 40 acres. This area was divided into 12 plots of about equal size. Five permanent sample sites outside and from 100 to 300 yards from the borders of the main area were also sampled.

The infestation data are included (Table 2.1) as a matter of record and for future reference since this area is one of our most valuable test sites. The crop was never abundant enough to yield the 50-fruit samples without searching. It maintained this level from September 4 to October 7, after which it declined gradually until November 19 by which time production had ceased completely in 25 percent of the area.

The data show that the mean infestations within the main area ranged from 3.3 to 36.3 larvae per pound (equivalent to from 20 to 85 percent infested fruit) on different dates and from 8.6 to 20.9 for the season between the various plots. In the main area, infestations were lowest in the windward corner plot (5), the highest most windswept plot (1), and were greatest in the lower centrally located plots most protected from prevailing winds (6, 7, 10).

The percent parasitism (based on emerging material only) ranged from 39 to 77 percent. In general, the infestation followed the pattern noted in many field tests though less exaggerated. This was the high initial index followed by a sharp decline after about 3 weeks. There is no reason to attribute this decline to parasite activity since the infestation in late October quadrupled that of October 14 during the period of maximum parasitism. The early decline in infestation appears most likely due to some brood separation between the adults initially attracted to the area with the onset of a crop and their first generation progeny. This needs further study but has been recognized as a factor that might influence the reliability of control data which uses pre-treatment infestations as the basis for evaluating treatment effectiveness.

The outside areas (13-17) sometimes used as controls had somewhat lower infestations than the main area but the fluctuations followed almost the same pattern.

Table 2.1--Dorsalis infestations in unsprayed guava. Larvae per pound guava fruit. Tripler Hospital area, Oahu, T. H. 1953.

Block	9/4	9/9	9/15	9/24	9/30	10/7	10/14	10/22	10/28	11/3	11/12	11/19	Mean
1	12.8	32.7	14.5	4.2	1.7	2.8	3.5	5.4	2.2	1.8	13.2	8.2	8.6
2	36.0	27.0	28.8	8.7	0.6	6.3	5.6	6.0	6.0	6.4	17.3	3.6	12.7
3	29.4	17.1	28.2	13.0	4.4	5.4	2.8	0.8	9.4	4.7	10.0	15.9	11.8
4	13.9	42.1	17.6	15.0	2.8	3.6	1.0	6.6	15.4	4.8	11.2	1.8	11.3
5	17.0	13.2	27.3	9.8	2.0	4.9	2.5	3.7	10.5	6.5	15.7	4.3	9.8
6	51.9	57.6	16.8	9.6	9.7	8.1	5.0	8.0	13.8	10.0	11.0	6.4	17.3
7	62.6	94.9	25.9	8.5	5.8	6.5	0.9	8.8	13.6	10.0	4.4	8.8	20.9
8	25.9	13.7	23.3	7.8	3.6	3.1	2.1	3.9	15.4	28.1	14.2	-	12.8
9	20.7	24.4	11.6	7.0	3.5	4.4	2.0	14.3	12.5	10.6	0	38.9	12.5
10	27.7	41.2	7.3	4.4	6.1	2.7	2.5	20.3	28.3	23.6	-	-	16.4
11	43.2	40.9	11.3	8.8	6.3	5.0	2.1	7.5	15.4	12.9	9.2	0	14.8
12	31.4	31.1	17.8	6.7	8.9	5.9	9.7	10.3	23.1	13.5	-	-	15.8
Ave. lv./lb.	31.0	36.3	19.2	8.6	4.6	4.9	3.3	8.0	13.8	11.1	10.6	9.8	13.4
% parasites	47.3	39.0	56.4	55.6	66.0	76.9	66.7	76.8	70.6	73.8	69.8	66.7	63.8
13-17	-	24.2	15.8	5.1	4.6	3.5	1.3	12.3	4.0	10.5	7.4	4.7	8.5
% Parasites	-	27.9	24.9	23.0	60.7	60.8	94.0	55.4	80.0	77.1	45.0	63.6	55.7

Effect of a Dieldrin Spray on Fruit Fly and Parasite Emergence
(Steiner, Lee, Kinoshita)

In field tests dieldrin, aldrin, and chlordane have consistently suppressed the percentage of parasites among the adults emerging from fruit samples. Such sprays also suppressed the percentage of total emergence when parasitized larvae were present but not in fruit sampled before establishment of cophilus. This suggested, as noted in previous reports, that these insecticides were selectively more toxic to parasitized than to unparasitized larvae.

In order to obtain more information on this point, under conditions similar to those previously noted, a 15-tree guava plot at Waimanalo was sprayed (to run off) with dieldrin 50 WP at 2 lb. toxidant per 100 gal. on November 12. A 35-fruit sample weighing 9 lb. was collected at random from the plot immediately before the spray and a similar sample from the same trees 1 hour after the spray. Subsequent "untreated" samples were taken from trees immediately adjacent to the sprayed plot.

The data are summarized in table 2.2.

Table 2.2--Effect of a dieldrin spray on oriental fruit fly and parasite, O. cophilus, emergence from picked guava fruit.

Time after spray	Sample source	Number			Mort.	Percent				
		Larvae per lb fruit	Total mature larvae	Pupae formed	mature larvae	Emerg. from pupae		Para. from all pupae	Identifiable in dead pupae	
						Flies & para.	Para.		Para.	Flies
1 hr.	Check	34	302	246	19	76	66	53	15	3
	Dieldrin	20 (-41%)	183	112	39	15	35	30	28	4
4 days	Check	31	191	165	14	47	54	34	16	11
	Dieldrin	12 (-61%)	98	81	17	36	38	28	23	0
11 days	Check	27	97	91	6	56	16	18	20	10
	Dieldrin	16 (-41%)	71	56	21	54	47	34	19	31
19 days	Check	129	581	334	43 1/	63	70	49	15	2
	Dieldrin	62 (-52%)	324	280	14	50	34	49	64	8
Average	Check				13 2/	60	52	38	16	6
	Dieldrin (49% control)				26 2/	39	38	35	34	11

1/ Overcrowding of 500 larvae in 1 emergence jar responsible for high mortality. That among the other 81 larvae from same source was only 7 percent.

2/ These means do not include the 19-day samples.

The dieldrin effected very poor control because of its slow action and the constant influx of flies into the small plot. The mean reduction of 49 per cent for the entire experiment was almost equalled by the initial reduction effected by absorption of the toxicant into the fruit picked 1 hour after spraying. These figures apply to larvae reaching maturity in the fruit and able to leave it. A further mortality among these larvae occurred before pupation. This averaged twice as great (26 percent) among larvae from dieldrin-sprayed fruit as from the controls. The mortality among pupae from sprayed fruit was also greater than from unsprayed (61 vs. 40 percent). The percentage of parasites that emerged from pupae was least where dieldrin had been used (38 vs. 52 percent). The percent of parasites in or from pupae (identifiable unemerged plus emerged) varied from date to date but averaged about the same. There was a higher percentage of dead parasites in pupae from the sprayed than from unsprayed plots. The difference in number of dead flies in unemerged pupae from the treated and untreated fruits probably was not significant.

Supplemental studies were conducted by Mr. Keiser in which guavas were held individually and the larvae removed from the fruit before they reached maturity without opportunity for them to contact external residues. Samples on 3 dates, 5, 11, and 21 days after the spray, showed 57 percent parasitism in the check whether based on live emergence only or on the total parasitization including that in the dead unemerged pupae. From the treated fruit, however, the live emergence included 31 percent parasites while the total parasitism based on living emergences plus dead parasites in unemerged pupae was actually 73 percent.

The data in table 2.2 support our earlier hypothesis that dieldrin sprays reduce total emergence from pupae as well as percent of parasites emerging, but the results do not indicate that the percent of initial parasitism was reduced because adult parasite mortality was greater than fly mortality during or before oviposition. However, in this test both parasites and flies in most instances made their first contact with the insecticide at the time they alighted on the fruit to oviposit and a differential effect from exposure to the toxicant would be less apparent than where larger areas are sprayed.

To recapitulate, dieldrin sprays (2 lb. toxicant per 100 gal.) effect a substantial control of larvae already present in guava fruit at time of application, they increase mortality in mature larvae after they leave the fruit and before pupation, they reduce total emergence from pupae as well as, or because of, a reduction in percent of parasites that emerge, and they had no depressive effect on total percent parasitism based on parasites emerged or dead in pupae when the sprays were applied to trees immediately adjacent to unsprayed areas. Dead pupae from dieldrin-sprayed fruit also have contained a higher percentage of parasites among identifiable adults than those from unsprayed fruits. The foregoing results of the dieldrin sprays were evident in fruit picked within 1 hour after application and held until eggs and larvae therein had hatched and matured as well as in fruit that was not picked until 19 days after spraying.

The mature larvae that failed to pupate were not dissected to determine if they were parasitized.

Residual Effectiveness of Surface Deposits on Guava Foliage
(Keiser, Ohinata, Fujimoto, Holloway, Lee)

A special field test was conducted during this quarter to determine the effectiveness of deposits from insecticidal formulations on guava foliage after different periods of weathering. The mean results (mortalities and deposits) are listed in table 2.3. Analyses were made of 100 disc samples from each of 3 trees per treatment. The total chlorine method of analyses was employed for both DDT and lindane.

Lindane emulsion at 1 lb. toxicant, and lindane suspension at 0.5 and 1.5 lb. toxicant per 100 gallons total spray was unsatisfactory against D. dorsalis by the time the second set of samples were taken (3 days after treatment). Against D.gucurbitae the dosage tested was unsatisfactory even on the day of spraying. The results with the dieldrin formulations are very interesting. The 2 emulsions tested, both at 1 pound toxicant per 100 gallons spray, were unsatisfactory against both species 3 days after treatment. The suspension sprays (0.5 and 1.5 lb. toxicant per 100 gal. spray) showed very toxic foliage residues, against D. dorsalis, for 3 days. The 1.5 lb. treatment was effective up to 17 days. Against D. cucurbitae, however, most of the effectiveness was lost after 3 days, but the 1.5 lb. dosage gave consistently higher mortalities than the 0.5 lb. concentration. DDT emulsion spray at 2 lb. toxicant per 100 gallons was completely unsatisfactory against D. cucurbitae but gave good control of D. dorsalis for 10 days. DDT suspension at 3 lb. toxicant per 100 gal. gave a high degree of control for at least 17 days against D. dorsalis. Apparently foliage deposits must exceed 3.5 µg. DDT/cm.^{2} to insure 80 percent or higher mortalities of D. dorsalis in 24 hours while deposits of as much as 16.4 µg. DDT/cm.^{2} gave poor control of D. cucurbitae.

Figures 2.1 and 2.2 present the data from the dieldrin and DDT suspension sprays, respectively. Table 2.4 presents data based on figures 2.1 and 2.2. While DDT suspension at 3 lb. gave a minimum of 90 percent kill of D. dorsalis for 13 days, the 1 lb. dosage was 90 percent effective for only 1 day. The 3 lb. treatment gave a minimum of 75 percent mortality of D. dorsalis for 18 days, while the 1 lb. dosage gave this minimum mortality for only 2 days. Against D.gucurbitae neither treatment was effective. Dieldrin 50 WP against D. dorsalis gave a minimum of 90 percent kill for 3 days at the 0.5 lb. dosage and 4 days at the 1.5. The greater effectiveness of the higher dosage was manifested at the 75 and 50 percent minimum mortalities effective for 9 and 16 days, respectively, for the higher dosage, and 3 and 4 days for the lower. This test shows the importance of a dosage above that required for initial high mortality as the higher concentrations appear to be effective for a duration almost geometric to the arithmetic increase in insecticide.

Table 2.3--Comparative effectiveness against adult fruit flies of different insecticides and formulations applied under field conditions when evaluated by exposure to guava foliage residues in the laboratory.1/

Treatment		Percent mortality (in 24 hours) from exposure to guava foliage collected indicated numbers of days following treatment. (Ug./cm² toxicant in red.)																	
Proprietary product	Pounds toxicant per 100 gallons total spray	D. dorsalis									D. cucurbitae								
		0	3	7	10	14	17	21	23	29	0	3	7	10	14	17	21	23	29
Lindane 20 EM	1.0	89 0.6	12 0.3	1 0.3	-	-	-	-	-	-	43	7	1	-	-	-	-	-	-
Lindane 25 EM2/	1.0	84 2.4	8 0.8	1 0.4	-	-	-	-	-	-	27	4	0	-	-	-	-	-	-
Lindane 25 WP	0.5	86 1.5	3 0.4	1 0.2	-	-	-	-	-	-	35	1	1	-	-	-	-	-	-
Lindane 25 WP	1.5	96 2.5	13 0.8	1 0.3	-	-	-	-	-	-	71	3	0	-	-	-	-	-	-
Dieldrin 24 EM	1.0	94	23	1	-	-	-	-	-	-	59	7	0	-	-	-	-	-	-
Dieldrin 15 EM	1.0	99	19	1	-	-	-	-	-	-	83	7	0	-	-	-	-	-	-
Dieldrin 50 WP	0.5	99	97	18	14	26	42	1	3	-	99	71	2	4	2	10	3	1	-
Dieldrin 50 WP	1.5	92	91	71	71	29	92	64	13	-	100	97	32	31	9	58	22	1	-
DDT 25 EM2/	2.0	87 7.8	77 3.0	53 2.8	82 2.3	43 1.9	71 1.9	14	22	11 2.1	38	13	10	6	4	9	8	1	1
DDT 50 WP	1.0	100 6.8	73 3.4	14 1.3	37 1.1	6 1.0	17 0.8	2	3	- 0.9	63	9	1	3	1	3	6	0	1
DDT 50 WP	3.0	97 16.4	81 9.1	81 5.2	98 5.3	81 3.6	94 3.6	49	63	24 3.2	69	43	27	47	13	37	6	4	1
Cumulative rainfall (inches)		.0	.07	.22	.24	.44	.44	1.99	3.20	4.12	.0	.07	.22	.24	.44	.44	1.99	3.20	4.12

1/ Each mortality listed based on average of 3 cages, 50 flies of each species in the same cage, plus 4 guava twigs with a total of approximately 50 leaves, or a total of 150 leaves plus 150 flies of each species for each mortality. The 18,000 flies used in this test were laboratory-reared and fed yeast protein hydrolysate since adult emergence.

