MEDITERRANEAN FRUIT FLY FEMALE ATTRACTANT STUDIES IN SUPPORT OF THE STERILE INSECT TECHNIQUE: TRAPPING EXPERIMENTS CONDUCTED ON THE ISLAND OF CHIOS, GREECE



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Abstract

This paper contains information on a four-year research programme co-ordinated by the International Atomic Energy Agency. The main objective of the programme was to develop a trapping system for females of the Mediterranean fruit fly (medfly), Ceratitis capitata (Diptera: Tephritidae), for practical use in Sterile Insect Technique (SIT) programs and to design and evaluate a trap to obtain eggs from wild female medflies in order to estimate sterility induction in the field population. The experiments were conducted from July to September 1994 -1997 on the island of Chios, Greece, in citrus orchards with low to medium medfly populations. Different trap types and several trap treatments consisting of sex and food based attractants were tested, following a standard coordinated experimental protocol. The most extensively tested were three food based "female" attractants (FA-3), namely ammonium acetate (AA), 1,4 diaminobutane (putrescine) and trimethylamine, all formulated in dispensers lasting one month. These attractants were evaluated in combinations of two (AA + putrescine, termed FA-2) or three (FA-3) dispensers in various traps, including dry (provided with DDVP) or wet (provided with water and 0.01% surfactant) plastic International Pheromone's McPhail traps (IPMT). Among the various traps and treatments tested, the most effective for medfly capture was the wet IPMT, baited with FA-3 attractants. This treatment captured predominantly females and was relatively selective for medflies. In dry IPMT traps, the FA-3 were as effective as the standard 300 ml aqueous solution of 9% of the protein NuLure and 3% borax, but much more medfly selective. Dry IPMT traps were also more selective than wet ones. FA-3 baited wet Tephri traps (a Spanish modification of the McPhail trap), performed somewhat poorer than IPMT traps. Other dry trap types tested were not effective. Additional experiments showed that certain insecticide formulations used in dry traps may have a repellent effect to the flies. Slightly modified wet or dry IPMT traps baited with the FA-3 could be readily used to capture live medfly females. The fertility of these females could later be assessed in cages with appropriate oviposition devises to measure the degree of sterility induced in the field populations by SIT programs.

1. INTRODUCTION

Although several trapping systems have been developed for the Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) [cf. 1, 6, 8, 19], a system selective for females has been unavailable until recently. Such a system could be used for mass trapping and also for capturing females to assess their fertility as a control method to verify effectiveness of Sterile Insect Technique (SIT) through induction of sterility in the wild population. To develop such a system, a research programme co-ordinated by the International Atomic Energy Agency (IAEA) was initiated in 1994. The program included extensive trapping experiments in several countries, using various trap types baited with promising female-targeted attractants.

Here, we report on the standard co-ordinated trapping experiments and on certain results of side experiments which were conducted. The full results are contained in the Annual Reports submitted to the Agency [10-13] and/or have been published elsewhere [16, 17].

2.1. Generalities, experimental area and orchards

The experiments were conducted from July to September 1994- 96 on the island of Chios, Greece. Chios is located in the central Aegean sea, about 10 km off the west coast of Turkey and has a typical Mediterranean climate (Table I). During experimental months, the weather was characterized by moderate to high temperature and humidity, slight to moderate winds usually blowing from the north, and an almost total lack of rainfall (a single rainfall occurred in September 1996). The experiments were conducted in an area located approximately 1 km west of the eastern coast of the island at an altitude of 1-2 m, in the middle of a 20 km² area cultivated mostly with various Citrus spp. trees, especially mandarin (Citrus nobilis Lour.), orange (Citrus sinensis Osbeck) and bitter orange (Citrus aurantium L.). In the same area were other medfly host trees such as apricot (Prunus armeniaca L.), pear (Pyrus communis L.), and fig (Ficus carica L.). Bitter oranges are the most preferred and severely attacked host during June to early July, while figs constitute a much preferred host in which the fly breeds from mid August to late October [15]. More detailed information on the climate, hosts and medfly phenology on Chios have been reported in recent publications [8, 15].

The co-ordinated experiments were conducted in two citrus farms each year (Site 1 and Site 2) which were approximately 100 m long and 100 m wide. The farms were located about 0.5 km apart (in 1994 and 1995) or were adjacent to one another (in 1996 and 1997). Both farms contained mandarin trees as the main culture and a few to several orange, bitter orange and lemon (Citrus limon Burm.) trees planted mostly between the mandarin trees. On the margins of the farms were a few other host trees, such as fig, and certain non host trees. The side experiments were mainly conducted in two other farms: Site 3 having similar size and tree species composition to Sites 1 and 2 and located 0.5 km from them; and Site 4 located about 1 km to the south. Site 4 was smaller (90 m long, 20 m wide) but had a tree species composition similar to the other sites. It is owned by the senior author and has been used extensively in the past for medfly trapping and other studies [e.g. 7, 8, 9, 15, 18].

High populations of adult medflies are usually present in the area from the end of June to early December [15]. The year 1996 was exceptional in that *C. capitata* adult populations remained at undetectable levels during early to mid July and at low levels during August. This was most probably due to the unfavorable weather conditions observed during February and March of that year [15].

