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MATING PERFORMANCE AND SPATIAL DISTRIBUTION OF MEDFLY (DIPTERA: TEPHRITIDAE) WHITE PUPA GENETIC SEXING MALES UNDER FIELD CAGE CONDITIONS

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ABSTRACT

In mixed populations of wild and males from T (Y;5) 1-61 white female pupa genetic sexing strain of *Ceratitis capitata* (Wiedemann), sterilized males of the genetic sexing strain expressed calling, lekking and mating compatibility with their wild counterparts. Nevertheless, their mating performance was most of time poor to very poor. For example, in a series of studies from June-October 1996, only 0- about ⅓ of expected matings (based on insect ratios) by genetic sexing sterilized males was recorded. Similar results were observed in the other years of this study. No substantial differences between gen. sex. male × wild female and wild male × wild female type copulations were detected in spatial distribution of couples in copula on the orange tree. Over 83% of both mating types were detected on the underside leaf surface.

Key Words: *Ceratitis capitata*, medfly, sexing strain, quality control, competitiveness

RESUMEN

En poblaciones mezcladas de machos salvajes y machos provenientes de pupas blancas de hembras T (Y;5) 1-61 de razas genéticamente sexadas de *Ceratitis capitata* (Wiedemann), los machos esterilizados de razas genéticamente sexadas expresaron la capacidad de llamamiento, la acción de seleccionar un lugar de apareamiento y apareamiento con su contraparte salvaje. No obstante, su capacidad de apareamiento fue en la mayoría de las veces de pobre a muy pobre, por ejemplo, en una serie de estudios llevados a cabo entre junio y octubre de 1996, solamente entre 0 y ⅓ de los apareamientos esperados (basados en proporciones de insectos) por machos estériles genéticamente sexados fueron registrados. Resultados similares se observaron en los otros años de este estudio. No se detectaron diferencias substanciales entre el tipo de copulación entre machos genéticamente sexados × hembras salvajes y machos salvajes × hembras salvajes en las distribuciones espaciales de parejas en cópulas sobre árboles de naranja. Mas del 83% de ambos tipos de apareamiento se detectaron en el lado inferior de la superficie de las hojas.

Artificial mass-rearing and sterilization may affect field effectiveness of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), drastically. Mass rearing conditions, selection genetic changes, irradiation and sterile insect technique (SIT) handling procedures may affect the vigor and behavior of sterile males and reduce strikingly their mating performance with the wild population in the field (Economopoulos 1996). Furthermore, recent evidence suggests development of behavioral resistance in the wild flies against sterile flies (McInnis et al. 1996). The limited success SIT had so far on several key insect pests was connected to a large degree with the quality of released insects. This became evident from the initial steps of the methodology and resulted in the development of tests which monitor the quality of sterile insects and the field effectiveness of the methodology (Calkins et al. 1996, Cayol et al. 1999). This effort culminated in the recent publication of a comprehensive manual of quality control for fruit flies (IAEA 1998).

The basic and closest to field conditions mating performance test available so far is that of field cage (Calkins & Webb 1983). Nevertheless, although the test is applied under natural conditions and involves a host tree, the fact that flies cannot freely fly away or "escape" from the host tree, or newcomers cannot mix with the caged tree flies reduces the value of the test. Recently, the interest on sterile insect competitiveness as deduced from egg hatch, first described in 1971 (Fried), has been renewed. Measurements of egg hatch from field oviposition in mock fruits are used for a more accurate evaluation of mating performance under completely natural conditions (Katsoyannos et al. 1999). Unfortunately, no practical method has been standardized so far on egg hatch measurement of field oviposited eggs in mock fruits.

In this study, the mating performance of a white female puparium strain has been evaluated under field cage conditions in citrus plantation.