2/ Laboratory prepared stock solutions using technical materials, xylene, and Triton B-1956 at a rate of 1/2 pt./100 gal. spray.

Figure 2.1.—Comparative effectiveness of dieldrin suspension spray at 2 concentrations applied in the field against adult fruit flies, when evaluated by exposure to guava foliage residues in the laboratory.

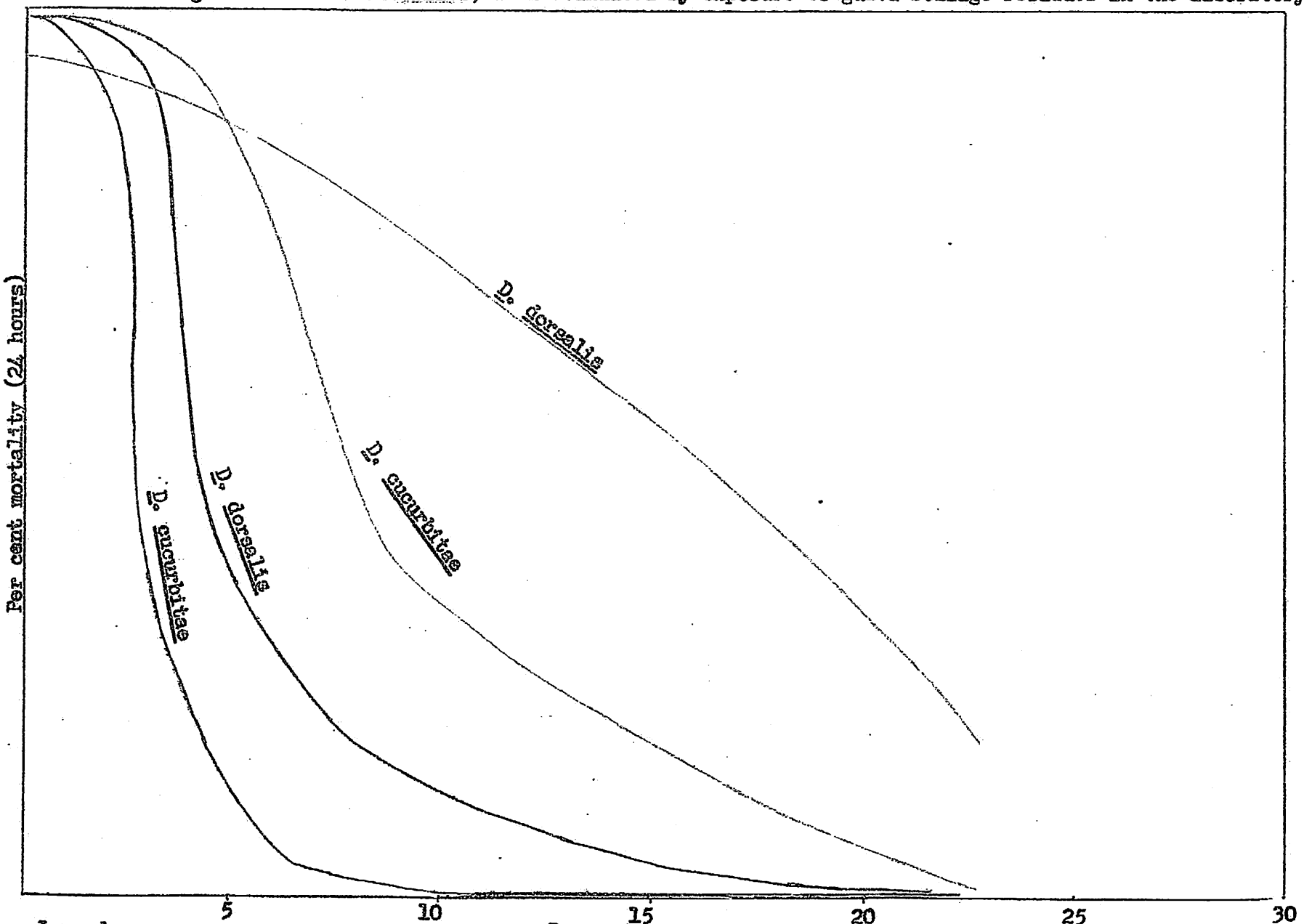
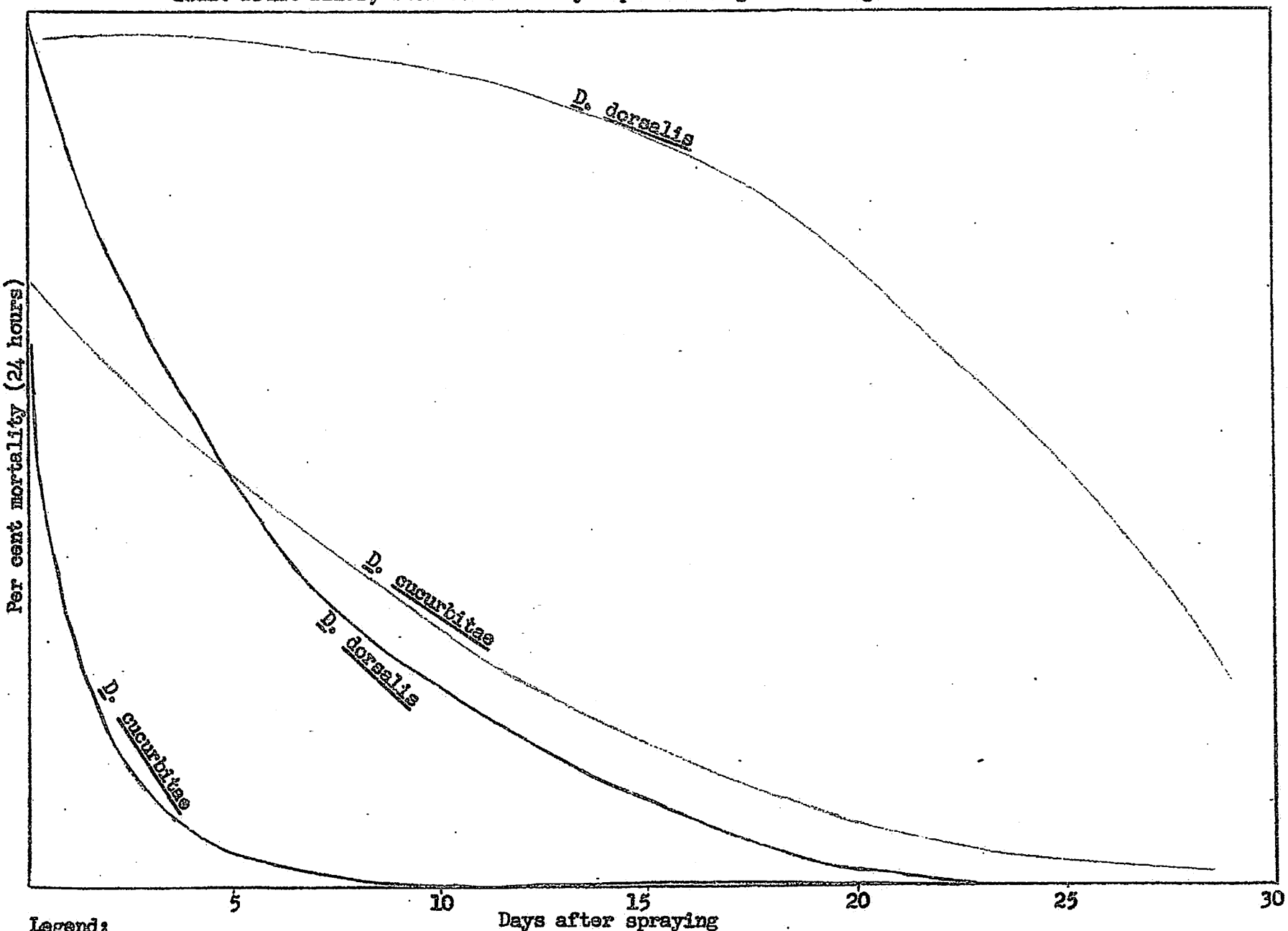


Figure 2.2.--Comparative effectiveness of DDT suspension spray at 2 concentrations applied in the field against adult fruit flies, when evaluated by exposure to guava foliage residues in the laboratory.



Legend:
——— DDT 50 WP - 3 lb. toxicant/100 gal. spray.
——— DDT 50 WP - 1 lb. toxicant/100 gal. spray.

Table 2.4—Duration of effectiveness of DDT and dieldrin applied under field conditions when evaluated in the laboratory by exposure of fruit flies to guava foliage residues.

Treatment		Number of days comparable field exposure yielding indicated percent mortalities of flies exposed in laboratory to guava foliage residues							
		D. dorsalis				D. cucurbitae			
Proprietary product	Pounds toxicant per 100 gallons total spray	90	75	50	25	90	75	50	25
DDT 50 WP	1.0	1	2	5	9	0	0	1	2
	3.0	13	18	24	29	0	0	5	12
Dieldrin 50 WP	0.5	3	3	4	7	2	3	3	4
	1.5	4	9	16	21	5	6	8	13

Further Studies with Demeton
(Keiser, Holloway, Fujimoto, Steiner)

During this quarter additional studies were conducted to determine the duration of effectiveness of demeton (Systox) against D. dorsalis larvae inside guava fruit. In the previous quarter, tests with 1, 2, and 4 lb. demeton per 100 gal. (per acre) were completed and these showed the importance of adequate dosage for effecting good kill. In this test, Systox 23 EM was applied with a Bean power sprayer at a dosage of 3 lb. demeton per 100 gallons spray using 1 gal. per tree or 6 lb. demeton per acre. The results are summarized in table 2.5. The treatment reduced the number of larvae already present in the fruit from 61 to 8, showed almost complete control of natural and artificial infestation during the period from 14 to 71 days after spraying. The 78-day collection yielded 9.1 larvae per fruit. However, this is far below the 52.4 in the check. Through the 78-day collection, a total of 449 guava were sampled, held individually, and examined 6 days later when the larvae were dissected. Of these, 312 received applications of 100 D. dorsalis eggs each.

Table 2.5--Comparative toxicity of demeton (Systox) applied to guava trees against D. dorsalis as evaluated by larval population from natural infestation and artificial egg deposition.1/

Test area	Mean number of larvae per fruit gathered from field on indicated numbers of days after spraying from natural infestation and from 100 D. dorsalis eggs placed on cut end.2/													
	0 3/	0 4/	2	8	14	21	28	35	42	50	57	65	71	78
Treated	60.6	7.5	2.6	2.8	0.0	0.0	0.0	0.0	0.06	0.0	1.0	1.0	0.3	9.1
Check	62.2		63.8	33.1	33.9	12.0 5/	3.7 5/	18.7 5/	29.9	26.5	42.3	56.3	32.5	52.4

1/ Systox 23 EM applied with Bean power sprayer (approximately 1 gallon total spray per tree) at rate of 3 lb. demeton per 100 gallons (6 lb. per acre). Trees sprayed to run-off.
2/ One hundred D. dorsalis eggs laid by laboratory-reared females fed (and fertilized by males fed) hydrolyzed protein from yeast and phytone (a soy hydrolysate). Eggs placed on cloth patch and inverted on cut calyx end. Larvae counted 6 days after egg patch application.
3/ Pre-treatment collection--1 hour before treatment.
4/ Post-treatment collection--with 1 hour after treatment.
5/ Poor egg hatch due to fly nutrition difficulties during the 3-week period noted.

Despite the present high cost of a treatment requiring 6 lb. demeton per acre the duration of effectiveness is long enough to span a crop season for some hosts, and the possibility of developing a control requiring only 1 application appears to justify further study with other systemics and with demeton on other hosts.

Table 2.6 presents detailed data from the pre- and post-treatment samplings as noted by the mean number of larvae recovered 6 days after each sampling. The presence of dead larvae in the fruit collected immediately after treatment indicates absorption of the poison as there was insufficient time for translocation. Also, the reduced number of larvae dissected from the fruit strongly indicates almost complete mortality of first- and second-instar larvae which disintegrated during the 6-day laboratory rearing period.

