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EFFECT OF AGE ON THE MATING PROPENSITY OF THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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Abstract

The effect of age on the mating propensity of both wild and laboratory-reared Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann) was investigated under laboratory and field cage conditions. The optimal age for wild flies ranged from 7 to 13 days, whereas in laboratory-reared flies it was between 3 and 5 days old. Virgin flies were selective and more prone to mate than flies that were held with both sexes combined and therefore, had a chance to mate before the test. The difference among ages in laboratory-reared flies was significant only in virgin flies. Virgin females showed a tendency to increase their mating propensity as they got older, whereas virgin males showed a bimodal pattern, with peaks at 4 and 11 days old. When flies of both strains and different ages were combined, laboratory-reared females accounted for 72% of the all the matings and most matings were by 4-day-old females. Wild males accounted for 67% of all the matings and the maximum number of matings were by 10-day-old males. For quality control purpose, flies should be virgin and at their optimal age, this will produce more robust data for statistical analysis. For control purpose, it is recommended to release sterile flies at 1-2 days old, because flies in the field will be at their maximum mating propensity. Our results support the concept that releasing males only will make the Sterile Insect Technique more effective, since sterile males will be virgin and therefore, more prone to mate.

Key Words: medfly, *Ceratitis capitata*, Sterile Insect Technique, quality control, mating behavior

Resumen

Se investigó el efecto de la edad en la propensión al apareamiento de moscas del Mediterráneo *Ceratitis capitata* (Wiedemann), silvestres y de cría masiva, en condiciones de laboratorio y jaula de campo. La edad óptima para las moscas silvestres estuvo entre los 7 y los 13 días de edad, para las moscas de laboratorio estuvo entre los 3 y los 5 días. Las moscas vírgenes fueron más selectivas y más propensas a aparearse que aquellas que se mantuvieron mezcladas antes de la prueba. Las diferencias entre edades solamente fueron significativas en moscas vírgenes de laboratorio. Las hembras vírgenes mostraron una tendencia a aumentar su propensión al apareamiento conforme aumentaba su edad, mientras que los machos mostraron un patrón bimodal, con picos a los 4 y 11 días de edad. Cuando se combinaron ambas cepas y diferentes edades, las hembras de cría masiva realizaron el 72% de todos los apareamientos y la mayoría fue por hembras de 4 días. Los machos silvestres realizaron el 67% de los apareamientos y el máximo fue por machos de 10 días de edad. Para fines de control de calidad, las moscas deben ser vírgenes y en su edad óptima, esto producirá datos más robustos para los análisis estadísticos. Para fines de control, se recomienda liberar las moscas estériles cuando tienen 1-2 días de edad, así las moscas estarán en el campo cuando sean más propensas a aparearse. Nuestros resultados apoyan el concepto de que liberar machos solamente hará mas efectiva la Técnica del Insecto Estéril, ya que los machos serán vírgenes y por lo tanto más propensos a aparearse.

The successful application of the Sterile Insect Technique (SIT) to suppress the Mediterranean fruit fly *Ceratitis capitata* (Wied.), as well as new developments to improve this technique, have motivated a wider use of SIT worldwide (Hendrichs et al. 1995). As the demand for more environmental friendly control methods increases, it is likely that the use of the SIT will expand. For wider and more efficient applications, new developments that improve it are required.

As with any other pest control method, it is generally accepted that a better knowledge and understanding of the biology, behavior and ecology of the target pest will result in improvements and more effective applications. One factor that is likely to improve the effectiveness and efficiency of the SIT is the development of new and better methods to estimate or evaluate male mating competitiveness and to determine the factors that are important for successful mating.