During the period of experimentation, the mandarin, orange, and bitter orange trees on all farms bore mostly unripe fruits unsuitable for oviposition and these remained uninfested. Only in the first weeks of July did sweet and bitter orange trees bear a small number of ripe or ripening fruits which were infested by *C. capitata*. The figs in the area matured and were highly infested from early to mid August. Hence, from July to early August the adult population originated from citrus fruits and from August to September from figs.

2.2. Treatments tested and experimental procedures

The trap types and materials used were purchased or provided by the co-ordinating Agency. The co-ordinated experiments were conducted according to a standard experimental protocol. In brief, the experiments consisted in comparing trap catches for each year for eight weeks in up to six trap treatments, in five experimental blocks per site, in a completely randomized block design.

TABLE I. MONTHLY AIR TEMPERATURE, PRECIPITATION, AND RELATIVE HUMIDITY RECORDED DURING THE YEARS 1991 - 93 AT A METEOROLOGICAL STATION LOCATED AT A DISTANCE OF ABOUT 1 - 2 KM FROM THE EXPERIMENTAL ORCHARDS

	Temperature ^O C		_	Mean	
Month	Avg. Avg.		- /g.	Precipitation	
		Min.	Max	mm	
Jan.	8.7	7.7	9.2	57.0	74.0
Feb.	8.4	5.0	11.2	66.3	72.3
Mar.	11.0	6.8	14.3	57.7	75.0
Apr.	14.2	9.3	18.0	48.3	77.0
May	17.5	13.1	21.3	19.0	74.7
June	22.9	17.8	27.0	0.0	69.3
July	24.8	20.4	28.8	0.0	57.3
Aug.	24.5	22.0	29.8	0.0	57.3
Sept.	20.7	17.0	26.0	0.0	63.3
Oct.	17.2	14.4	23.4	16.3	69.3
Nov.	13.7	10.0	16.9	102.3	73.0
Dec.	9.9	7.0	12.1	121.3	71.0

2.2.1. 1994 experiments

Site 1 and Site 2 (Co-ordinated experiments): The experiments were conducted from July 7 to September 9. At each site, twenty traps were deployed in five blocks of four traps each. Each block consisted of the following two treatments, replicated two times within each block. 1) Trimedlure (TML) baited Jackson traps (JT). These traps contained a white sticky insert and were baited with a TML impregnated plug ("Magnet" Trimedlure 70-0 Plug, 2 g active, supplied by AgriSense Ltd., Fresno, CA 93722, USA), placed in a special plastic basket hung inside the trap, in the middle of the horizontal wire hanger (JT, TML). 2) Closed Bottom Dry Trap (CBDT), FA-2. The CBDT [2, 4], was a dry trap consisting of a fluorescent green painted plastic sheet that formed a 15 cm long, 9 cm diameter, cylindrical container having three 2 cm diameter holes for insect access. The bottom and the top of the trap consisted of two removable plastic petri dishes. The traps were baited with two dispensers containing two synergistically acting, food based females attractants (FA-2), one dispenser with ammonium acetate (AA)(BioLure, Consep Inc., Bend, OR, USA), and the other with 1,4 diaminobutane (putrescine, P), prepared using a polyethylene membrane system [2]. The traps also contained a small toxicant strip of the insecticide methomyl to kill the attracted insects. (CBDT, FA-2, dry).

Site 3 (Side experiment 1): At this site, a total of 40 traps in five blocks (lines) of eight traps each were deployed on July 7, and were checked twice per week until September 9. Each block consisted of the following four treatments replicated twice within each block: 1) JT, TML; 2) CBDT, FA-2, dry; 3) CBDT, FA-2, wet (The CBDT traps, in addition to the FA-2 dispensers, were provided with a small plastic container filled with water and replaced as needed. This container was about 0.5x2x4 cm and was provided with a 3 mm diameter hole for the evaporation of water); and 4) IPMT, NU+B (International Pheromone's McPhail traps, baited with the standard 300 ml of an aqueous solution of 9% NuLure and 3% borax).

Site 4 (Side experiment 2): Field cage experiments [13] showed that wet (provided with water) IPMT traps baited with FA-2 were much more effective than dry IPMT or CBDT traps baited with the same attractants [13]. Apparently the presence of water in this trap enhanced its effectiveness. Other field cage experiments demonstrated a strong attraction of female medflies to orange juice [13, 14].

The purpose of this experiment was to compare the attractiveness of the FA-2 attractants in IPMT traps provided with water to a known effective attractant, NuLure, and with orange juice. The selectivity of these attractants and their impact to certain beneficial insects was also evaluated.

The experiment was conducted between July 28 and August 6. Two blocks of four different treatments each, were deployed in mandarin and orange trees. The distance between the traps was about 15 m. We tested the following treatments: 1) IPMT, NU+B; 2) IPMT, NU+B and FA-2 (in addition to the NU+B, two dispensers with the FA-2 were attached on the interior sides of the upper part of the trap); 3) IPMT, FA-2, wet (the trap bottom was filled with water); and 4) IPMT, orange juice (the bottom of the trap was filled with 300 ml of commercial pure orange juice of the local trade name "Amita").

The traps were checked daily and their position within each block re-randomized. NU+B and orange juice were renewed every 2-3 days. The total daily captures of the two traps of each treatment were considered a replicate.