Table 2.6--Toxicity of demeton (Systox) applied to guava trees against D. dorsalis larvae present in fruit at time of field application.1/

Sampling3/	Mean number of larvae recovered2/									
	Natural infestation plus 100 eggs					Natural infestation only				
	Live		Dead			Live		Dead		
	Second instar	Third instar	Second instar	Third instar	Total	Second instar	Third instar	Second instar	Third instar	Total
Pre-treatment	1.0	60.6	0.7	0.1	62.4	0.0	47.2	0.0	0.0	47.2
Post-treatment	0.2	7.5	4.1	5.3	17.0	0.0	13.3	0.0	1.0	14.3
Check	0.0	62.2	0.0	0.0	62.2	0.0	33.8	0.0	0.0	33.8

1/ Systox 23 EM at rate of 3 lb. demeton per 100 gal. total spray applied to run-off (6 lb. demeton per acre).
2/ Larvae dissected from fruit 6 days after insertion in individual rearing cans. Where eggs were placed on cut end, 100 D. dorsalis eggs from laboratory-reared flies used.
3/ Pretreatment--fruit collected within one hour prior to treatment. Post-treatment fruit collected within 1 hour after completion of spray.

Prior to the application of the sprays, guava fruit in all stages of development--from 1/2 inch in diameter to completely ripe--were bagged in pliofilm and tied securely so that the spray would not contact these fruit. Fruit of all stages was used in order to extend the sampling period as much as possible. The 1/2-inch diameter fruit was bagged in clusters; all of the others were bagged singly. After the spray was applied the bags were not disturbed until they were cut open the following morning, 18 hours later, and the first sampling made. Subsequent samples were taken whenever ripe fruit was available, and comparable fruit collected from the same tree. (This latter fruit was not bagged, and received the direct spray.) The results are shown in table 2.7.

Table 2.7--Comparative toxicity of demeton (Systox) applied to guava trees against D. dorsalis as evaluated by larval population from natural infestation and artificial egg deposition.1/

Treatment		Mean number of larvae per fruit gathered from field on indicated numbers of days after spraying, from natural infestation and from 100 D. dorsalis eggs placed on cut end.2/							
		3/4	2	8	14	28	35	65	78
Treated	Bagged	20.5	16.0	0.0	0.0	0.0	25.0	49.0	80.0
	Not bagged	9.2	0.9	0.7	0.0	0.0	0.0	0.0	6.7
Check - Not bagged		69.5	63.8	33.1	33.9	3.7 3/	19.5 3/	56.3	52.4

1/ Systox 23 EM at rate of 3 lb. demeton per 100 gal. total spray applied to run-off (6 lb. demeton per acre). Fruit of all sizes bagged in pliofilm and collected, when ripe, on indicated numbers of days after treatment.
2/ Eggs from laboratory-reared flies, fed yeast and soy hydrolysates.

These data show that a considerable degree of translocation occurred during the 18-hour period from the time the trees were sprayed to the time the pliofilm bags were cut open and the first sampling made. Natural infestations prior to treatment plus the 100 eggs per fruit yielded a mean number of larvae of 69.5 in the check (not bagged), 9.2 in the treated (not bagged), and 20.5 in the bagged treated. The collection made 2 days after treatment also showed substantial control from translocation only. (The results from bagged and exposed fruit as listed in table 2.7 were obtained from the same treated trees.) Collections made 8, 14, and 28 days after treatment showed 100 percent control in the bagged fruit. By 35 days, however, there was no control in the bagged fruit. This was also noted in the 65 and 78-day collections. Fruit (not bagged) from the same trees, however, showed 100 per cent control 35 and 65 days after treatment and a high degree of control 78 days later. As mentioned above, the 1/2 inch diameter fruit (and also some 3/4 and 1 inch) were bagged in clusters. This covered not only the fruit proper but portions of the stems and stem ends leading to the fruit. These may be important storage areas for the demeton since when such areas did not receive the direct spray, fruit which ripened 35 or more days later did not contain sufficient demeton to effect control of D. dorsalis larvae.

An additional study was made (summarized in table 2.8 to determine the extent of translocation from a treated portion to an untreated part of the same tree. In the previous test the foliage spray of 3 lb. demeton per 100 gallons meant the application of approximately 14 grams toxicant per guava tree on the basis of 1 gallon spray liquid per tree. Accordingly, this quantity of actual toxicant (as contained in Systox 23 EM) was used to paint the main trunk and main lateral branches of other trees. As evident in table 2.8, there was significant control of larvae in fruit on branches of trees, the main trunk of which was painted with Systox 23 EM at a dosage of 14 grams demeton per tree. This is the first record of such control in guava trees, and it was effective from 12 to 69 days following treatment, or a total of 57 days. In the lateral branch treatments a single major branch was treated with the same dosage near its junction with the trunk. There was significant control in fruit from the treated branch from 6 to 69 days following treatment, or a total of 63 days. However, there was no control in the fruit on untreated branches. Evidently there is translocation upward only, from the main trunk to all branches, or from one scaffold branch to its smaller branches only. The paint treatment was not as effective as when the same quantity of toxicant was applied to the foliage, branches, and entire tree as a regular spray.

The data summarized in table 2.8 involved the sampling of 235 guava reared individually in tuna cans plus 100 D. dorsalis eggs each.

Table 2.8—Comparative toxicity of demeton (Systox) applied to guava foliage against D. dorsalis as evaluated by larval population from natural infestation and artificial egg deposition.[1]

Treatment[1]		Mean number of larvae per fruit gathered from field on indicated numbers of days after spraying from natural infestation and from 100 D. dorsalis eggs placed on cut end.[2]											
		1-2	6	12	19	26	33	40	48	55	63	69	76
Main trunk		64.8	64.0	16.3	8.4	7.2	0.0	9.7	0.0	2.3	30.1	3.7	32.1
On bark of scaffold branches	Treated	31.5	17.5	—[3]	0.0	5.3	5.6	0.0	—[3]	8.5	19.2	4.5	42.3
	Untreated	95.7	43.0	48.3	8.4	34.0	24.8	45.8	48.3	54.3	58.5	50.2	69.7
Check		55.6	44.0	42.3	11.0[4]	1.4[4]	17.0[4]	42.6	38.0	42.3	56.3	32.5	52.4

1/ Individual guava trees painted with 14 grams demeton per tree, applied either to main trunk or scaffold branch (60 ml. Systox 23 EM).

2/ One hundred D. dorsalis eggs laid by laboratory-reared females fed (and fertilized by males fed) hydrolyzed protein from yeast and phytone (a soy hydrolysate). Eggs placed on cloth patch and inverted on cut calyx end. Larvae counted 6 days after egg application.

3/ Mature fruit not available on particular sampling date.

4/ Poor egg hatch due to fly nutrition difficulties during the 3-week period noted.

Bait Spray Development Studies
(L. F. Steiner, R. K. S. Lee, R. Kinoshita)

These studies were continued throughout the quarter in the same manner as described in previous reports (see page 73, July-Sept. 1953 Quarterly Report).

Measured quantities of bait-spray mixtures were applied to the top and bottom surfaces of guava foliage held in position a few inches above 3'x3' screened-bottomed trays on which flies that were attracted and killed dropped and were counted. Each treatment was replicated 6 times on small guava trees planted 200 per acre. The replicates were distributed at the rate of about 1 per 20 trees or 10 per acre. The application rate throughout the current quarter was 5 ml. mixture per tree. This seldom resulted in the use of more than 1 to 2 grams of attractant and 4 grams of insecticide in any one acre and was not sufficient to exhaust the fly population which was subject to constant replenishment from surrounding areas. The highest returns per pound of yeast hydrolysate applied were recorded late in the quarter when rates in the first 24 hours after application were 333,000 cucurbitae and 185,000 dorsalis and the combined rate for the first 5 days was nearly 1.4 million flies per pound of the hydrolysate applied.

The comparative performances of different formulas are given in table 2.9.

As usual, the percentage of females in the catches was high, generally near 60 per cent and no significance is attached to the infrequent deviations from this performance.

In Expt. 53-14, applications were made on October 12 and 21, and flies collected from October 12 to November 9. Light rains totaling 1.04" fell on 13 of the days. With malathion as the toxicant NBC sample No. 2, a liquid yeast hydrolysate containing 42.8 percent solids, was not significantly superior in performance to the finished yeast hydrolysate (YH) from the same source. The comparison was made on an approximately equal solids basis. The partially hydrolyzed yeast protein (PHYP) at twice the concentration of the YH caught more flies than the latter but the differences were not significant. It was, however, significantly more effective against each species at the higher than at the lower concentration. At equal concentrations the catches made by the PHYP was almost identical with those made with the fully hydrolyzed finished protein hydrolysate (PH) from the same source.

In Expt. 53-15, which extended for 2 weeks, the NBC #2 formulation (with parathion) was less attractive than an equal quantity (but more than 55 percent less solids) of the PHYP to dorsalis and at 0.20 lb. per gal. was inferior to 0.10 lb. of dry yeast hydrolysate to each species. A PHYP-parathion dry mix was again inferior to the freshly prepared product but not significantly so. However, if the earlier tests are also considered it must be concluded that the dry-mix PHYP-parathion makes a somewhat less effective bait spray than a fresh tank mix. Because of the larger amount of NBC #2 required to equal the dry YH, there would be no saving in cost in use of the former. Among the enzymatic yeast hydrolysates therefore the most practical from the standpoint of cost per unit of effectiveness is the yeast hydrolysate (Nutritional Biochemicals Corp.) and the partially hydrolyzed yeast protein (Marvin R. Thompson Co.).

Table 2.9.--Results of replicated tests of experimental bait spray formulas using enzymatic yeast hydrolysates.

Expt. and treat.	(Quantities in pounds per gal. water) Hydrolysate[1]	Insecticide	Other	Dosage ml./ tree	Dead flies per tree dor.	Dead flies per tree cuc.	Percent females dor.	Percent females cuc.
53-14	Oct. 12-Nov. 9. Applied 10/12, 21. Rainfall 1.04" (over 13 days)							
A	NBC #2--0.47	Malathion 25WP 0.60		5 x 2	169	511	58	52
B	PHYP 0.20	do. 0.60		5 x 2	70	307	62	57
C	YH 0.20	do. 0.60		5 x 2	122	393	54	54
D	PH 0.20	do. 0.60		5 x 2	71	307	58	55
E	PHYP 0.40	do. 0.60		5 x 2	148	557	62	56
		LSD 5%			55	197		
53-15	Nov. 10-24. Rainfall 0.21" (over 4 days)							
A	NBC #2--0.10	Parathion 25WP 0.20		5	35	56	65	64
B	do. 0.20	do. 0.20		5	79	79	65	62
C	PHYP 0.10	do. 0.20		5	63	62	68	61
D	Same as C except dry mix prepared 8/15/53			5	48	41	70	62
E	YH 0.10	Parathion 25WP 0.20		5	92	112	66	63
		LSD 5%			18	30		
53-16A	Nov. 24-Dec. 7. Rainfall 0.62" (over 5 days)							
A	PHYP--0.10	Parathion 25WP 0.20		5	41	50	73	68
B		do. 0.20	+CGS[2] 0.30	5	2	1	73	100
C	PHYP--0.10	do.	do.	5	11	4	55	52
D	do.	do.	+raw sugar 0.30	5	51	35	71	70
E	do.	do.	+CRS[3] 0.30	5	7	4	63	75
		LSD 5%			15	13		
53-16B	Dec. 7-21. Rainfall 1.77" (over 6 days)							
A	PHYP--0.20	Parathion 25WP 0.20		5	103	174	51	60
B	do.	do.	+CGS 0.30+ salicylic acid	5	34	57	52	63
C	do.	do.	+CGS 0.30	5	35	53	56	69
D	do.	do.	+CRS 0.30	5	38	64	54	61
E	do.	do.	+raw sugar 0.30	5	95	109	49	64
		LSD 5%			18	30		
53-17	Dec. 23-Jan. 4. Rainfall 1.74" (3 days)							
A	YP--0.20	Parathion 25WP 0.20		5	130	288	52	58
B	do.	Diazinon-xylene 1:1 0.20		5	6	27	51	58
C	do.	do. 0.10		5	6	30	47	60
D	do.	Chlorthion 50 Em. 0.20		5	92	39	50	70
E	do.	do. 0.10		5	123	82	52	72
		LSD 5%			28	61		

1/ See text for identification.
2/ Caramalized granulated sugar.
3/ Caramalized raw sugar.

In Expt. 53-16A with parathion, raw sugar did not significantly change the effectiveness of PHYP in attracting dorsalis but did reduce attraction to cucurbitae. Caramelized granulated sugar plus parathion was ineffective and this sugar greatly depressed the catches of the PHYP-parathion when included in the formula. Caramelized raw sugar had a more adverse effect on catches of each species than regular raw sugar. The adverse effects were less pronounced in the subsequent experiment but here also caramelization of raw and granulated sugars before use with the PHYP-parathion made them less effective than raw sugar which again significantly depressed cucurbitae but not dorsalis catches as compared to the standard PHYP-parathion formula.

In the final experiment, parathion proved far superior to Diazinon against each species when used with yeast hydrolysate. It was superior to chlorthion against both species at an equivalent concentration but the use of only half as much chlorthion in this test made the latter equally effective against dorsalis. Tests are needed with a lower range of concentrations of toxicant since some repellency is indicated for the chlorthion.