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Materials and Methods

Cylindrical field cages 2m h × 2.9m d were used (Synthetic Industries, Dalton, GA 30720, USA). Each cage was installed over a Navel orange tree in a mixed plantation with Navel and Valencia orange trees. The cage ceiling was covered by thick white fabric to provide shade. For the study of 1996 (Table 1), most wild flies were raised from larvae in sour oranges and loquats (early summer) and figs (late summer). The genetic sexing males were of the white female pupa strain T (Y;5) 1-61(95) (Franz et al. 1994) at generations 5-10. They were gamma-sterilized 1-2 days before adult emergence. Eight experiments were performed from June till October (see also caption of Table 1). Flies for the experiments in 1998 (Tables 2 and 3) were similar except that the genetic sexing males were of generations 33 and 36, respectively, and were not gamma-sterilized. In the June experiments, high mortality was observed on the second and third experiment days because of air-born toxicity due to near-by bait spraying. In both 1996 and 1998 matings were recorded from 09:00-18:00, every half hour.

In all experiments trees were pruned to fit the cages and make easy the census of fly activities and copulations. Males and females were separated soon after emergence and kept on standard adult diet (unless indicated differently in the table) prior to introduction into the field cage. Water was sprayed on the caged trees on the hot hours of the day to provide the flies with drinking water.

Results and Discussion

In 1996 mating performance experiments are presented in Table 1. All genetic sexing males included were gamma sterilized. In June-August (highest daily temperatures under shade between 30-36°C), the genetic sexing males produced only 0-33% of observed matings while expected values according to insect type ratios were 50-90%, i.e. 13 observed instead of 87 expected matings in total. In 3 out of the 5 experiments organized in this period, the genetic sexing males contributed zero to near zero of mating activity observed, while in the other 2 experiments their mating share was 1/2.7 and 1/2.5 of expected values, respectively. It is noted that the reduced performance of genetic sexing males in the June 18 experiment could had been intensified by the young age of laboratory flies mixed in this test (refer to Table 1 caption). On October 10 and 17 (when daily temperatures were between 16-29°C), genetic sexing males succeeded in getting 1/3.4 to 1/3.1 of their expected mating activity, i.e. 8 observed instead of 28 expected matings in total. That is, although the results in October were not as bad as in July, the genetic sexing males competed again poorly with wild males for matings with wild females. In all experiments the difference was highly significant ($P < 0.001$). Both type matings were recorded almost exclusively on the underside leaf surface, with sterile matings located relatively more at lower canopy than the wild type matings. The majority of both type matings was recorded at the same tree canopy sectors.

The results of the 1998 experiments are shown in Tables 2 and 3. All genetic sexing males involved were not sterilized. The genetic sexing strain was already at generations 33 or 36 with extensive break down. In the June experiment (warm weather) the genetic sexing males, at 1:1:1 ratio (wild males: wild females: gen. sex. males) and fed complete diet, obtained more than expected matings, while at 1:1:3 ratio they obtained significantly fewer than expected matings. In September (cooler weather) at both insect ratios they obtained mating percentages lower than expected. It was observed that their performance was higher in the second and third experiment days as compared with the first one. At the ratio of 1:1:3, sugar fed laboratory flies obtained fewer than expected matings in both June and September. The difference was not significant in June, while in September it was highly significant. When insect ratios of 1:1:3 are compared with 1:1:1 ratios in June, the genetic sexing males of the high overall male: female ratio (4:1) obtained mating values lower than expected, while in the low overall male: female ratio (2:1) they obtained mating values higher than expected ones. In September the genetic sexing males of the low male: female ratio did not perform as well. It could be that the reduced male: female ratio improves the sterile male performance under warm weather. Also, if we compare the mating performance of genetic sexing males fed complete diet with sugar fed ones at 1:1:3 ratio, we observe that in the June experiments the sugar fed males had superior mating performance as compared with the complete diet fed ones. The opposite was true in September. In conclusion, the mating performance of non-irradiated genetic sexing males was considerably improved as compared with the performance of irradiated males in the 1996 experiments. This could be the result of no irradiation-damage and/or even of strain performance improvement because of sexing break down between 1996-98. The mating performance of genetic sexing males fed complete adult diet as compared with sugar only feeding, did not clearly prove superiority of any of the treatments.