2.2.2. 1995 experiments

Because, during the 1994 experiments the CBDT, FA-2 proved ineffective under the conditions of Chios, this trap type was excluded from the 1995 experiments.

Site 1 and Site 2 (Co-ordinated experiments): The experiments were conducted from July 7 to September 1. At both sites, the following five treatments were tested: 1) JT, TML; 2) OBDT, FA-2 (the OBDT trap was an opaque, green, open-bottom cylinder (similar to CBDT), provided with a yellow sticky paper insert with a pre-applied new, special adhesive instead of the standard Bird Tanglefoot [5]); 3) IPMT, FA-2, dry (the IPMT was baited with FA-2 and included two small strips impregnated with the insecticide methomyl as a killing agent.); 4) IPMT, FA-2, wet (in the bottom part of the IPMT trap, 300 ml of water were added to enhance the effectiveness of the attractants and kill the attracted insects). In the first trap check, we observed that many insects did not drown into the water. To break the surface tension of the water and facilitate insect drowning, during each of the following trap services 0.01% of the surfactant Agral-90 (alkylphenolethoxylate, Prometheus Ldt. Greece) was added in each trap.); and 5) IPMT, NU+B.

Site 4 (Side experiment). The initial trap catch data from the coordinated experiments showed that treatments 4 and 5 were the best. To gain more data on these treatments, a two-choice test was conducted in Site 4 between July 27 and August 2 involving five pairs of traps of the treatments 4 and 5 described above. The two traps in a pair were suspended at about 5 m from one another and at 5-10 m from other pairs. They were checked every second day (3 times in total).

2.2.3. 1996 experiments

Data from the 1995 experiments showed that the OBDT was ineffective under the conditions of Chios, and this trap type was excluded from the 1996 experiments. This year we mainly evaluated the FA-2 attractants alone or in combination with trimethylamine (TMA). This compound acts synergistically with the FA-2 attractants and is formulated in dispensers

lasting one month (BioLure Consep Inc., Bend, OR, USA). The attractants were evaluated in combinations of two (FA-2) or all three (FA-3).

The co-ordinated experiments were conducted from July 23 to September 17. The following treatments were tested: 1) JT (TML); 2) IPMT, FA-2, wet (in wet traps, 0.01% of a wetting agent (Triton X -100, Union Carbide, Danbury, Connecticut, USA) was added); 3) IPMT, FA-3, wet (water and surfactant were added as described above); 4) IPMT, NU+B; 5) IPMT, FA-2 and NU+B; 6) Tephri, NU+B (The Tephri trap, a Spanish trap, was a cylindrical, smaller modification of the McPhail trap (Agro Alcoy, Alcoy, Spain). Apart from having a bottom opening, it has four lateral holes of 2 cm diameter in the upper part. It was baited with 250 ml aqueous solution of NU+B.); and 7) Frutect (The Frutect trap (RonPal Ltd., Rishpon, Israel), was a yellow sticky coated 40x40 cm panel with a dark red, 12.5 cm diameter sphere, attached in the center of the panel. The sphere was pre-baited with a long lasting (evaporating) proteinaceous attractant placed in its interior, which was dispersed to the exterior via a sponge which formed a 1.0 cm band on the outside periphery of the sphere.)

The treatments tested were not the same in the two sites of the standard coordinated experiment.

Site 1. The following six of the above treatments were tested: 1) JT, TML; 2) IPMT, FA-2, wet; 3) IPMT, FA-3, wet; 4) Tephri, NU+B; 5) IPMT, NU+B; and 6) Frutect trap.

Site 2. The following five of the above treatments were tested: 1) JT, TML; 2) IPMT, FA-2, wet; 3) IPMT, FA-3, wet; 4) IPMT, NU+B and FA-2; and 5) IPMT, NU+B.

Site 4 (Side experiment). The FA-2 and FA-3 treatments, which were the best in the coordinated experiments, were further evaluated in a two-choice test conducted between August 25 and September 15 at Site 4, using five pairs of traps. The traps in a pair were suspended at about 5 m from one another and 5-10 m from other traps. They were checked every 2-3 days (8 times in total). After a check, the positions of the traps in a pair were interchanged. Two trap-checks of a pair were considered as replicate.

2.2.4. 1997 experiments

The FA-3 attractants in McPhail type traps provided with water were found to be the most effective treatments during 1996 [3, 16]. To gain more information on the effectiveness of these attractants we tested them again in wet and dry IPMT and Tephri traps in the standard coordinated experiments conducted from July 7 to September 1. In the water of IPMT traps, 2 drops (concentration about 0.01%) of the surfactant Triton were added. In the dry traps as well as in the wet Tephri traps, we placed a 15x25x5 mm piece of a DDVP plug (Biological Control Systems, provided by IAEA). The following treatments were tested in both Sites: 1) JT, TML (only two such traps at Site 2); 2) IPMT, NU+B; 3) IPMT, FA-3, wet (as in 1996); 4) IPMT, FA-3, dry with DDVP; 5) Tephri, FA-3, dry with DDVP (the DDVP plug was placed in the special basket of upper part of the trap); and 6) Tephri, FA-3, wet with DDVP (250 ml of water was placed in the bottom of the trap).