Figure 2.3 charts the comparative day-to-day performance of the best bait-spray formula in terms of fly returns per 1/1000 lb. of hydrolysate used, also the per-trap-day catches (dorsalis only) made by bait traps in the same areas. The traps were serviced weekly with 235 ml. of fresh bait containing 20 gm. raw sugar plus yeast and vinegar. Cucurbitae catches in the traps were too low to chart. It is evident from figure 2.3 that the return per 0.45 gram of the protein hydrolysate as a poisoned bait spray on foliage was far greater than that from 20 gm. sugar in the traps and that as previously reported, the loss of effectiveness of the bait sprays are rapid though they generally were still attracting and killing flies 9 to 19 days after their application. Throughout this quarter they caught more cucurbitae than dorsalis.

Control of Fruit Flies Attacking Passion Fruit

(L. F. Stolmer and R. K. S. Lee)

As stated in the last report, efforts are being made in Hawaii to develop a passion fruit industry. Both dorsalis and cucurbitae attack this fruit, causing it to become deformed or to drop prematurely if stung when immature. Few flies develop in the fruit since the eggs are deposited in the thick rag. (See Figure 2.4.)

A test of a malathion bait-spray formula was conducted on a 1/3-acre block of passion fruit adjacent to one of the guava plantings at the University of Hawaii experimental farm at Waimanalo where there was a continuous heavy influx of flies of both species. The vines were trained on tall scrap-iron supports.

Twelve sprays were applied (at weekly intervals) of a formula containing partially hydrolyzed yeast protein 0.5 lb., malathion 25 WP, 1 lb., and water, 40 gal. The entire 1/3-acre was sprayed since the attraction of the bait spray to fruit flies would render any nearby unsprayed area useless for check purposes. Applications were made with a conventional power sprayer at a rate of 4 gal. per min. at 400 lb. p.s.i. through a broom equipped with 2 nozzles that provided a flat fan-shaped spray. (See figure 2.5.) No attempt was made to thoroughly wet all foliage; however, distribution on the outer leaves and fruit was very even. The first spray was applied August 26 and the last, November 12.

Figure 2.3.--Comparative performance of bait sprays and traps. Oct.-Dec. 1953.

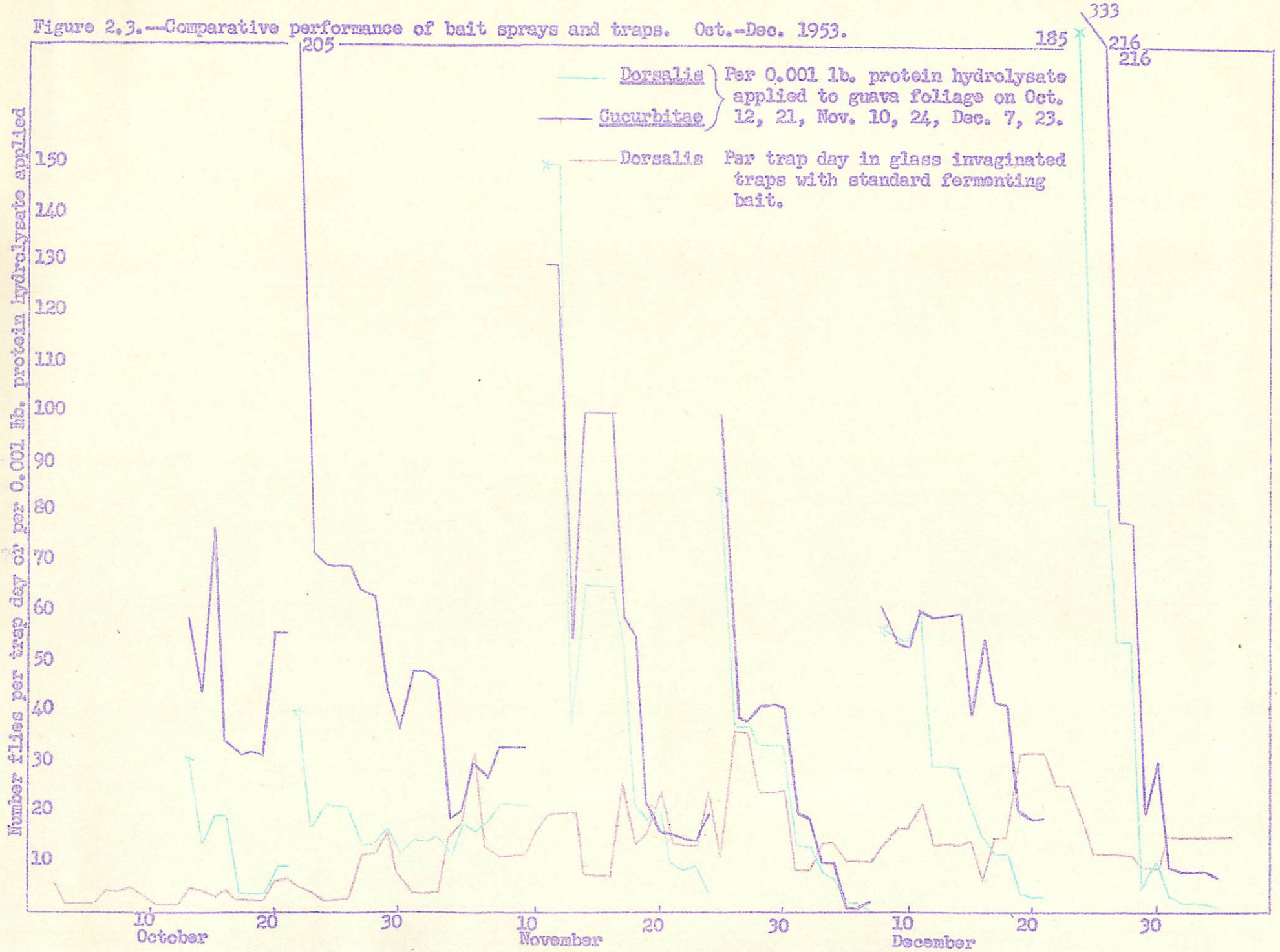


Figure 2.4.--Oriental fruit fly attempting oviposition near old oviposition sits on nearly mature passion fruit.

Figure 2.5.--Applying protein hydrolysate-malathion bait-spray to passion fruit with 2-nozzle broom throwing a flat-fan-shaped spray at rate of 4 gal. per min. (10 min. per acre). Both sides of each row was sprayed.

From 300 to 500 fruits were examined on the vines at 2-week intervals starting immediately before the first spray and at 1-week intervals for 6 weeks after the last spray by which time the vines had stopped producing. The results of these examinations are given in table 2.10.

Table 2.10.--Fruit fly injury to passion fruit. Waimanalo, Oahu.

Sprayed 12 times at weekly intervals 8/26 to 11/12/53	Percent fruit with fruit fly oviposition punctures (300-500 examined)	
Date	Immature 1/2" or less	Mature green to ripe
Pre-spray 8/26	67.0	68.0
9/11	15.3	72.6
9/23	0	30.0
10/8	0	18.0
10/22	0	9.0
11/5	0.4	0.8
11/18	1.5	1.5
11/27	0	1.8
12/2	0	2.5
12/10	0	3.0
12/16	0	1.5
12/23	no fruit	0.8

At the time of the first spray flies were abundant in the block and 67-68 percent of the fruit on the vines was stung. The weekly sprays brought about an almost complete cessation of further egg-laying and stinging but more than 8 weeks passed before all the immature injured fruit had matured and been removed. After spraying ceased there was a slight amount of fly injury evident once more on older fruits, but the production ceased because of maturity of the vines before any pronounced increase in attack occurred.

Reference to figure 2.3 will show that the bait sprays in the adjacent guava block along with the traps were attracting as many flies in December as in August.

Four glass traps located among the treated vines and 5 in nearby guava and papaya plantings were baited with the standard raw sugar, yeast, vinegar lure. Mean per-trap-day fly catches were as follows:

	In passion fruit vines		In nearby guava	
	dor.	cuc.	dor.	cuc.
Before sprays	11.3	3.1	14.2	2.3
After sprays	2.6	0.6	8.0	1.7

These data indicate that there was a considerable suppression of the fly population in the treated area and some depression in the adjacent unsprayed guava, but not all flies entering the area were attracted and killed by the poison residues before some had responded to the liquid baits.

Twenty metal trays, each covering 1.5 sq. ft. of ground area were scattered under or near to the passion fruit vines and examined frequently for the purpose of estimating the total kill of flies in the area. These estimates on a per-acre basis are given in table 2.11.

Table 2.11.—Estimated number flies killed by bait sprays.

Date of application	Spray No.	Estimated number flies killed (per acre) during week after each spray	
		dorsalis	cucurbitae
8/26	1	37,800	21,150
9/3	2	10,350	8,100
9/11	3	6,750	7,200
9/17	4	900	450
9/23	5	4,500	4,500
10/2	6	4,050	4,950
10/8	7	4,950	4,050
10/15	8	6,750	4,950
10/22	9	11,700	4,500
10/29	10	9,000	4,050
11/5	11	20,250	6,300
11/12	12	10,350	3,150
Totals		127,350	73,350

Accurate fruit production records and the effect of reduced oviposition on set could not be determined but for a few weeks after spraying started there was a noticeable increase in newly set fruit. No pollinating bees were ever found affected by the sprays. The estimated numbers of flies killed far exceeded the total number of fruit produced.

Malathion Deposits on Passion Fruit

(K. Ohinata and L. F. Steiner)

Malathion residues on passion fruit foliage and fruit were checked after each of the 12 weekly sprays on the 1/3-acre passion fruit block at Waimanalo sprayed with a bait-spray formulation of 3 lb. malathion 25 WP and 1.5 lb. partially hydrolysed yeast protein per acre.

Fresh spray samples (25-30 single fruit samples) were picked as soon as the foliage was dry. For later samples, replicates of 5-7 fruits (approximately a pound) were taken. Foliage deposits were checked by analyses of leaf discs.

Results are shown in tables 2.12 and 2.13. A residue loss of over 60% from foliage as well as fruit is indicated one day after spray, and by the 4th day after spray only an insignificant amount of malathion is detectable. Frequent light showers in the Waimanalo area probably contributed toward some of this residue loss, although it is known that the rate of malathion breakdown is influenced by sunlight and temperature as well as by rainfall.

Table 2.12.—Malathion deposits on passion fruit foliage.

Spray No.	0 day after spray		1 day after spray		2 days after spray		4 days after spray		5 days after spray		6 days after spray		8 days after spray	
	Range	µg/cm²	Range	µg/cm²	Range	µg/cm²	Range	µg/cm²	Range	µg/cm²	Range	µg/cm²	Range	µg/cm²
1	0.4–2.9	1.3							-	0.3				
2	1.0–1.5	1.1							-	0.0			-	0.0
3	1.0–1.8	1.6									-	0.0		
4	1.7–2.0	1.8									0.2–0.3	0.3		
5	1.2–1.8	1.5	0.8–1.1	0.9	0.3–0.5	0.4								
6	1.5–1.8	1.6					0.1–0.3	0.2			-	0.0		
7	1.0–1.7	1.3	0.3–0.5	0.4										
8	1.4–1.9	1.7	0.2–0.6	0.4			0.0–0.2	0.1						
9	1.3–1.7	1.5	0.3–0.6	0.5			0.0–0.3	0.2			-	0.0		
10	1.8–2.0	1.9	0.4–1.0	0.7					-	0.0				
11	2.1–2.7	2.4												
12	-	1.8	0.9–1.4	1.1			-	0.1			-	0.0		
Mean µg/cm²	1.6		0.7		0.4		0.1		0.1		0.0		0.0	

Table 2.13.—Malathion deposits on passion fruit.

Spray No.	0 day after spray		1 day after spray		2 days after spray		4 days after spray		5 days after spray		6 days after spray		8 days after spray	
	PPM range	Mean	PPM range	Mean	PPM range	Mean	PPM range	Mean	PPM range	Mean	PPM range	Mean	PPM range	Mean
1	0.0-21.2	4.0							0.0-1.0	0.5				
2	0.0-5.0	2.0							0.0-0.3	0.2				0.0
3	2.0-4.8	2.8									0.0-0.5	0.3		
4	1.2-8.5	4.8									0.1-0.9	0.5		
5	0.4-5.9	2.9	1.5-2.7	2.2	0.3-1.0	0.6								
6	0.3-6.0	2.2						0.0				0.0		
7	0.7-5.1	2.1	0.3-0.4	0.4										
8	0.6-4.3	2.4	0.3-1.1	0.6			0.0-0.2	0.1						
9	1.8-6.0	3.6	0.8-1.8	1.3				0.0				0.0		
10	0.7-4.5	2.3	0.3-1.8	0.9						0.0				
11	1.5-5.2	3.4												
12	1.5-4.5	3.1	0.8-2.5	1.5			0.0-0.1	0.1				0.0		
Mean PPM	3.0		1.1		0.6		0.1		0.2		0.1		0.0	

Post Treatment Investigations in Areas Used for Large-Scale Tests of Methyl Eugenol-Poison Bait Stations for Control of D. dorsalis (L. F. Steiner, R. K. S. Lee)

Ookala Area (Hamakua Coast), Hawaii

This large-scale 6-sq.-mile experiment on guava was terminated May 8, 1953, except for quarterly determinations of infestation indexes at the 30 regular (plus several supplementary) permanent fruit sample sites.