After pioneering work by Prokopy & Hendrichs (1979), the "Field Cage Test" (Calkins & Webb 1983) has been used as a research and quality control tool to characterize and understand the mating behavior of fruit flies and to evaluate the mating competitiveness of laboratory-reared ster-

ile flies (i.e. Chambers et al. 1983, Hendrichs 1986, Guerra et al. 1986, Robinson et al. 1986, Orozco & Lopez 1993, McInnis et al. 1996, Lance et al. 1996, Cayol et al. 1999, Calcagno et al. 1999, IAEA 1999). However, there is not a detailed protocol on how the test should be run for quality control purposes, and factors such as density, sex ratio, and age of the flies vary widely. Fine tuning of these factors is important to compare results from different locations and strains, to standardize quality control procedures and make them more efficient, and to determine the conditions that make the test more sensitive, so it can be used as an early warning for mass rearing decision making.

The general goal of this research project was to investigate the effect of age on the mating propensity of both wild and laboratory-reared Mediterranean fruit flies. Our specific goals were to determine: 1) if mating propensity changes with age, and 2) if there is an effect of the mating status (virgin vs. non-virgin) on the mating propensity of males and females.

Materials and Methods

Five different studies were carried out. In all cases, laboratory-reared flies (L) were obtained as irradiated pupae from the Moscamed facility in Metapa, Mexico. Wild flies (W) were obtained as larvae from infested coffee berries collected in Southwestern Guatemala. After leaving the fruit, mature larvae were placed in screened plastic containers for pupation. For both strains, at eclosion, adults were sorted by sex and placed in plastic cages with food (sugar + yeast hydrolyzate 3:1 ratio) and water. Adult flies were kept virgin before the tests, except in those cases in which the effect of the mating status was investigated. In these cases, in one group males and females were held together, so they had a chance to mate before the test (mixed). In the other group, males were held in one cage and females in another cage, so they could not mate before the test (virgin).

When different age groups or strains (laboratory-reared or wild) were tested in the same cage, flies were marked with a small spot of water paint on the thorax the day before the test. A different color was used for each age group and strain. So far we have not detected that these color markings have any effect on the mating performance of the flies.

Field cage studies were carried out in the standard 3 m in diameter by 2 m high cages, with a coffee bush inside (Calkins & Webb 1983). In those cases in which wild flies were used, the tests were done in a coffee plantation in Southwestern Guatemala. When only laboratory-reared flies were used, the cages were located in the "coffee garden" of ECOSUR, in Tapachula, Chiapas, Mexico.

During the test, the behavior of the flies was observed and mating pairs were detected. When a mating was observed, the pair was collected in a vial and the following information was recorded: 1) Time in copula. The time at which the mating was formed was recorded and the vials with the mating pairs were observed frequently to record the time at which the copulation was finished; 2) Site of mating, whether it was on the cage screen or over the coffee plant. In the case of matings on the plant, whether they were on the top or bottom part of the leaf, on a branch or on a fruit; and 3) Kind of mating, recording the age, and strain (where applicable) of the male and the female in each mating, according to the colors used.

Field and laboratory observations were made from 07:00 to 13:00 h. Temperature conditions ranged from 22 to 32°C, relative humidity from 65 to 90%, and a photoperiod of 12:12 (L:D).

Wild Flies, Mixed Ages (W1)

This was a field cage study with only wild flies. The ages of the flies were 7, 9, 11 and 13 days old. This age range was selected based on our previous unpublished observations and due to the limiting number of flies available. Adult flies were released in the cages between 07:00 and 08:00 h. In each cage, 10 males and 10 females of each age group were released. Flies that could not fly or died during the observation period were replaced. The experiment was repeated 10 times (5 cages per day, two days).

Laboratory-Reared Flies, Mixed Ages (L2)

This was a field cage study similar to the previous one but with laboratory-reared flies. The effect of the mating status (virgin vs. mixed) was evaluated by running two sets of tests, one with virgin flies (L2v) and the other with mixed flies (L2m).