2.2.5. Experimental procedures

At all sites and in all years, the traps were hung on citrus trees (mostly mandarin), at a distance of about 10-20 m between the lines (blocks) and 10-20 m within blocks (the distance depending on the number of treatments), with all treatments randomly distributed within each block. They were checked twice a week in a regular schedule for eight consecutive weeks. In each check, the position of the traps within each block was re-randomized.

TML dispensers were renewed every 15 days whereas the FA-2 and FA-3 dispensers and the plugs with the DDVP were renewed only once, one month after the initiation of the

experiment. Frutect traps (used in 1996) were replaced by new ones after one month. After every second check, the liquid in the IPMT and Tephri traps was replaced with fresh solution. Sticky inserts were renewed as often as needed. The pH of the NuLure solution was checked several times at the beginning and the end of each two-check periods, and was always found to be about 8.0.

At each check, we recorded the number and sex of medflies captured and the total number of other insects captured. In certain treatments, we also recorded the number of individuals of certain other important insect species. These included: the olive fruit fly, Bactrocera oleae (Gmelin); the celery fly, Euleia (Philophylla) heraclei L.; a leaf miner of celery (Tephritidae); Chrysopa sp. (Chrysopidae); and yellowjacket wasps, Vespula germanica (F.). Evidence of medfly predation by yellowjackets was recorded. At regular intervals (usually every second trap check) samples of medfly females from certain treatments were dissected and the presence of mature oocytes determined. The few females showing degenerated or otherwise decomposed oocytes were not considered.

Data from the co-ordinated experiments are given in the tables as mean numbers of captures per trap per day. However, for the statistical analysis, data were grouped in captures of two weeks per trap per treatment (N=20), transformed to $\ln (x + 0.5)$ and submitted to Analysis of Variance. Means were separated according to Tukey's HSD test. When two treatments were compared, data were analyzed using Student's t test.

3. RESULTS AND DISCUSSION

3.1. 1994 experiments

The numbers of medflies captured during the entire experimental period in Sites 1 and 2 of the co-ordinated and in Site 3 of the side experiment 1, are given in Table II. CBDT traps with or without a water reservoir captured about twelve times fewer medflies than the NU+B baited IPMT traps, and in a similar sex ratio (about two times more females than males). Compared to the JT, TML, the CBDT traps were about 30-40 times less effective in total captures.

These results may not reflect the actual efficacy of the FA-2 attractants because the attracted insects in the CBDT traps were subjected to extremely high predation by ants and especially by yellowjacket wasps. However, in the very first replicates, no apparent signs of predation were observed, probably because the wasps had not located the traps yet or had not learned to find and predate them easily. If we exclude predation by taking into account the results pertaining to the first trap check only (Table III), then the CBDT traps again prove to be much less efficacious than the traditional traps.

The addition of a water reservoir to enhance humidity inside the CBDT traps had no effect on trap captures (Tables II and III). However, it should be considered that the water reservoir used had a very small (3 mm diameter) opening, allowing only a limited amount of water to evaporate. This was obvious by the fact that water replenishment in the reservoirs was usually not necessary during most of the trap checks. In additional experiments conducted in field cages using the FA-2 attractants in IPMT traps, the presence of water vapor enhanced the efficacy of the FA-2 attractants considerably [13]. Concerning the medfly selectivity of the traps, no conclusions can be made from the results of Table II, since the treatments tested are not comparable.

The results of the side experiment 2 conducted at Site 4, are given in Table IV. Medfly captures were similar in all treatments, except for the combination of NU+B with FA-2. In that case the captures were higher (although not statistically different) indicating a possible synergistic effect of NU+B with the FA-2 attractants. Remarkable, also, was the high

TABLE II. MEAN NUMBERS OF MEDFLIES AND OTHER INSECTS CAPTURED PER TRAP CHECK IN THE 10 TRAPS OF EACH TREATMENT (5 BLOCKS X 2 TRAPS OF EACH TREATMENT/BLOCK), DURING THE ENTIRE EXPERIMENTAL PERIOD OF 1994, IN THE STANDARD, CO-ORDINATED EXPERIMENTS AND IN THE SIDE EXPERIMENT 1. THE NUMBER OF TRAP CHECKS WAS 22 PER SITE FOR THE STANDARD CO-ORDINATED EXPERIMENT AND 20 FOR THE SIDE EXPERIMENT.

Treatments ^a	Num	Number of medflies captured ^b			
	Males	Females	Total	Insects ^c	
Standard experim	ient				
Site 1					
JT, TML	14.2	0.006	14.3	-	
CBDT, FA-2	0.14	0.3	0.43	1.99	
Site 2					
JT, TML	12.4	0.01	12.41	-	
CBDT, FA-2	0.1	0.29	0.4	4.2	
Side experiment 1					
JT, TML	7.8	0.007	7.8	- -	
CBDT, FA-2	0.08	0.2	0.3	5.8	
CBDT, FA-2, wet	0.08	0.2	0.3	5.9	
IPMT, NU+B	1.1	2.2	3.3	16.2	

^a For treatments see text.

TABLE III. NUMBER OF MEDFLIES AND OTHER INSECTS CAPTURED IN THE 10 TRAPS OF EACH TREATMENT DURING THE FIRST TRAP CHECK OF 1994, IN THE STANDARD CO-ORDINATED EXPERIMENTS (JULY 12), AND IN THE SIDE EXPERIMENT 1 (JULY 11). PREDATION BY YELLOWJACKET WASPS WAS NOT APPARENT DURING THE FIRST CHECK.