The results of the August determinations reported last quarter indicated that populations had leveled off within 90 days after removal of the bait stations, in treated and untreated areas at 700, and 1100 ft. The summer crop at higher elevations had not started to ripen.

On Nov. 2, 3, 4, full holding-box samples of 45 to 60 fruit were obtained from all 30 regular sites, plus 3 supplementary sites at 1900, 700, and 300 ft. in the treated area. Fruit production at the higher elevations had just passed its peak. At 300 ft. it was nearing its end. The samples were left at Hilo where they were secreened by Messrs. Nakagawa and Farias.

The mean infestations by elevations and areas are given in table 2.14. Also included are comparable mean data for the same locations 1 year before while the male annihilation experiment was in progress. At that time no control was indicated at 300 ft. near the ocean, probably because the almost constant on-shore winds prevented attractive odors from reaching many infested hosts along the coastal pali. However, reductions of 65, 75, 91, and 100 percent were indicated at 700, 1100, 1500, and 1900 ft. In 1953 infestations at these levels in the treated area ranged from 49 percent less (at 1100') to 126 percent more (at 1500') than in the controls. The average for all elevations was 15.1 larvae per pound for the former treated area and 15.6 for the controls. The latter was 4 times the infestation of November 1952. The percentage parasitism in November 1953 was less than the year before but there was no significant difference between treated and control areas. Capitata infestations were negligible except at 1900'.

These data, as did those obtained in August, indicate that the area used for the treatment was neither more nor less favorably situated with respect to oriental fruit fly attack than those areas with which it was compared in arriving at the estimates of treatment effectiveness and there is no reason to question the validity of those estimates.

Kilauea Area

After termination in May 1953, of the small methyl eugenol control experiment in this area where extensive fly movement appeared to blanket the entire treated and adjacent areas, a reduced number of feeding station traps were maintained. Fly movement as evidenced by trap catches in non-host areas was low in July, August, September, and October, 1953, but was very great in November with a further increase in December. While the control test was in progress the catches in non-host areas were greatest in January and February, May and June, November and December of 1952, and January to April, inclusive, of 1953.

Table 2.14--Infestation indexes in guava. Hamakua Coast. Nov. 1952 vs. Nov. 1953.

Location		Oct.-Nov. 1952					Nov. 2-4, 1953				
		Control areas			Treated area	% Diff. in tr.	Control areas			Treated area	% Diff. in tr.
		NW	SE	Mean			NW	SE	Mean		
300'	Lv./lb. Dor.	17.0	6.9	12.0	10.2	- 8	27.4	18.6	23.0	12.8	- 44
	Cap.	0.3	0.1	0.2	0.2	0	0	0	0	0	0
	% Para.	51	52	52	59		58	70	64	32	
700'	Lv./lb. Dor.	3.4	4.2	3.8	1.1	-65	29.5	16.8	23.2	31.8	+37
	Cap.	0.1	0.02	0.1	1.1	+1000	1.0	0	0	0	-100
	% Para.	59	64	62	66		52	36	44	58	
1100'	Lv./lb. Dor.	2.0	2.9	2.4	0.6	-75	20.3	20.9	20.6	10.6	- 49
	Cap.	0.4	0.8	0.6	1.1	+83	1.5	0	0.8	0	-100
	% Para.	74	64	69	90		58	56	57	55	
1500'	Lv./lb. Dor.	0.4	1.4	0.9	0.08	-91	2.5	10.8	6.6	14.9	+126
	Cap.	0.2	1.0	0.6	1.0	+67	0.1	0.2	0.2	0.9	+350
	% Para.	92	68	80	50		42	46	44	52	
1900'	Lv./lb. Dor.	1.4	0.05	0.7	0	-100	8.1	1.4	4.8	5.5	+ 15
	Cap.	4.0	1.5	2.8	0	-100	2.0	0.1	1.0	5.1	+410
	% Para.	96	100	98	-		62	42	52	37	

The catches for certain feeding station traps are given in table 2.15.

Table 2.15. Comparative fly catches by certain poison-bait stations during and after control experiment with methyl eugenol.

Trap and location	Mean no. dorsalis males per trap mon. 16 months Jan. '52 - Apr. '53	8 months Apr. - Dec. '53	Per cent difference
1.[1/] Center of treatment area. Elevation 2900'. (17 such stations in 80-acre square during first 16 months.)	486	3,250	+ 568
9 & 12 1/4 mile north and 1/4 mi. south of No. 1. Just outside treated area. El. 2800-2900'.	1,710	2,538	+ 48
15 & 14 1/2 mile north and 1/2 mi. south of No. 1. El. 2800-3000'.	4,540	3,590	- 21
55 3-1/2 mi. north of No. 1. Non-host area. El. 3800'	6,180	3,500	- 43
52 On SE rim of Kilauea caldera. Desert. Non-host area 5 mi. N.E. of No. 1. El. 4000'	980	895	- 55

1/ The total catch made by the 17 stations, plus Nos. 9 and 12 approximated 304,000 flies during the 16 months period. The grand total for all traps (25) for the 2 years was approximately 1 million flies with a mean of 1,940 per trap month prior to termination of the control test and 2,450 per trap month in the last 8 months.

With the removal of 16 competing stations in the 80-acre treated area, the per trap-month catch of the one remaining increased 568 per cent, and that of the nearest trap outside the area 48 per cent while the more distant traps were making monthly catches 21 to 55 per cent less than during the first 16 months. The combined monthly catches made by traps 1, 9, and 12 in the 8 month post-treatment period averaged 29 per cent less than the monthly catches made by all 19 stations during the control test. This strongly suggests that 3 traps in an area of about 120 acres would be almost as effective in trapping all males present as would 19. The 3 traps represented about the same density as had been used in the 6-square mile treated area on the Hamakua Coast.

Line Project I-a-6-3. INACTIVE.

Line Project I-a-6-4. Development of Resistance to Insecticides in Fruit Flies. (S. Maeda, S. Shimono, L. F. Steiner)

Selection of Parathion Strains

Only two generations of "parathion" exposed strains were produced this quarter. The low temperature in the insectary lengthened the life cycle of the fly from a month to a month and a half. The Strain I and II larval stages were 12 to 16 days and the pupal stages required 12 to 14 days. The temperature in the insectary fluctuated from 62° to 84° F. in December. The temperature is now controlled at a range of 70° to 84° F.

For F_{17} Strain I, eight per cent of the 266,000 eggs set on the medium survived the 0.105 p.p.m. parathion level and when compared to control larval cultures, it is estimated that 77 per cent of the larvae were killed by parathion. The three-day old flies were exposed in a 12-liter flask coated with residue obtained from 1.2 mg. parathion per 100 ml. ethyl alcohol. Of the 16,000 flies treated, 7% survived. Eggs were collected from the survivors for the following generation.

The F_{18} larvae when cultured in a medium with 0.11 p.p.m. parathion allowed only 2,000 of the larvae to mature. These larvae pupated and only about 1,000 flies emerged. The fly population for this generation was too low for selection and topical tests. These flies were saved as stock flies for the succeeding generation.

Concentrations of 0.1 to 0.105 p.p.m. parathion in the medium were used to select Strain II F_{17} and F_{18} larvae. The adults in this strain are not being exposed. There were sufficient F_{18} flies for a topical test. The LD_{50} values of the F_{18} parathion resistant and unexposed laboratory flies were of the same order, indicating that the selected strain has not acquired any tolerance after 18 generations of larval exposure. The records covering the larval selection of Strain II and larval and adult selections of Strain I are tabulated in table 4.1 and 4.2.

Table 4.1.—Selection of F_{17} and F_{18} *D. dorsalis* larvae and adults for parathion resistance.

Strain I								
	Larval Selection				Adult Selection			
Generation	P.P.M. parathion in medium	No. of eggs set	Percent larval recovery	Percent larval mortality	Dose mg./para. 100 c/ROH	No. of flies treated	No. of flies survived	Percent mortality
F_{17}	0.105	266,000	8	77	1.2	15,900	1,200	93
F_{18}	0.11	110,000	2	96	Adults not selected			

Table 4.2.—F_{17}, F_{18} larval selection for parathion resistance.

Strain II						
	Larval Selection				Adult Stage	
Generation	P.P.M. parathion in medium	No. of eggs set	Percent larval recovery	Percent larval mortality	Topical Test	LD_{50} value in µg. parathion/g. wt.
						Control
F_{17}	0.1	108,000	4	89		
F_{18}	0.105	129,000	19	64	0.76	0.72

Selection of DDT Strains

X-Strain (Larvae and immature adults selected)

Records for the selection of F_{14} and F_{15} larvae and adults of X-strain are summarized in table 4.3. DDT at 225 p.p.m. in the medium containing F_{14} larvae resulted in a 70% mortality level while a residue prepared from 1 g. of DDT in 100 ml. acetone by the 12-liter flask method resulted in an adult mortality of 90 percent. Increasing the concentration of DDT in the medium to 250 p.p.m. allowed only 5% of the 178,000 eggs set to mature as full-grown larvae. The larval selection was so severe that most of the flies emerged had to be saved as stock flies. The remaining flies were treated topically to determine the degree of tolerance of these flies to DDT.

Table 4.3.--Selection of F_{14}, F_{15} *D. dorsalis* larvae and adults for DDT resistance.

X-Strain										
	Larval Selection				Adult Stage					
					Adult Selection				Topical Test LD_{50} µg. DDT/g. female fly	
Generation	P.p.m. DDT in medium	No. of eggs set	% larval recovery	% larval mort.	Dose g. DDT/100 ml. acetone	No. of flies treated	No. of flies survived	% mort.		Control
F_{14}	225	237,000	11	69	1.0	9,900	1,000	90		
F_{15}	250	178,000	5	91	Adults not selected				570	19

The F_{15} flies and control laboratory flies were treated topically with DDT and kept in the insectary with temperature fluctuating between 62° to 74° F. for 24 hours. Usually, the insectary temperature fluctuates between 70° to 86° F. This low temperature gave increased mortalities for all the flies treated with DDT resulting in a LD_{50} value lower than previous values. Comparing the LD_{50} values, it took 30 times more DDT for the X-strain than the laboratory unexposed strain. The last evaluation for tolerance was done on 12th generation flies. At that time the X-strain had a 6-fold tolerance, and within the last three selections this strain has increased in tolerance to 30-fold when compared to the unexposed strain.

P-Strain (Mature adults selected)

Four concentrations of 50% wettable DDT were used in selecting F_{29}, F_{30}, and F_{31} mature adults by the petri dish-cage method. For each generation selection, a population ranging from 6,000 to 8,000 flies were treated with an average mortality of 85% and 600 to 1,500 survivors. The mortalities obtained for different residues for three generations are listed on table 4.4.

The 10-day old F_{31} adults were treated topically along with the unexposed strain of comparable age with recrystallized technical DDT. The 24-hour mortalities were higher than usual when incubated at temperatures 8° F. lower than the average insectary temperature. Dosage-mortality curves were drawn and LD_{50} values read off from the curves. The P-strain LD_{50} value of 85 µg. DDT/g. female wt. was five times the unexposed strain LD_{50} value of 19. This P-strain has not increased in tolerance to DDT since the 10th generation, and it appears that it will not develop any further tolerance by selecting in this manner.

Table 4.4.--Adult selection of D. dorsalis for DDT resistance.

P-Strain	Adult Stage										
	Percent mortalities									Topical Test	
Generation	µg. per cm.²/						No. of flies treated	No. of flies survived	Percent mort.	LD_{50} µg./DDT per gm. female	
	12.7	25.5	32.0	51.0	64.0	128.0					control
F_{29}	57	73	79		88		6,750	1,400	79		
F_{30}		78	85		93	90	7,500	986	87		
F_{31}		80	87		92	91	5,850	600	90	85	19

Tolerance of Wild Flies

Studies on the evaluation of resistance to parathion and DDT in wild flies are still continuing. There were nine collections of guavas and kamani from Maui, Hawaii, and Oahu. Guavas collected from the Hana and Nahiku, Maui, areas were very lightly infested with fruit flies, and parasitism was heavy. There were not enough adult flies reared from these collections for topical tests. False kamani nuts collected from Hana and Iao Valley, Maui, were heavily infested; however, 80 to 90 percent of the infestation was parasitized and there were insufficient fruit fly populations for topical tests.

Flies emerging from guavas and laboratory flies were fed sugar and water for a few days and were tested topically with acetone solutions of DDT and parathion. In some cases only two concentrations of insecticides in duplicate were run depending on the available flies, otherwise at least three concentrations were used and were tested in triplicate. Although the LD_{50} values may be approximate in some cases it gave some idea as to the nature of tolerance of the wild flies to DDT and parathion. Topical tests on flies from two guava collections from Sacred Falls on Oahu and on flies emerged from guavas collected from the Hamakua Coast (two separate collections), Kilauea, and Kalapana on Hawaii indicated that these flies were no more tolerant to parathion and DDT than the unexposed laboratory flies as shown below:

D. dorsalis reared in guavas collected from:	LD_{50} values in µg. insecticide/g.	
	Parathion	DDT
Hamakua Coast I, Hawaii	0.4	23
Sacred Falls, Oahu	0.7	33
Laboratory flies	0.5	21
Kilauea, Hawaii	0.5	21
Hamakua Coast II, Hawaii	0.6	25
Kalapana, Hawaii	0.6	20
Laboratory flies	0.9	16

Protein hydrolysate was withheld from the diet of these flies to avoid confusing natural tolerance with that acquired as a result of diet. Most of the tests were made within 2 or 3 days after emergence. The tests indicate that the laboratory strain now used as a control for the selected DDT and parathion strains is representative of normal wild populations.