The density in the cage was greater than the one with wild flies as was the age range tested. This was done to increase the number of potential matings during the test and given the greater availability of flies. Four different age groups of flies were released in each cage. Twenty five males and 25 females of each age group, so the total number of flies per cage was 200 (100 males and 100 females). The ages of the flies for the first day were: 2, 5, 8, and 11 days old; the second day the ages were: 3, 6, 9, and 12; and the third day were: 4, 7, 10, and 13. Both mated and dead flies were replaced with individuals of the same sex, age and mating status, so the density, as well as the sex and age ratios in the cages were constant. Four replicates were done for both virgin and mixed flies.

Laboratory-Reared Flies, Same Age (L3)

This was a field cage study with laboratory-reared flies in which all the flies in the cage were the same age (cohort). One hundred pairs (100 males and 100 females) of 2-day-old flies were released initially in the field cage. Flies were ob-

served from 07:00 to 13:00 h every day. Mated pairs were vial collected and records on time and location of mating were taken. At 13:00 h all the flies from the cage were collected and transferred to the laboratory where they were maintained in glass cages provided with food and water. The sexes were sorted out to prevent matings during the time when flies were not observed (13:00 to 07:00 h). Two different approaches were followed. In one case, both mated and dead flies were replaced with virgin individuals of the same age and sex, observations were made during 15 consecutive days (L3a). In the other case, both mated and dead flies were not replaced (L3b), so the test was finished when no more females were available. Four replicates of each test were done.

Laboratory-Reared Flies, Mixed Ages for Males and Fixed Age for Females (L4)

This was a laboratory cage study with laboratory-reared flies. In a $1.0 \times 0.6 \times 0.9$ m screened cage with a potted coffee plant inside, 20 males and 5 females were released. Males were of 4 different age groups (5 males per age group) and females were of a fixed age, older than the young males and younger than the old males. The first day of the test, males were: 2, 5, 8, and 11 days old and female age was 7 days old. The experiment was conducted over 3 consecutive days, so the age range for the males was from 2 to 13 days, and for the females was from 7 to 9 days. Both mated and dead flies were replaced with individuals of the same age and sex. This experiment was carried out with virgin females and with females that were exposed to males before the test. Males were always virgin. Four replicates of each approach were done.

Wild and Laboratory-Reared Flies, Mixed Ages (WL5)

This was a field cage study. In each cage, 60 laboratory-reared sterile flies (30 males and 30 females) and 60 wild flies (30 males and 30 females) were released. Flies were sorted in 3 age groups and the ages of the flies were the same for both strains. The ages of the flies for the first day were 2, 5, and 8 days old; for the second day were 3, 6, and 9 days old; the third day were 4, 7, and 10 days old, the fourth day were 5, 8, and 11 days old, and the fifth and last day were 6, 9, and 12 days old (range tested was 2 to 12 days old). All flies were virgin before the test. Three replicates were done.

RESULTS

Wild Flies, Mixed Ages (W1)

A total of 260 matings were recorded in the 10 replicates. Although differences among ages were not significant, 7-day-old flies showed the lowest mean number of matings for both sexes. Males showed a gradual increase in the number of matings as they aged. The maximum number of matings was achieved by 13-day-old males. Females showed the greatest number of matings at 11 days old (Table 1). The most common combination was between 13-day-old females with of 7-day-old males (9.3% of all combinations), but was very similar to other combinations, such as 11-day-old females with 11-day-old males (8.8%) and 13-day-old females with 13-day-old males (8.4%). The least common combinations were between 7-day-old males and females (2.6%) and 9-day-old females with 7-day-old males (3.5%).

Laboratory-reared Flies, Mixed Ages (L2)

There was a significant effect of the mating status on the mating propensity of both, males and females ($P = 0.0001$). Greater number of matings were recorded from virgin flies than from mixed flies at all ages, except on 2-day-old females, where the mean number of matings was the same (Fig. 1A).

Differences among ages in both, virgin and mixed females, were not significant ($P = 0.057$ for virgin females, and $P = 0.497$ for mixed females). However, in both types of females there was a tendency to increase mating propensity from 2 to 4

TABLE 1. MEAN (SE) NUMBER AND PERCENTAGE OF MATINGS, PER SEX AND AGE OF WILD MEDITERRANEAN FRUIT FLIES UNDER FIELD CAGE CONDITIONS. AGE GROUPS WERE COMBINED AND FLIES WERE VIRGIN (N = 260).