Treatments		Number of medflies	Other				
	Males	Females	Total	insects ^b			
Standard experiment							
Site 1							
JT, TML	144.2a	0.0a	144.2a	-			
CBDT, FA-2	3.9b	6.1b	9.7b	83.0			
Site 2							
JT, TML	105.0a	0.1a	105.3a	-			
CBDT, FA-2	1.4b	2.8b	4.2a	118.2			
Side experiment 1							
JT, TML	65.7a	0.0b	65.7a	-			
CBDT, FA-2	0.4c	0.6b	1.0c	119.9b			
CBDT, FA-2, wet	0.5c	0.4b	0.9c	130.1b			
IPMT, NU+B	5.3b	6.9a	12.2b	272.9a			

^a For statistical analysis see text.

^b Because of high predation in CBDT, statistical analysis was not possible.

^c The few other insects captured by Jackson traps were not counted.

^b The few other insects captured by Jackson traps were not counted.

attractiveness of commercial orange juice, which was comparable to both that of NU+B and FA-2. Orange juice is inexpensive and readily available for implementation in medfly trapping. However, it was quite unselective, attracting a very large number of a wide variety of other insects species, including beneficials such as chrysopid predators. Isolation of the attractive compounds of orange juice could possibly lead to the detection of more selective medfly attractants. The rather high number of other insects captured by FA-2 baited traps in this experiment was due to the fact that these traps captured large numbers of an unidentified brown colored fly species. When used in IPMT traps provided with only water, the FA-2 attractants were of similar effectiveness with NU+B. Concerning female selectivity of the FA-2, this did not differ from NU+B or orange juice baited traps, the sex ratio being about 1:1.

TABLE IV. MEDFLIES AND OTHER INSECTS CAPTURED IN IPMT TRAPS, AVERAGE NUMBER OF INSECTS CAPTURED/DAY DURING TEN CONSECUTIVE DAYS (10 REPLICATES) BY TWO TRAPS OF EACH TREATMENT, IN THE SIDE EXPERIMENT 2 - 1994

Treatments		Other		
	Males	Females	Total	insects
NU+B	8.9a	9.6a	18.5a	95.6a
NU+B & FA-2	11.6a	1 8.0a	29.6a	254.4a
FA-2, wet	10.1a	11.3a	21.4a	197.5a
Orange juice	10.5a	12.8a	23.3a	619.8b

^a Means in each column followed by the same letter are not significantly different at the 0.05 level, Tukey's HSD test.

3.2. 1995 experiments

3.2.1. Medfly captures

The mean numbers of medflies and other insects captured during the entire experimental period in the co-ordinated experiments and the side experiment are given in Table V. The OBDT traps were ineffective, but showed somewhat higher captures than the dry, FA-2 baited IPMT traps. In contrast, the captures of medflies (especially of females) were quite high in the treatments IPMT, FA-2, wet, and IPMT, NU+B. At Site 1 and in the side experiment, female captures in treatment IPMT, FA-2, wet, were somehow higher than in IPMT, NU+B, whereas at Site 2 the captures in IPMT, NU+B, were about 40% higher than in IPMT, FA-2, wet. Apparently, the presence of humidity inside the IPMT traps is a decisive factor affecting effectiveness.

IPMT, FA-2, wet, traps were generally more female selective than IPMT, NU+B traps. The lack of humidity inside the dry traps, either the OBDT or the IPMT, FA-2, dry, is apparently one of the reasons for their inefficiency. In 1994 experiments, the sex ratio in NU+B baited or FA-2 baited IPMT traps was about 1:1 or slightly in favor of females. In 1995, however, more females were captured.

Dissections of samples of females captured in the side experiment (July 27 to August 2) showed that from a total of 168 females captured by the IPMT, FA-2,wet traps, 76.2% contained mature oocytes as compared to 68.5% out of 149 females captured by the IPMT, NU+B traps. In samples of 75 females per treatment from the standard coordinated

TABLE V. MEAN NUMBERS OF MEDFLIES AND OTHER INSECTS CAPTURED/TRAP/DAY IN THE TWO SITES OF THE CO-ORDINATED EXPERIMENT AND IN THE SIDE EXPERIMENT DURING 1995. IN THE STANDARD CO-ORDINATED EXPERIMENTS, THERE WERE FIVE TRAPS PER SITE PER TREATMENT AND THEY WERE CHECKED 16 TIMES DURING THE ENTIRE EXPERIMENTAL PERIOD. IN THE SIDE EXPERIMENT, FIVE TRAPS OF EACH TREATMENT WERE USED AND THEY WERE CHECKED THREE TIMES.