The study of copulation site (1998, Table 3) concluded that the preferred site by both type matings is the underside of the leaf. There were some differences on mating site preference between June (higher temperatures) and September (lower temperatures). In June the preferred mating site by both type matings was the lower canopy while in September, matings moved higher in the canopy, the phenomenon been more striking with the genetic sexing type matings. As to tree sector and although differences were not significant, in June both type matings appeared to concentrate in the north and west of the tree canopy, the phenomenon

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TABLE 1. MATING PERFORMANCE OF GAMMA-STERILIZED, MASS-REARED T (Y;5) 1-61/95 GENETIC SEXING MALES (LS) WHEN MIXED WITH WILD FLIES FROM SOUR ORANGES, LOQUATS OR FIGS (W), IN A SERIES OF ORANGE-TREE FIELD CAGE STUDIES FROM JUNE-OCTOBER 1996.

Experiment dates[a]		Temp. range (°C)	Insect combinations tested W:W:Ls	Total no. of medflies per field cage	Total no. of matings observed	Type of matings % Ls × W		Chi-square test		
						O[b]	E	χ^2	df	*P*
June	18 (4)	17-30	1:1:9	220	23	4	90	192.56	3	<0.001
July	5 (2)	17-36	1:1:9	220	9	33	90	32.67	3	<0.001
	25 (2)	18-34	1:1:1	60	21	0	50	21.00	3	<0.001
August	1 (3)	21-34	1:1:3	100	37	3	75	103.25	3	<0.001
	8 (3)	23-35	1:1:3	100	27	30	75	35.29	3	<0.001
October	10 (3)	16-25	1:1:3	100	18	22	75	26.80	3	<0.001
	17 (3)	17-29	1:1:3	100	17	24	75	24.56	3	<0.001
	24 (3)[c]	10-18	1:1:3	100	1	0	75	—	—	—

[a]In parenthesis is the duration of the experiment in days.

[b]O = observed, E = expected % matings based on insect ratios. Except Oct.24 when no statistical comparison was made because only 1 mating was recorded, in all other experiments observed matings were found significantly fewer from expected at $P = 0.001$ (χ^2 test).

[c]Due to cold, rainy weather only one mating (between wild flies) was observed.

[d]Upon mixing, wild flies were 6-13 days old in the different experiments, while genetic sexing males were 3-5 days old or older, except the first 2 days of the June 18 experiment when they were 1-3 days old. In each experiment 2 field cages were used, each with the indicated total flies and insect ratios. The total number of matings recorded are for both cages of each experiment. All flies were fed complete diet except Experiments Oct.10 and Oct.17 in which half of sterilized males were fed only sugar, with no substantial effect observed on their mating performance.

TABLE 2. MATING PERFORMANCE OF NON-STERILIZED, MASS-REARED T (Y;5) 1-61/95 GENETIC SEXING MALES (LN) WHEN MIXED WITH WILD FLIES FROM SOUR ORANGES OR FIGS (W), IN A SERIES OF ORANGE-TREE FIELD CAGE STUDIES ORGANIZED IN JUNE AND SEPTEMBER 1998.

Experiment dates[a]		Temp. range (°C)	Insect combinations tested W:W:Ln[b]	Total no. of medflies per field cage	Total no. of matings observed	Type of matings% Ln × W		Chi-square test		
						O[c]	E	χ^2	df	P
June	18/21 (3)	22-35	1:1:1 (CD)	100	22	72.7	50	4.60	3	$P < 0.25$
June	18/21 (3)	22-35	1:1:3 (CD)	100	10	40.0	75	6.53	3	$P < 0.10$
June	18/21 (3)	22-35	1:1:3 (S)	100	19	57.9	75	2.97	3	$P < 0.25$
September	22/25 (3)	16-29	1:1:1 (CD)	100	42	30.9	50	11.06	3	$P < 0.025$
September	22/25 (3)	16-29	1:1:3 (CD)	100	42	59.5	75	9.08	3	$P < 0.05$
September	22/25 (3)	16-29	1:1:3 (S)	100	47	48.9	75	23.69	3	$P < 0.001$

[a]Two successive experiments were organized in June and another two in September at the indicated starting dates. In parenthesis is the duration of each experiment in days.