Line Project I-a-6-5. Fruit Fly Attractants and Repellents. (P. L. Gow, D. H. Hayashi, and L. F. Steiner)—by P. L. Gow.

Comparative Field Tests of Lures

Since olfactometer tests with sucrose were showing a significant response which appeared to be olfactory, it was decided to test whether sucrose solutions exposed in the field and protected against fermentation with salicylic acid would be attractive. In Field Experiment 92, white (commercial granulated) sugar was compared with raw sugar. Included in the experiment was a raw sugar-vinegar-yeast fermenting lure which differed from the standard fermenting lure in containing 20% sugar instead of the usual 8%. Also included was a bait consisting of salicylic acid in water at a concentration of 1 to 1000 such as was used in the two sugar baits to prevent fermentation, since olfactor screening tests had indicated some attractiveness for salicylic acid.

Field Experiment No. 92

Lure	Material
A	Raw sugar (20%) + vinegar (1.3%) + yeast
B	White sugar (20%) + salicylic acid (1/1000)
C	Raw sugar (20%) + salicylic acid (1/1000)
D	Salicylic acid (1/1000)

Lure	Percent of mean catch for Lure A		
	1-4 days	5-7 days	Entire week
A	100.0	100.0	100.0
B	0.8	0.6	0.7
C	3.3	5.9	4.9
D	0.3	0.5	0.6
LSD 5%	38.6	19.5	25.6
Mean catch Lure A	40.8	66.8	107.6

Apparently sucrose is not attractive at enough distance from the trap to be effective in the field. This experiment also shows that salicylic acid can be used to prevent fermentation in field lures without effecting the accuracy of the test since it fails to show any attraction at a dilution of 1/1000 although this concentration proved somewhat attractive in the olfactometer.

Field Experiment 93 was to test the effect of preconditioning bacterium No. 14 to soy meal before preparing the SM-14 culture. The strain of bacterium No. 14 was carried in shallow 10% soy meal with transfer to fresh soy meal medium every week until 9 such transfers had been made. A second strain was cultured in 10% soy meal medium with transfer every 48 hours until 17 transfers had been made. Both strains showed increased activity over the original culture which was maintained on stock culture agar under refrigeration. No other change was noted in the strain transferred at weekly intervals, but the strain transferred at 48-hour intervals lost the ability to produce the red color characteristic of SM-14 cultures.

SM-14 cultures were prepared from each strain and from the original refrigerated agar culture by culturing in trypticase soy broth for 48 hours. These SM-14 cultures were used to prepare these lures for Field Experiment 93.

Field Experiment No. 93

Lure	Material
A	Standard.
B	SM-14. Bacteria from original refrigerated agar culture.
C	SM-14. Bacteria from strain cultured in 10% soy meal medium with weekly transfers.
D	SM-14. Bacteria from strain cultured in 10% soy meal medium with 48-hourly transfers.

Lure	Percent of Standard Lure mean catch		
	1-4 days	5-7 days	Entire week
A	100.0	100.0	100.0
B	112.1	69.0	96.1
C	73.7	59.5	67.5
D	76.0	52.7	67.2
LSD 5%	28.3	29.6	26.4
Mean catch standard lure	57.9	33.5*	95.5*

*One replication had to be dropped from the experiment during the last half of the week because of broken traps.

It is apparent that preconditioning the bacteria in this fashion resulted in decreased attractiveness in the SM-14 lures. Since the preconditioned strains showed more vigor in the SM-14 cultures it appears likely that the decrease in attractiveness of the lures prepared with the preconditioned bacteria was due to increased production of repellents which are known to be present in SM-14 cultures rather than to decreased production of attractants.

Quantitative Olfactometer Screening Tests

Following is a summary of the results of olfactometer screening tests. No Mediterranean fruit flies were present in the olfactometer cage during the period when these tests were made.

	D. dorsalis	D. cucurbitae
Materials tested	21	21
Attractants	9	4
Enhancers	4	6
Repellents	0	0
Obscurants	0	5
No Effect	8	7

Results for the various materials is presented in table 5.1. The indices show the ratio of catches for each material as compared with the catches in water or in the standard fermenting lure. An index of more than one indicates attraction, and an index of less than one indicates repellence. A dash (-) indicates the difference was not significant. The mean catch in water and mean catch in Standard lure for both sexes are shown. Mean catches in water and in standard for females alone are not given. When the mean catch in water was zero, no ratio could be calculated, so in such cases the actual mean catch is given, if significant, and the value is marked with an asterisk (*).

If a material is found to be attractive in water, it is classed as an attractant; if not attractive in water but attractive when combined with the standard lure, it is classed as an enhancer. If a material is found to be repellent in water, it is classed as a repellent, while a material which showed no significant repellence in water but decreased the catch in the standard is classed as an obscurant. Where the water catch is low, a material may actually be repellent yet fail to show a significant difference from the water catch, so it is probable that some of the materials classed as obscurants are actually true repellents.

Olfactometer Concentration Tests of Materials Previously Found to be Attractive

To date, 636 materials have been screened in quantitative olfactometer tests for D. dorsalis, 238 for D. cucurbitae, and 127 for C. capitata. Most of these materials were tested at only one concentration, 0.1%. Of these materials, 221 showed some attraction for D. dorsalis, 72 for D. cucurbitae, and 47 for C. capitata.

It is known that most insect attractants have a peak concentration for attractiveness with the attractionfalling off on either side of the peak concentration and perhaps even become repellent as the concentration is further increased. There is no way of predicting at what concentrations an untested material may be attractive, and in screening a large number of materials the time involved in testing each material over a range of concentrations is prohibitive, so some kind of compromise must be made in the matter of concentration. A considerable number of preliminary olfactometer tests when this screening program was first undertaken indicated that to screen all materials at a concentration of 0.1% would probably hit somewhere in the attractive range of concentration of most materials likely to be useful in the field. However, it was recognized that a material showing only a slight attraction at 0.1% might be very attractive at some other concentration and that all materials showing attraction at this initial concentration should be retested over a considerable range of concentrations before any idea could be obtained of their true attractiveness. Rather than continually interrupt our screening program, the purpose of which was to eliminate the materials which had no promise at all as attractants, by making concentration tests on attractants as they appeared, it was considered preferable to screen a large number of compounds and then make concentration tests after a considerable number of attractants had been found.

In November, 1953, we finished screening all materials which we had previously collected for this purpose, and began making olfactometer concentration tests on materials shown to be attractive in screening tests.

Table 5.1 Olfactometer Screening Tests.

Material	Conc. %	Water Index ♀	Water Index Both sexes	Water Mean water catch	Standard Index		Standard Mean Std. catch
Dacus dorsalis							
[illegible]							
Acetamide	0.1	2.7	2.4	6.7	1.6	1.5	182.7
Allyl formate	0.1	-	8.0	0.3	1.6	-	33.7
Amyl chloride	0.1	4.0*	17.0	0.3	1.3	-	188.0
Ethyl acrylate	0.1	-	*8.0	0.3	-	-	33.7
Ethyl vinyl ether	0.1	4.67	28.0	0.3	1.3	1.3	188.0
Glycerine acetaldehyde	0.1	-	5.9	2.3	-	-	301.7
Morpholine	0.1	16.0	15.0	1.0	-	-	128.0
Phenol	0.1	3.2	3.1	6.7	1.6	1.4	182.7
Tannic acid	0.1	12.0	11.9	2.3	-	-	301.7
[illegible]							
Dimethyl carbonate	0.1	-	-	0.3	2.2	1.7	13.7
Ethylene diformate	0.1	-	-	0.3	1.5	1.6	13.7
Methyl acetate	0.1	-	-	0.3	1.3	-	188.0
3,4,5 Trimethoxybenzoic acid	0.1	-	-	6.7	1.8	1.5	182.7
[illegible]							
None							
[illegible]							
None							
[illegible]							
Acetyl acetone	0.1	-	-	0.3	-	-	33.7
Allyl acetate	0.1	-	-	0.3	-	-	51.0
Allylomine	0.1	-	-	0.3	-	-	51.0
Allyl ethyl ether	0.1	-	-	0.3	-	-	51.0
Glycine	0.1	-	-	2.3	-	-	301.7
3-Hydroxy-2-butanone	0.1	-	-	0.3	-	-	13.7
Nitroethane	0.1	-	-	1.0	-	-	128.0
n-Propyl formate	0.1	-	-	1.0	-	-	128.0
Dacus cucurbitae							
[illegible]							
Acetamide	0.1	9.0*	13.7*	0.0	-	-	95.0
Allyl acetate	0.1	15.5	33.7*	1.0	1.4	1.5	68.3
Ethyl acrylate	0.1	30.3	18.3*	1.0	1.3	1.4	79.3
Tannic acid	0.1	-	4.33*	0.0	0.6	0.7	26.7

Table 5.1 Cont'd

Material	Conc. %	Water Index ♀	Water Index Both sexes	Water Mean water catch	Standard Index ♀	Standard Index Both sexes	Standard Mean Std. catch
Dacus cucurbitae cont'd							
Ethanals							
Allyl ethyl ether	0.1	-	-	1.0	1.4	1.5	68.3
Amyl chloride	0.1	-	-	0.3	1.5	-	19.3
Ethyl vinyl ether	0.1	-	-	0.3	-	1.3	19.3
Morpholine	0.1	-	-	0.0	-	2.0	6.0
Nitroethane	0.1	-	-	0.0	-	2.0	6.0
n-Propyl formate	0.1	-	-	0.0	2.4	2.6	6.0
Propanols							
None							
Glycerols							
Acetylacetone	0.1	-	-	1.0	0.7	0.7	79.3
Glycerine acetaldehyde	0.1	-	-	0.0	0.7	0.7	26.7
Methyl acetate	0.1	-	-	0.3	-	0.8	19.3
Tannic acid	0.1	-	4.33*	0.0	0.6	0.7	26.7
3,4,5-Trimethoxybenzoic acid	0.1	-	-	0.0	0.3	0.4	95.0
Alcohols							
Allyl amine	0.1	-	-	1.0	-	-	68.3
Allyl formate	0.1	-	-	1.0	-	-	79.3
Dimethyl carbonate	0.1	-	-	0.3	-	-	73.7
Ethylene diformate	0.1	-	-	0.3	-	-	73.7
Glycine	0.1	-	-	0.0	-	-	26.7
3-Hydroxy-2-butanone	0.1	-	-	0.3	-	-	73.7
Phenol	0.1	-	-	0.0	-	-	95.0

Seven materials indicated as attractive by screening tests were retested at concentrations of 0.01%, 0.1%, and 1.0%. Five of these materials were again tested at concentrations of 0.01%, 1.0%, and 100% to find whether the 100-fold range or the 10,000-fold range of concentration would be most likely to indicate whether a material deserved further testing over a more limited range of concentrations. To obtain the 100% concentration the test material was not combined with the liquid (water or standard lure) in the trap but was soaked into a cotton plug suspended from the neck of the trap. Where the material was a solid, it was dissolved in a suitable solvent such as acetone or ether, the cotton plugs then being soaked in the solution, and the solvent allowed to evaporate before the plugs were placed in the traps. The results of these concentration tests are presented in table 5.2.

The tests were made by combining the test material with water and with the Standard fermenting lure using polyethylene glycol 400 monolaurate as emulsifying agent. In the index columns the values indicated in red were found to be significant at the 5% level by analysis of variance. Values which were not shown to be significant by statistical analysis are presented in purple. In some cases these values appear to fall into series indicating concentration curves and thus to gain significance from their position in series. It was also thought that presenting such values would be useful for comparing the two concentration ranges over which tests were made. In the columns labeled "mean water catch" and "mean Standard catch" are shown values for females alone and for both sexes. Since both D. dorsalis and D. cucurbitae were caught in these tests, results are given for both species whether or not attraction was previously shown in screening tests.

In the case of allyl phthalate the tests over both ranges of concentration indicate for D. dorsalis an optimum concentration between 1% and 100% with the test over the shorter range of concentrations best indicating a concentration curve. Results for D. cucurbitae showed no significant attraction but suggest that there may be some attraction at concentrations in excess of 1%.

The tests with civet indicate for D. dorsalis an optimum concentration at some value in excess of 1% and again the shorter range of concentration provides a clearer picture. There is a suggestion that a lower concentration than 0.01% may be attractive to D. cucurbitae, but due to the low catch of flies the results cannot be regarded as very reliable.

o-Diethoxybenzene is definitely more attractive to males than to females but cannot be regarded as a specific male attractant for D. dorsalis. The tests indicate an optimum concentration between 1 and 100% with both ranges of concentration giving a clear picture. The material evidently has no attraction for D. cucurbitae.

Diethyl malonate appears to have an optimum concentration between 0.1% and 1.0%, with the shorter concentration range giving the better concentration. It is possible that the presence of the 100% concentration in the longer range test had an effect on the performance of all the lures in this test since the traps are so close together. This material showed no attraction for D. cucurbitae.