Traps and	Mean number of medflies captured ^b			Sex ratio	Mean no.° other
Treatments ^a	Females	Females Males		(F:M)	insects captured
Site 1					
JT, TML	0.007c	11. 171a	11.178d	-	-
OBDT, FA-2	0.011c	0.000c	0.011c	-	0.451c
IPMT, FA-2, dry	1.454b	0.033c	1.4 8 7b	44.4	2.687b
IPMT, FA-2, wet	11.444a	0.956b	12.407a	11.9	12.400a
IPMT, NU+B	10.153a	1.258b	11.411a	8.1	14.535a
Site 2					
JT, TML	0.105d	29.571a	29.676ab	-	-
OBDT, FA-2	0.429c	0.007e	0.436d	-	0.382d
IPMT, FA-2, dry	5.116b	0.135d	5.250c	35.2	4.433c
IPMT, FA-2, wet	18.069a	1.553c	19.622b	11.7	21.451b
IPMT, NU+B	29.418a	4.855b	34.273a	6.1	37.462a
Side experiment					
IPMT, FA-2, wet	12.00a	4.60a	16.60a	2.6	18.73b
IPMT, NU+B	10.56a	6.13a	_16.70a	1.7	38.43a

^a For treatments, see text.

experiment (Site 2) taken at August 25, 47.2% of those captured by the IPMT, FA-2,wet traps contained mature oocytes, as compared to 32.4% of those captured in the IPMT, NU+B traps. These results suggest that

both treatments capture mature as well as immature females, in relative proportions that change with the season according to the composition of the adult population.

3.2.2. Other insects captured

As shown in Table V, the IPMT, FA-2, wet traps were slightly to much more medfly selective than the IPMT, NU+B ones. Also, they captured lower numbers of the beneficial Chrysopa sp., the generalist predator V. germanica, and the very important olive pest, B. oleae (olive fruit fly), which was present in high numbers in the citrus orchards, most probably foraging for adult food. These results show that the FA-2 attractants are more medfly selective than NU+B, attracting fewer non-target insect species.

The results concerning maturity of captured females in treatments FA-2, FA-3 and NU+B showed, respectively, 80.8, 80.7 and 73.5% females with mature oocytes and were not statistically different (c²). These results were similar to those obtained in 1995 for FA-2 and NU+B.

^b For statistical analysis, see text.

^c The few other insects captured by Jackson traps were not counted.

3.3. 1996 experiments

3.3.1. Medfly captures

The mean numbers of medflies and other insects captured during the entire experimental period at the two sites of the coordinated experiment and the one side experiment are given in Table VI. As can be seen from these results, in all cases and for females as well as for total captures, wet IPMT traps baited with FA-3 were, by far, the most attractive, followed by FA-2 baited traps. In contrast to the findings of previous year, FA-2 baited traps were more attractive than IPMT, NU+B traps. The increase in captures by the combination of NU+B and FA-2 suggested by our 1994 side experiments was only in part confirmed (Site-2, Treatment 4).

Tephri traps without insecticide, baited with NU+B (Site 1, treatment 4) were 3 - 4 times inferior to similarly baited IPMT traps, whereas Frutect traps (Site 1, treatment 6) captured as many medflies as the NU+B baited IPMT traps. However, this trap type has several disadvantages (laborious service, capture of large numbers of beneficial insects) which render it unsuitable for use and maybe even dangerous for the agro-ecosystem. Jackson traps baited with TML were slightly more attractive for males than FA-3 baited IPMT traps.

In addition to being highly attractive, FA-3 traps (as also FA-2 baited ones), were more female selective than the other trap systems tested, capturing 2 - 4 times more females than

TABLE VI. MEAN NUMBERS OF MEDFLIES AND OTHER INSECTS CAPTURED PER TRAP PER DAY AT THE TWO SITES OF THE STANDARD, CO-ORDINATED EXPERIMENTS, AND IN ONE SIDE EXPERIMENT DURING 1996

Traps and	Mean number of medflies captured ^b			Sex ratio	Mean no.c
Treatments ^a	Females	Males	Total	(F:M)	other insects captured
Site 1					
JT, TML	0.003e	1.13a	1.14bc	0.003	0.9a
IPMT, FA-2, wet	0.69b	0.33ab	1.01ab	2.13	14.2c
IPMT, FA-3, wet	1.69a	0.77a	2.46a	2.19	4.3b
Tephri, NU+B	0.06d	0.08c	0.15d	0.75	5.3b
IPMT, NU+B	0.32c	0.19c	0.51c	1.73	10.8c
Frutect	0.29c	0.26c	0.55c	1.2	4.7b
Site 2					
JT, TML	0.00d	0.35a	0.35c	0.0	0.05c
IPMT, FA-2, wet	0.41ab	0.09b	0.51b	4.5	12.3a
IPMT, FA-3, wet	0.98a	0.24a	1.21a	4.1	4.3b
IPMT, NU+B & FA-2	0.42c	0.12ab	0.54b	3.6	15.3a
IPMT, NU+B	0.12c	0.05b	0.18c	2.3	9.1a
Side experiment					
IPMT, FA-2, wet	2.15a	0.85a	3.0a	2.5	10.5a
IPMT, FA-3, wet	6.66b	2.15b	8.8b	3.1	4.7b

^a For treatments, see text.

^b For statistical analysis, see text.

^c Many small insects (parasitoids etc.) captured by Frutect traps were not counted.

males. These sex ratios are inferior to those observed in 1995 (for FA-2) but the same is true for NU+B. This discrepancy might be due to the differences in the population size and composition between the two years. FA-3 baited traps performed better than FA-2 baited ones throughout the experimental period in all experiments and apparently are suitable also for early detection of the population presence as shown by the trap captures in the first check days, when the population was very low. For example, during the first trap check, FA-3 baited traps captured 5 - 7 times more medflies than the JT, TML.