[b]CD: laboratory males fed on complete diet, S: laboratory males fed on sugar only.

[c]O = observed, E = expected % matings based on insect ratios. Except June 1:1:1 (CD) and 1:1:3 (S) when no significant differences were detected in all other experiments observed matings were significantly different from expected (chi-square test). Within the O column when 1:1:1(CD) was compared with 1:1:3 (CD) the difference was found significant at $P = 0.10$ in both June and September; when 1:1:3 (CD) was compared with 1:1:3(S) the difference in June was found significant at $P = 0.05$ while in September the difference was not significant (t-test).

[d]Upon mixing, wild flies were 8-12 days old in the different experiments, while genetic sexing males were 4-6 days old. In each experiment 3 field cages were used, each with the indicated total flies and insect ratios. The total number of matings recorded is for the two successive experiments of each month and for all 3-days of the specific cage experiment. In the experiments of June, high mortality of both wild and mass-reared flies occurred due to pesticide application near the experimental area.

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TABLE 3. PERCENT OF TOTAL MEDFLY COPULATIONS OBSERVED ON THE DIFFERENT ORANGE TREE CANOPY SITES IN 1998. CAGED-TREE EXPERIMENTS WITH WILD FLIES (W) FROM SOUR ORANGES OR FIGS MIXED WITH NON-IRRADIATED GENETIC SEXING MALES OF STRAIN T (Y;5) 1-61/95 (LN) AT GENERATIONS 33-36.

	June 1998		September 1998	
Tree-canopy site	W × W	Ln × W	W × W	Ln × W
Leaf surface				
Top	0.0 a	3.3 a	5.6 a	1.6 a
Bottom	85.7 b	83.3 b	88.7 c	90.2 c
Other sites (fruit, tree trunk, cage screen and floor)	14.3 a	13.4 a	5.6 a	8.1 a
Height				
High	5.3 a	3.4 a	14.9 ab	23.7 bc
Middle	15.8 a	10.3 a	40.3 c	47.5 c
Low	78.9 bc	86.2 c	44.8 c	28.8 bc
Tree sector				
West	30.0	35.7	20.9	11.5
South	10.0	3.6	35.8	36.1
East	10.0	3.6	13.4	24.6
North	40.0	50.0	7.5	8.2
Center	10.0	7.1	22.4	19.7

[a]The above data are based on 181 matings recorded in total. Data of different sex ratio and adult feeding treatments were pooled together because no substantial differences were observed. In the columns and the rows means followed by different letter are significantly different: $P < 0.001$, F = 9.033, df 11, 24 (Leaf surface) or $P < 0.05$, F = 2.182, df 11,24 (Height). No significant difference was found in Tree sector: $P < 0.166$, F=1.433,df 19,40 (Tukey's test).

[b]Upon mixing, virgin wild flies or genetic sexing males were 8-12 or 4-6 days old, respectively. Experiments were organized in June and September with 2 three-day replicates of 3 field cages each time. One hundred total flies per field cage were always used at W:W:Ln 1:1:3 or 1:1:1 sex ratios, the first sex ratio tested with Ln flies fed either complete or sugar only diet and W flies fed always complete diet. In June environmental conditions were 25-35°C, 32-48% RH and 2000-15000 Lux, while in September the conditions were 16-29°C, 36-78% RH and 600-12500 Lux, respectively.

been again more intense with the genetic sexing male matings. In September, wild type matings concentrated primarily in south, west and center while genetic sexing type matings concentrated in south, east and center of tree canopy. It is interesting to note that in June about half of total both-type matings concentrated in the cooler northern sector of the tree, while in September only 8% of matings preferred this part of the tree canopy.

In conclusion, genetic sexing sterilized males were found much inferior than their wild counterparts in mating performance under field cage conditions. Nevertheless they performed sexual activity mostly on the same canopy sites as the wild flies. Further research is needed, especially to elucidate the effect of protein feeding before releasing on the survival and mating effectiveness of sterile males.

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