Sex	Age	Mean Number of matings (SE)	Percent
Females[a]	7	4.8 (0.70)	18.46
	9	6.3 (0.77)	24.23
	11	8.3 (1.22)	31.54
	13	6.7 (0.42)	25.77
Males[b]	7	5.5 (0.40)	21.15
	9	6.7 (0.67)	25.77
	11	6.5 (0.56)	25.00
	13	7.3 (0.42)	28.08

[a]F = 2.38, D.F. = 3, $P = 0.070$.
[b]F = 1.88, D.F. = 3, $P = 0.134$.

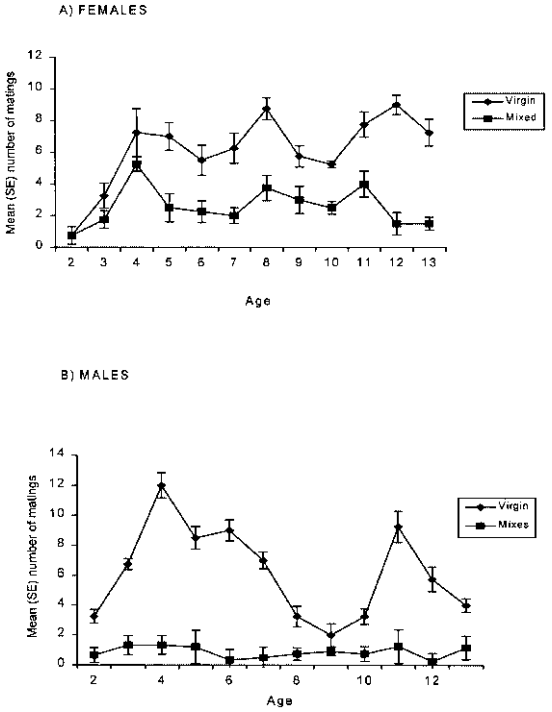

Fig. 1. Age-specific mean number (SE) of matings by laboratory-reared virgin flies and flies in which the two sexes were mixed before the test. A) Females, B) Males.

days. Later, in the case of mixed females, their mating propensity decreased. In the case of virgin females, there was a second peak when females were 8 days old and the third and greatest peak was recorded when females were 12 days old.

There was a significant difference ($F = 2.92$, $P = 0.007$) among ages in virgin, but not in mixed males ($F = 0.81$, $P = 0.63$). The maximum mean number of matings was attained by 4-day-old virgin males (Fig. 1B). Virgin males showed a bimodal pattern in their mating propensity, increasing from 2 to 4 days old, then decreased to a minimum at age 9 and peaking again when they were 11 days old. The mean number of matings by mixed males was never greater than 2 and without any clear pattern associated with age.

Laboratory-reared Flies, Same Age (L3)

There were significant differences among ages for both replacement ($F = 4.39$, $P = 0.0001$) and non-replacement ($F = 10.28$, $P = 0.0001$) tests. The maximum number of matings was recorded when flies were 3 days old (Fig. 2). Mating activity gradually decreased with age. In the non-replacement test, by age 9 all the flies had mated or died. In this test, over 70% of all matings were by 3 and 4-day-old flies. When mated and dead flies were replaced, the fraction of matings at these same ages was only 39.9% of all matings.

Laboratory Cage, Mixed Ages for Males, Fixed Age for Females (L4)

There was a significant differences among male ages when females were virgin, but not when females were mixed. The greatest number of matings were achieved by 5-day-old males (Table 2). The total number of matings with mixed females was 34, whereas in the test with virgin females was 66. Although this greater mating propensity, virgin females apparently were more selective than mixed females, regarding the age of the males and based on the statistical analysis.