Table 5.2 Olfactometer Concentration Tests. Dacus dorsalis
Index values in red are significant at 5% level

Material	Conc. %	Water Index ♀	Water Index Both sexes	Water Mean water catch ♀ both sexes	Standard Index ♀	Standard Index Both sexes	Standard Mean Std. catch ♀ both sexes
Allyl phthalate	1.0	11.0	14.7	0.7	0.7	0.6	117.3
	0.1	7.0	9.0	1.0	0.8	0.8	234.7
	0.01	1.5	2.0		0.8	0.8	
	100.0	3.6	2.7		1.0	1.1	
	1.0	3.5	3.5	3.3	1.1	1.1	85.7
	0.01	2.8	2.2	7.3	1.0	1.0	161.7
Civet (synthetic)	1.0	20.00*	43.7		0.8	0.8	
	0.1	3.67*	6.7	0.0	0.7	0.7	209.0
	0.01	1.33*	1.7	1.0	0.6	0.6	448.0
	100.0	2.5	1.9		0.9	0.8	
	1.0	12.0	9.8	2.7	1.1	1.1	92.3
	0.01	0.5	0.6	6.0	0.9	0.9	199.7
o-Diethoxybenzene	1.0	3.4	22.3		1.4	3.0	
	0.1	3.1	14.5	5.0	1.7	3.2	63.7
	0.01	1.3	2.3	8.0	1.1	1.3	122.3
	100.0	15.0	20.7		1.2	1.6	
	1.0	13.0	61.0	0.3	1.7	2.5	106.0
	0.01	11.0	6.0	1.0	1.1	1.1	205.3
Diethyl malonate	1.0	9.0	7.3		0.7	0.7	
	0.1	11.4	6.9	2.3	1.1	1.1	203.0
	0.01	1.6	1.2	6.3	0.9	0.9	378.7
	100.0	1.0	0.9		0.3	0.3	
	1.0	0.4	0.8	2.7	0.3	0.1	25.3
	0.01	0.5	0.7	5.0	0.3	0.3	50.3
Diethyl phthalate	1.0	16.3	15.2		1.1	1.0	
	0.1	10.3	10.1	2.0	1.1	1.1	214.7
	0.01	4.8	5.2	4.3	0.9	0.8	440.3
	100.0	3.6	3.6		1.2	1.1	
	1.0	3.0	3.4	4.0	1.4	1.2	70.7
	0.01	1.8	1.7	6.3	1.3	1.1	141.3

Table 5.2 Olfactometer Concentration Tests. Cont'd

Dacus dorsalis cont'd

Material	Conc. %	Water Index ♀	Water Index Both sexes	Mean water catch ♀ / Both sexes	Standard Index ♀	Standard Index Both sexes	Mean Std. Catch ♀ / Both sexes
2,4-Dihydroxybenzaldehyde	1.0	3.4	3.4	10.3 / 21.7	0.5	0.6	141.7 / 297.7
	0.1	3.5	3.1		0.8	0.8	
	0.01	3.8	3.4		0.8	0.8	
2,4-Dihydroxybenzoic acid	1.0	0.2	0.7	3.0 / 5.3	0.8	0.6	52.7 / 133.7
	0.1	0.8	1.1		0.8	0.8	
	0.01	0.7	0.6		0.9	0.8	
Dacus cucurbitae							
Allyl phthalate	1.0	4.0	8.0	0.3 / 0.3	0.8	0.8	24.7 / 46.3
	0.1	4.0	7.0		0.7	0.7	
	0.01	1.0	2.0		0.7	0.7	
	100.0	4.00*	8.5	0.0 / 0.7	0.4	0.5	16.7 / 31.3
	1.0	7.00*	12.0		0.4	0.5	
	0.01	2.3*	4.5		0.5	0.4	
Civet (Synthetic)	1.0	1.0	1.0	0.3 / 0.3	0.08	0.1	100.0 / 171.7
	0.1	4.0	5.0		0.03	0.3	
	0.01	1.0	3.0		0.04	0.4	
	100.0	1.00*	1.67*	0.0 / 0.0	0.1	0.1	13.0 / 22.0
	1.0	0.00*	0.00*		0.05	0.05	
	0.01	3.00*	3.00*		0.6	0.5	
o-Diethoxybenzene	1.0	4.0	3.0	0.3 / 1.0	0.4	0.4	17.3 / 43.0
	0.1	5.0	2.0		0.7	0.5	
	0.01	4.0	2.0		0.8	0.8	
	100.0	1.00*	1.5	0.0 / 0.7	0.4	0.3	25.0 / 45.3
	1.0	1.00*	3.5		0.2	0.2	
	0.01	2.67*	6.0		0.4	0.5	

Table 5.2 Olfactometer Concentration Tests (cont'd)

Dacus cucurbitae (cont'd)

Material	Conc. %	Water Index ♀	Water Index Both sexes	Mean Water Catch ♀ Both sexes	Standard Index ♀	Standard Index Both sexes	Mean Std. catch ♀ Both sexes
Diethyl malonate	1.0	5.0	2.7	0.3	0.4	0.6	29.0
	0.1	8.0	4.0	1.0	0.7	0.8	53.3
	0.01	3.0	2.0		0.6	0.7	
	100.0	1.0*	3.0	0.0	0.9	1.0	95.3
	1.0	0.33*	1.0	0.7	0.5	0.5	192.0
	0.01	0.33*	1.0		1.0	1.0	
Diethyl phthalate	1.0	11.7	15.5	1.0	0.5	0.6	44.3
	0.1	13.3	13.8	1.3	0.5	0.5	74.3
	0.01	7.0	8.8		0.6	0.6	
	100.0	15.00*	27.0	0.0	0.8	0.8	18.7
	1.0	14.67*	34.5	0.7	1.1	1.2	32.3
	0.01	2.0*	4.0		0.6	0.8	
2,4-Dihydroxybenzaldehyde	1.0	0.8	0.9	1.7	0.3	0.3	22.3
	0.1	0.4	0.7	3.0	0.6	0.6	40.7
	0.01	6.6	5.7		0.9	1.0	
2,4-Dihydroxybenzoic acid	1.0	21.0	18.0	0.3	0.6	0.7	47.3
	0.1	17.0	11.0	0.7	0.7	0.8	74.0
	0.01	6.0	4.0		0.6	0.8	

With diethyl phthalate an optimum concentration for D. dorsalis at a level higher than 1% is indicated. Again the shorter concentration range gives a clearer picture. The response of D. cucurbitae to this material was much like that of D. dorsalis except that it tends to obscure the attraction of the standard lure for D. cucurbitae.

Tests were made only over the shorter concentration range for 2,4-dihydroxybenzaldehyde and 2,4-dihydroxybenzoic acid. These materials did not attract D. dorsalis but 2,4-dihydroxybenzaldehyde was attractive for D. cucurbitae with indication that the optimum concentration is less than 0.01%.

It is difficult to draw conclusions as to the relative value of the two concentration ranges from so few tests. However, there is some indication that the shorter range is safer to use as an initial concentration test for materials showing promise in screening tests. With all traps in a test grouped as close together as they must be in olfactometer testing, it is very possible that the presence of traps representing a 100% concentration may keep the air in the vicinity of all the traps pretty well saturated with the odor of the test material and thus affect the performance of all the traps. It is possible that for testing high concentrations the spot test technique might be advantageously employed, diluting the test material for concentrations less than 100% with a neutral material such as mineral oil. Certainly concentrations greater than 10% are not suitable for use in glass traps in the field, but should be employed in some sort of poison feeding station. Results at high concentrations in glass traps cannot be used to predict behavior of such concentrations on poison feeding stations, since a concentration that would build up to a repellent level in the dead air space inside a glass trap might be highly attractive on the well-ventilated surface of a poison feeding station.

Olfactometer Tests with Sugar

In the report for the previous quarter it was reported under olfactometer screening tests that sucrose at a concentration of 10% in water in invaginated glass traps was attractive to all three species of fruit flies. Sucrose was tested as an attractant not in the hope that it would be useful as a bait but in the hope that some basic information on fruit fly attractants might be derived from a study of this material. The vapor pressure of sucrose at room temperature is too low to be determined, and this compound may be regarded as being practically non-volatile. As has been previously reported, lyophilization experiments on SM-14 cultures strongly indicated that the attractants contained therein are so non-volatile as not to be distilled at room temperatures under a pressure of a fraction of millimeter of mercury. This and other experimental results lead to formulation of the hypothesis that these attractants get into the air not by volatilization but by a kinetic process somewhat analogous to what takes place in a steam distillation, water molecules evaporating from the surface tending to knock the non-volatile molecules of attractant out of the surface and then push them away from the vicinity of the surface out into the air. When a solution of sucrose appeared to have some attraction, it appeared that here we might have a compound that can be easily obtained in very pure form and can be detected by chemical means at very low concentrations, and that by working with such a substance it might be possible to test the validity of our hypothesis. Moreover, if sucrose

could be demonstrated to exert an olfactory attraction—the only alternative explanation for increased catches would be a gustatory response on the part of flies entering the traps during random movements—the conclusion would be justified that appreciable volatility is not always a prime necessity in insect attractants, thus opening up the whole field of non-volatile organic compounds to study in this connection. It was therefore believed that it would be worth devoting time to studies to determine whether the comparatively non-volatile sucrose is itself attractive and whether the apparent attractiveness as determined by the glass traps is truly the result of an olfactory response.

The question immediately arose as to whether the attraction exerted by a sucrose solution was due to the sucrose or to an impurity. The sucrose used in the original test was ordinary commercial granulated sugar which is known to have impurities present in very low concentration. An average figure on organic non-sugars in ordinary granulated sugar obtained from a report of California and Hawaiian Sugar Refining Corporation, Ltd., is 0.01%. Confectioners' A grade sugar, however, is reported to have an average organic non-sugar content of about 0.001% or 1/10 that of the granulated grade. A test was made to compare the two grades of sugar. The test consisted of 3 lures with 4 traps containing each lure. These were tested in the olfactometer 3 times on the same day so that the test contained 12 replications. Results are shown in Olfactometer Test No. 441. In all olfactometer tests with sugars, solutions were made up just before exposing and exposure periods were too short for any appreciable fermentation to develop.

To insure good response to sugar it was found necessary to withhold sugar from the flies in the olfactometer cage for at least 24 hours before sugar tests were made. During the period that the following tests were made, sugar was placed in the cage only over week-ends, and sugar tests were not made until Tuesday of the following week. Some sugar was present in the diet of the flies, however, since fruit juices and tomato sections were continuously supplied, so that the diet of the flies during the time these tests were made was probably closer to a field diet than when raw sugar was available to them.

Olfactometer Test No. 441

Lure	Material
A	Water.
B	Water + 10% sucrose (Confectioners' A).
C	Water + 10% sucrose (ordinary granulated).

Lure	Percent of mean water catch			
	D. dorsalis		D. cucurbitae	
	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	580.5	615.5	606.7	703.7
C	639.9	646.6	566.7	611.1
LSD 5%	103.1	104.8	165.6	162.2
Mean water catch	21.3	41.4	1.3	2.3

The fact that differences between the two grades of sucrose fall far short of significance and that the differences are in opposite directions for the two species of flies indicates that the two grades are equally attractive. Since the organic non-sugar impurities were approximately ten times as high in the granulated as in the confectioners' A it appears quite unlikely that the attraction in the latter is due to an impurity.

Two tests were then made to determine the approximate optimum concentration of sucrose. These are reported as Olfactometer Tests No. 442 and 445.

Olfactometer Test No. 442

Lure	Material
A	Water.
B	Water + 2% sucrose.
C	Water + 10% sucrose.
D	Water + 50% sucrose.

	Percent of mean water catch			
	D. dorsalis		D. cucurbitae	
Lure	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	142.2	145.5	400.0	270.0
C	581.3	590.4	2500.0	1130.0
D	518.4	537.9	2250.0	1070.0
LSD 5%	83.9	81.2	864.0	291.6
Mean water catch	16.2	33.0	0.3	1.7

Olfactometer Test No. 445

Lure	Material
A	Water.
B	Water + 10% sucrose.
C	Water + 20% sucrose.
D	Water + 40% sucrose.

	Percent of mean water catch			
	D. dorsalis		D. cucurbitae	
Lure	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	828.6	899.0	1300.0	1292.3
C	1234.7	1330.0	2014.3	1976.9
D	898.0	1036.0	1771.4	1584.6
LSD 5%	333.9	402.7	882.9	709.8
Mean water catch	8.2	16.7	1.2	2.7

The results of these tests indicate that the optimum concentration is probably in the neighborhood of 20%.

The question still remained as to whether the response to sucrose is olfactory or gustatory. We know that with ordinary glass invaginated traps, whether of standard size or the small traps used in our olfactometer tests, there is some re-emergence of flies. Comparing a material known to be acceptable as food with water in such traps might show an attraction which was wholly gustatory, since greater re-emergence from the water traps than from the sucrose traps where there was food to hold the flies longer and increase their chances of being drowned might show differences in number of flies caught, even though an equal number of flies entered both sets of traps.