3.3.2. Other insects captured

The results concerning other insects captured (Table VI) clearly show that from the most promising treatments, the FA-3 baited IPMT traps were, by far, the most medfly selective, capturing up to 4 times fewer non-target insects than the FA-2 baited traps which were the next less selective. Tephri traps (without insecticide), baited with NU+B captured not only fewer medflies than NU+B-baited IPMT traps, but also fewer non-target insects. The Frutect trap, apart from the numbers given in the tables, captured a large number of small insects (probably parasitoids), as well as several lady beetles (Coccinellidae) which are predators of the citrus scale and other harmful homopterans. Hence, this trap type can be considered as detrimental to the citrus agro-ecosystem and its use should be avoided in citrus plantations.

3.4. 1997 experiments

3.4.1. Medfly captures

The mean numbers of medflies and other insects captured during the entire experimental period in the two sites of the coordinated experiment are given in Table VII. At both sites and for females as well as for total captures, wet IPMT traps baited with FA-3 were the most attractive followed by FA-3 baited wet Tephri traps. Since, in FA-3 baited wet IPMT traps stronger yellowjacket predation was observed than in other treatments, the real effectiveness of this treatment is actually higher than that appearing in the table. Similar was the situation for the males, with the exception of the JT, TML which, this year, captured many more males than the other treatments. Large differences in male captures were observed during the first four weeks of experimentation, whereas, later in the season, the differences in male captures between FA-3 baited IPMT wet traps and JT traps converged. In 1996, FA-3 baited IPMT traps captured slightly fewer males than TML baited JT traps. This may be due to the fact that, in 1996, trap installation coincided with the beginning of the fly activity, whereas, in 1997, by the time the experiments were initiated, the population was already high. Dry traps (IPMT or Tephri), captured similar numbers of medflies but represented about half of those captured by wet traps. NU+B baited IPMT traps captured almost as many flies of both sexes as did dry traps. In additional experiments [12], we found that the DDVP used in the traps of the coordinated experiments had a certain repellency to the flies. Hence, the real effectiveness of dry traps was actually higher than that shown in the table. Other side experiments conducted without using insecticide showed that FA-3 baited dry IPMT traps actually captured about 74% of the females compared to similarly baited wet ones [12]. It is interesting to note that wet Tephri traps at both sites captured fewer, but not significantly different, numbers of medflies than did wet IPMT traps.

FA-3 baited wet IPMT traps this year captured significantly more mature females (79.4%) (c² test) than did NU+B baited traps (71.7%), but the difference between the two treatments was not high (7.7%).

TABLE VII. MEAN NUMBERS OF MEDFLIES AND OTHER INSECTS CAPTURED PER TRAP PER DAY IN THE TWO SITES OF THE STANDARD, CO-ORDINATED EXPERIMENTS DURING 1997

Traps and	Mean nu	Mean number of medflies captured ^b			Mean no. other
Treatments ^a	Females	Males	Total	F:M	insects captured
Site 1					
JT, TML	0.003d	6.8a	6.8a	0.0005	0.11f
IPMT, NU+ B	1.3c	1.2c	2.5c	1.1	49.2a
IPMT, FA-3, wet	3.0a	1.9b	4.9a	1.6	10.2b
IPMT, FA-3, dry	1.4b	0.9c	2.3bc	1.5	2.3d
Tephri, FA-3, dry	1.4b	0.8c	2.2bc	1.7	1.2e
Tephri, FA-3, wet	2.4ab	1.7bc	4.1 a b	1.4	6.3c
Site 2					
JT, TML°	(0.0)	(9.1)	(9.1)	-	(0.07)
IPMT, NU+B	2.7d	2.2c	4.5c	1.2	24.4a
IPMT, FA-3, wet	5.3a	3.2a	8.5a	1.6	5.9b
IPMT, FA-3, dry	2.9bc	2.1ab	5.1ab	1.4	1.5 d
Tephri, FA-3, dry	2.1cd	1.3bc	3.4bc	1.6	0.7e
Tephri, FA-3, wet	3.1 a b	1.9bc	5.0b	1.6	3.4c

^a For treatments, see text.

3.4.2. Other insects captured

The results concerning other insects captured are presented in Table VII and clearly show that the FA-3 baited IPMT traps were much more medfly selective than NU+B baited traps, and dry traps were much more selective than wet traps. NU+B baited IPMT traps were, by far, more attractive to the olive fruit fly, and FA-3 baited IPMT traps and NU+B baited ones captured similar numbers of celery flies. During spring and early summer of this year, citrus trees were highly infested by aphids and, as a result, high populations of beneficials such as *Chrysopa sp*. were present in the orchards. NU+B baited IPMT traps captured large numbers of these beneficials, hence were detrimental to the biological equilibrium of the agroecosystem, while FA-3 baited traps were not. Large to moderate numbers of yellowjackets were captured only by NU+B baited traps.

4. CONCLUSIONS

Our findings show that the newly developed, dry CBDT and OBDT traps [2, 4, 5] baited with the two synergistically acting attractants, (FA-2), were ineffective, at least under the summer field conditions of Chios (moderate to high temperature, moderate to strong wind, low to moderate relative humidity, high predation). In contrast, when the FA-2 attractants were used in appropriate traps (such as wet IPMT traps) they were as attractive and female selective as the traditional NU+B baited traps but more medfly selective. Effectiveness of FA-

^b For statistical analysis, see text.

^c Data derived from only two traps (results not included in the statistical analysis).

2 was increased considerably by addition of TMA, a third synergistically acting attractant. The IPMT trap baited with all three attractants, water, and a surfactant was found to be the most effective, female targeted trapping system existing today. In comparison to other traps tested, it captured the highest total number of medflies, the lowest total number of other insect species, and the highest female to male ratio. The wet Tephri trap baited with FA-3 and provided with a DDVP plug followed in efficacy. Dry IPMT traps baited with the FA-3 were about 20% less effective than wet ones but much more medfly selective. Dry traps, in general, are much easier to service (no need to replace the water) and are considered more practical to use than wet ones, especially for mass trapping purposes. Special attention should be directed to the selection of a proper insecticide for dry traps, since our results showed that some formulations may be repellent to the flies. Wet or dry IPMT traps, with slight modifications [17], could be readily used to capture live females in order to assess their fertility as a method of verifying success of SIT programs.

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REFERENCES

- [1] BAKER, P.S., et al., Improvement of attractant dispensing systems for the Mediterranean fruit fly (Diptera: Tephritidae) sterile release program in Chiapas, Mexico, J. Econ. Entomol. **81** (1988) 1068-1072.
- [2] EPSKY, N.D., et al., Visual and chemical cue interactions in a dry trap with food-based synthetic attractant for *Ceratitis capitata* and *Anastrepha ludens* (Diptera: Tephritidae), Environ. Entomol. **24** (1995) 1387-1395.
- [3] EPSKY, N.D., et al., Field evaluation of female-targeted systems for *Ceratitis capitata* (Diptera: Tephritidae) in seven countries, J. Econ. Entomol. (1998) (in press).
- [4] HEATH, R.R., et al., Development of a dry plastic insect trap with food-based synthetic attractant for the Mediterranean and Mexican fruit flies (Diptera: Tephritidae), J. Econ. Entomol. 88 (1995) 1307-1315.
- [5] HEATH, R.R., et al., Systems to monitor and suppress *Ceratitis capitata* (Diptera: Tephritidae) populations, Florida Entomol. **79** (1996) 144-153.
- [6] HENDRICHS, J., et al., Behaviour of female and male Mediterranean fruit flies, Ceratitis capitata in and around Jackson traps placed on fruiting host trees, Insect Sci. Appl. 10 (1989) 285-294.

- [7] HENDRICHS, J., et al., Sex differences in movement between natural feeding and mating sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae), Oecologia 86 (1991) 223-23.
- [8] KATSOYANNOS, B.I., Evaluation of Mediterranean fruit-fly traps for use in the sterile insect-technique programmes, J. Appl. Entomol. 118 (1994) 442-452.
- [9] KATSOYANNOS, B.I., HENDRICHS, J., Food bait enhancement of fruit mimics to attract Mediterranean fruit fly females, J. Appl. Entomol. 119 (1995) 211 213.
- [10] KATSOYANNOS, B.I., KOULOUSSIS, N., Medfly female attractant studies in support of the Sterile Insect Technique. Experiments conducted during summer 1995 in Chios, Greece. Annual Report (1995) IAEA, 42 pp.
- [11] KATSOYANNOS, B.I., PAPADOPOULOS, N., Medfly female attractant studies in support of the Sterile Insect Technique Experiments conducted during summer 1996 in Chios, Greece. Annual Report (1996) IAEA, 49 pp.
- [12] KATSOYANNOS, B I., PAPADOPOULOS, N., Medfly female attractant studies in support of the Sterile Insect Technique Experiments conducted during summer 1997 in Chios, Greece. Annual Report (1997) IAEA, 30 pp.
- [13] KATSOYANNOS, B.I., et al., Medfly female attractant studies in support of the Sterile Insect Technique. Experiments conducted during summer 1997 in Chios, Greece. Annual Report (1994) IAEA, 35 pp.
- [14] KATSOYANNOS, B.I., et al., Response of *Ceratitis capitata* to citrus chemicals under semi-natural conditions, Entomol. Exp. Appl. **82** (1997) 181 -188.
- [15] KATSOYANNOS, B.I., et al., Seasonal and annual occurrence of Mediterranean fruit flies (Diptera: Tephritidae) in Chios island, Greece: Differences between two neighboring citrus orchards, Ann. Entomol Soc. Am. 26 (1998) (in press).
- [16] KATSOYANNOS, B.I., et al., Field evaluation of Mediterranean fruit fly (Diptera: Tephritidae) female selective attractants for use in monitoring, mass trapping and sterile insect technique programs, J. Econ. Entomol. (1998) (in press).
- [17] KATSOYANNOS, B.I., et al., Development of a system of assessing the fertility of *Ceratitis capitata* in SIT programs, J. Econ. Entomol. (1998)(submitted).
- [18] PAPAJ, D.R., et al., Use of fruit wounds in oviposition by Mediterranean fruit flies, Entomol. Exp. Appl. 53 (1989) 203-209.
- [19] VILLEDA, M.P., et al., Mediterranean fruit fly *Ceratitis capitata*: Behavior in nature in relation to different Jackson traps, Florida Entomol. **71** (1988) 154 -162.