An attempt was made to determine whether the response is olfactory by suspending spirals of blotting paper soaked in sucrose solution from the stoppers of standard glass traps and screening the blotting paper spirals so that flies entering the traps would be unable to contact and taste the solution. The surface area of the spirals of blotting paper were 28 square inches. It was found that with no water in the glass traps practically all flies entering the traps re-emerged. When water plus a wetting agent (polyethylene glycol 400 monolaurate) was used in the traps most of the flies entering were caught. The sucrose traps failed to attract more flies than the traps containing only water, but, if the hypothesis is valid that evaporating water is necessary to carry the sucrose out into the air, this result was not entirely unexpected. Water in the blotting paper moistened with sucrose solution could have been prevented from evaporating by the water placed in the bottom of the traps to drown the flies. Undoubtedly the latter must have saturated the atmosphere in the traps, and the sucrose solutions in the moistened blotters, having a lowered vapor pressure due to the dissolved sugar, would actually take up water from the atmosphere within the trap rather than evaporate.

Standard glass traps were then provided with screen domes which covered the trap entrances. Each screen dome was provided with a 1/2-inch hole to serve as trap entrance. Observation of such traps when placed in the olfactometer showed very little re-emergence of flies. A test was made with these traps comparing water with 20% sucrose. The results are shown as Olfactometer Test No. 456. Another test using the same traps was made which included 20% sucrose soaked into pieces of sponge placed in the traps and solid granulated sugar placed in the traps. Results are shown in Olfactometer Test No. 461.

Olfactometer Test No. 456

Lure	Material
A	Water) Standard size glass traps with entrance
B	Water + 20% sucrose) holes screened to 1/2-inch entrances.

Lure	Percent of water mean catch			
	D. dorsalis		D. cucurbitae	
	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	795.0	737.6	2385.7	2762.4
LSD 5%	298.5	325.5	1817.5	2223.4
Water mean catch	17.2	40.7	10.5	15.5

Olfactometer Test No. 461

Lure	Material	
A	Water.	Traps screened to 1/2-inch entrances.
B	20% sucrose soaked into pieces of sponge	
C	Solid granulated sugar	
D	20% sucrose solution	

Lure	Percent of water mean catch			
	D. dorsalis		D. cucurbitae	
	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	1814.3	1080.0	971.4	966.0
C	1095.9	1008.9	533.3	522.7
D	732.7	624.5	433.3	437.6
LSD 5%	855.8	817.7	334.6	305.6
Mean water catch	8.2	15.0	10.5	23.5

Test 456 showed the same order of differences for both species as was noted in previous tests with full-size trap openings where no effort was made to decrease re-emergence. In Test 461 there was no liquid standing in the B and C lure traps to entrap and drown the flies, so re-emergence would certainly be greater from these traps if it were indeed a factor in determining the size of the catch. However, these two lures caught more flies than the D lure. These two tests strongly indicate that the attraction is olfactory and not gustatory in nature. The high catch with the solid granulated sugar in Test 461 indicates that enough sucrose volatilizes from the crystalline form to attract flies over a short distance. Since the vapor pressure of this compound is too small to be determined, the amount of airborne sucrose actually encountered by the flies' olfactory receptors must be extremely small, indicating that the compound is actually extremely attractive in the sense that a very low concentration in the air is sufficient to elicit an olfactory response. The fact that 20% sucrose soaked into small pieces of sponge was more attractive than the same solution present as a pool of liquid in the traps was probably due to the increased area of liquid exposed by the sponge pieces. This can be taken as further evidence that the sucrose is in fact carried out of the traps in the air and that the response is therefore olfactory. The high response to the solid sugar indicates that the hypothesis that evaporating water plays a major part in causing diffusion of sucrose molecules into the air may not be valid. However, it should be noted that the granules of solid sugar exposed a much greater surface area to the air than did the liquid pools in the traps. It is impossible to estimate the relative surface exposed in the solid sugar traps as compared with the traps containing pieces of sponge soaked in sucrose solution.

Olfactometer tests were made with other sugars as compared to sucrose. The results of these tests are presented in table 5.3. The column labeled "Index to water" gives the ratio of the sugar solution catch to water catch. The column labeled "Index to sucrose" shows the ratio of sugar solution catch to sucrose catch. Indices in red were significant by analysis of variance.

Table 5.3. Olfactometer Tests with Various Sugars.

Sugar	Conc. %	Mean water catch both sexes	Index to water		Index to sucrose	
			♀	Both sexes	♀	Both sexes
Dacus dorsalis						
Dextrose	10	40.5	3.4	3.1	0.8	0.8
Levulose	10	40.5	4.9	4.2	1.2	1.0
Galactose	10	11.2	2.4	3.3	0.4	0.3
Mannose	10	11.2	0.7	0.9	0.1	0.1
Xylose	10	31.3	1.1	1.2	0.1	0.1
Arabinose	10	31.3	4.6	4.5	0.4	0.4
Sorbose	10	26.7	2.5	2.2	0.3	0.3
Maltose	10	26.7	7.4	7.2	0.9	0.8
Glycerose	10	7.3	10.4	10.3	1.7	1.8
Dacus cucurbitae						
Dextrose	10	3.3	3.3	3.3	0.7	0.7
Levulose	10	3.3	6.8	6.6	1.4	1.3
Galactose	10	2.2	5.7	4.3	0.2	0.1
Mannose	10	2.2	0.7	0.8	0.02	0.03
Xylose	10	2.2	2.6	3.1	0.2	0.2
Arabinose	10	2.2	6.5	7.8	0.4	0.4
Sorbose	10	1.3	7.0	5.8	0.5	0.5
Maltose	10	1.3	13.5	15.8	1.0	1.3
Glycerose	10	3.9	5.0	4.1	0.6	0.6

The material listed as glycerose was prepared by oxidising glycerol with hydrogen peroxide according to the method of Witzemann, J. A. C. S. 36, 2223-2234 (1914). The reaction mixture after oxidation was neutralized with $CaCO_3$ to precipitate ferric ions used as catalyst and to neutralize any acids produced. The result was a mixture of glyceraldehyde and dihydroxy acetone in unknown ratio and with an unknown yield. The reaction mixture was diluted to represent a 10% original concentration of glycerol and was undoubtedly less than 10% glycerose. This material was more attractive than sucrose to D. dorsalis but less attractive than sucrose to D. cucurbitae.

With the other sugars the results were much the same for both species. The results are summarized as follows:

Summary of Sugar Tests

	Sugar	No. of C atoms	Chemical nature
1. As attractive as sucrose	(Sucrose	12	Aldose+ketose
	(d-Levulose	6	Ketose
	(Maltose	12	Aldose+aldose
2. Less attractive than sucrose	(d-Dextrose	6	Aldose
	(d-Galactose	6	Aldose
	(l-Arabinose	5	Aldose
3. Not attractive	(d-Mannose	6	Aldose
	(l-Xylose	5	Aldose
	(l-Sorbose	6	Ketose

Glycerose, which was more attractive than the first group to D. dorsalis and fitted into the second group for D. cucurbitae, was not included in the summary since it is not a single sugar. It is a mixture of d- and l-glyceraldehydes which are aldotrioses with dihydroxyacetone which is a ketotriose. It was thought that the smaller sugar molecules might be more volatile and therefore more attractive. However, these results show that there is no apparent correlation of relative attractiveness with number of carbon atoms in the molecules, nor with the chemical nature of the sugar.

To determine whether caramelization increased attractiveness of sucrose white granulated sugar was caramelized by cautiously heating in an evaporating dish until a deep brown color developed. No effort was made to control the degree of caramelization other than depth of color produced, since such control could only be achieved by careful control of temperature and time. The caramelized sucrose was tested against both white sugar and raw sugar. Results are reported as Test No. 449.

Raw sugar was considerably more attractive than pure sucrose for both species. Caramelization made the sucrose approximately as attractive as the raw sugar for D. dorsalis, but did not significantly increase the attraction of sucrose for D. cucurbitae. Test No. 450 shows results of a second test in which caramelized raw sugar replaces the raw sugar in the previous test.

Olfactometer Test No. 449

Lure	Material
A	Water.
B	Water + sucrose (20%).
C	Water + raw sugar (20%).
D	Water + caramelized sucrose (20%).

Lure	Percent of mean water catch			
	D. dorsalis		D. cucurbitae	
	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	1850.0	2233.3	1550.0	1523.0
C	5425.0	7604.8	5950.0	6967.0
D	6391.7	7304.8	1950.0	2400.0
LSD 5%	1841.3	1550.0	1822.5	1221.0
Mean water catch	4.0	7.0	0.7	1.0

Olfactometer Test No. 450

Lure	Material
A	Water.
B	Water + sucrose (20%).
C	Water + caramelized sucrose (20%).
D	Water + caramelized raw sugar (20%).

Lure	% of mean water catch		Mean water catch	
	D. dorsalis		D. cucurbitae	
	♀	Both sexes	♀	Both sexes
A	100.0	100.0	0.00	0.00
B	662.5	570.6	2.00	4.00
C	6050.0	6017.6	22.67	45.00
D	9387.5	8647.1	21.67	41.33
LSD 5%	1155.8	1099.4	7.04	10.89
Mean water catch	2.7	5.7	0.0	0.0

In this test caramelization has greatly increased the attraction to D. cucurbitae as well as to D. dorsalis. The caramelized raw sugar is considerably better than the caramelized sucrose for D. dorsalis but not for D. cucurbitae. While somewhat inconsistent with respect to D. cucurbitae response, these two tests show that caramelization of sugar introduces new and considerably more powerful attractants, and tend to indicate that the superior attractiveness of raw sugar to sucrose is probably due to impurities other than caramelization products produced during the processing of the raw sugar.

Miscellaneous Olfactometer Tests

As usual, olfactometer tests were made with lure remnants from each field experiment reported herein. Results are reported under Olfactometer Tests No. 451 and 454.

Olfactometer Test No. 451

Lure	Material
A	Raw sugar (20%), vinegar (1.3%), yeast.
B	Water + white sugar (20%) + salicylic acid (1/1000).
C	Water + raw sugar (20%) + salicylic acid (1/1000).
D	Water + salicylic acid (1/1000).

(These were the same lures as used in Field Expt. 92.)

	Percent of A lure mean catch			
	D. dorsalis		D. cucurbitae	
Lure	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	28.2	29.7	37.0	34.0
C	50.0	52.0	22.2	30.0
D	9.6	8.7	9.9	8.0
LSD 5%	20.6	17.6	30.1	16.3
A lure mean catch	180.0	356.3	27.0	50.0

Comparison of the results for D. dorsalis with those from Field Experiment 92 indicate a considerable difference in performance of sugars in the field and olfactometer. It is probable that this is due to distance effect and that sugars are attractive only over a range of a few feet.

Olfactometer Test No. 454

Lure	Material
A	Standard.
B	SM-14 bacteria from original refrigerated agar culture.
C	SM-14 bacteria from strain cultured in 10% soy meal medium with weekly transfers.
D	SM-14 bacteria from strain cultured in 10% soy meal medium with 48-hourly transfers.

	Percent of Standard lure mean catch			
	D. dorsalis		D. cucurbitae	
Lure	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	103.6	104.5	206.9	194.7
C	71.7	74.3	171.6	163.0
D	46.7	46.8	151.0	136.5
LSD 5%	50.2	45.6	76.8	45.9
Mean catch in standard	130.7	280.0	34.0	63.0

These results compare very well with those of Field Experiment 93, indicating that preconditioning of the bacteria to soy meal results in a poorer lure.

A test with methyl eugenol was made in which only melon flies were counted to see if this material has any attraction for melon flies. Results are shown as Olfactometer Test No. 443.

Olfactometer Test No. 443

Lure	Material
A	Water + cotton plugs suspended from neck of traps.
B	Water + cotton plugs containing 1 drop methyl eugenol.

Lure	Percent of A lure mean catch	
	D. cucurbitae	
	Female	Both sexes
A	100.0	100.0
B	483.0	655.6
LSD 5%	335.0	296.0
Mean catch in water	1.0	1.5

It is indicated that methyl eugenol does have some attraction for both sexes of melon flies with more males responding than females. However, the test was run in the cage containing dorsalis, and the rapid entrance of dorsalis males into the B trap may have resulted in some forced entrance of cucurbitae.

A test was made in which various concentrations of clorox were used to oxidize SM-14 lure. All concentrations used were sufficient to remove the color and objectionable odor of the lure. Results of this test are presented as Olfactometer Test No. 462.

Olfactometer Test No. 462

Lure	Material
A	SM-14.
B	SM-14 + 2% clorox.
C	SM-14 + 3% clorox.
D	SM-14 + 4% clorox.
E	SM-14 + 5% clorox.

Lure	Percent of A lure mean catch			
	D. dorsalis		D. cucurbitae	
	♀	Both sexes	♀	Both sexes
A	100.0	100.0	100.0	100.0
B	70.3	66.8	29.9	29.6
C	53.1	47.2	16.6	15.6
D	31.0	25.7	9.1	8.3
E	17.9	17.4	3.5	4.0
LSD 5%	18.8	20.7	20.5	23.8
Mean catch in A lure	48.3	88.3	160.7	252.3

This test indicates that even a concentration of 2% clorox results in loss of attractiveness which is somewhat at variance with earlier olfactometer and field tests which indicated that a concentration of 2% was permissible. Further study of results of oxidation by clorox is indicated.

Line Projects I-a-6-6, I-a-6-7, and I-a-6-8. INACTIVE.