Field Cage—Wild and Laboratory-Reared Flies (WL5)

There was a significant difference between laboratory-reared and wild flies, both in the number of matings achieved and the age for maximum mating activity. Laboratory-reared females showed greater mating propensity and the age of maximum mating activity was earlier in life, compared to wild females (Fig. 3). These females accounted for 72.0% of all the matings and the greatest number of matings were recorded when they were 4 days old.

In the case of males, wild males achieved more matings (67.1%) than laboratory-reared males and the greatest number was recorded when they were 10 days old (Fig. 4). The maximum mean number of matings was by 4-day-old lab females with 10-day-old wild males.

Time in Copula

The time in copula was recorded in all field cage tests. A summary of these data is presented in Figure 5. There was not a clear pattern or con-

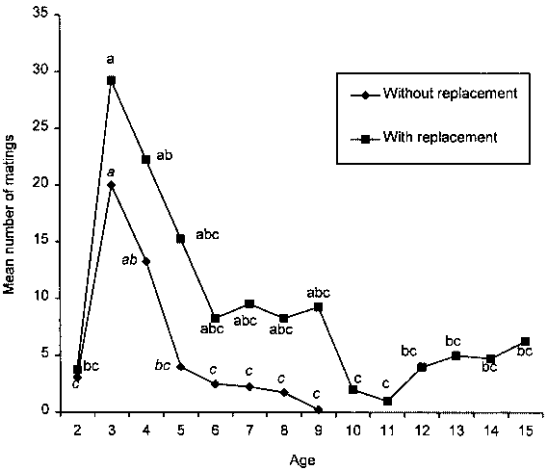

Fig. 2. Age-specific mean number of matings by laboratory-reared flies in tests with and without replacement of mated and dead flies. Same letters at each point (mean) indicate non significant differences within each test by the Tukey Multiple Range Test.

TABLE 2. MEAN (SE) NUMBER AND PERCENTAGE OF MATINGS BY MALES OF DIFFERENT AGES UNDER LABORATORY CONDITIONS. FEMALES WERE OF A GIVEN MEDIAN AGE, AND THEY WERE KEPT MIXED WITH MALES OR KEPT VIRGIN BEFORE THE TEST (N = 34 AND 66 MATINGS FOR MIXED AND VIRGIN, RESPECTIVELY).

	Mean (SE) number of matings		Percentage of matings	
Age	Mixed[a]	Virgin[b]	Mixed	Virgin
2	0	0.25 (0.29) b	0	1.51
3	0.25 (0.29)	0.75 (0.87) ab	2.94	4.54
4	0	2.00 (2.31) ab	0	13.63
5	1.25 (1.44)	3.50 (4.04) a	14.70	21.21
6	0.75 (0.87)	1.75 (2.02) ab	8.82	10.61
7	0.75 (1.73)	1.25 (1.44) ab	8.82	7.58
8	1.00 (1.15)	2.00 (2.31) ab	11.76	12.12
9	1.00 (1.15)	1.25 (1.44) ab	11.76	7.58
10	0.50 (0.57)	1.00 (1.15) ab	5.88	4.54
11	1.00 (1.15)	0.75 (0.87) b	11.76	7.57
12	1.00 (1.15)	0.75 (0.87) b	11.76	1.51
13	1.00 (1.15)	1.25 (1.44) b	11.76	7.57

[a]Differences were not significant by the Chi square test, $P = 0.218$, d.f. = 11). Data were transformed by √X for analysis.
[b]Means followed by the same letter are not significantly different (F = 2.85, $P = 0.0086$) by the Tukey Multiple Range test. Data were transformed by √X for analysis.

sistent effect of age on the duration of copula. However, this parameter was consistently affected by the strain. Generally, wild flies showed greater mean time in copula than laboratory-reared flies. Considering all the tests together, the mean time in copula for wild and laboratory-reared females was 125.8 and 103.5 minutes, respectively. For males, the means were 140.6 and 104.8 minutes

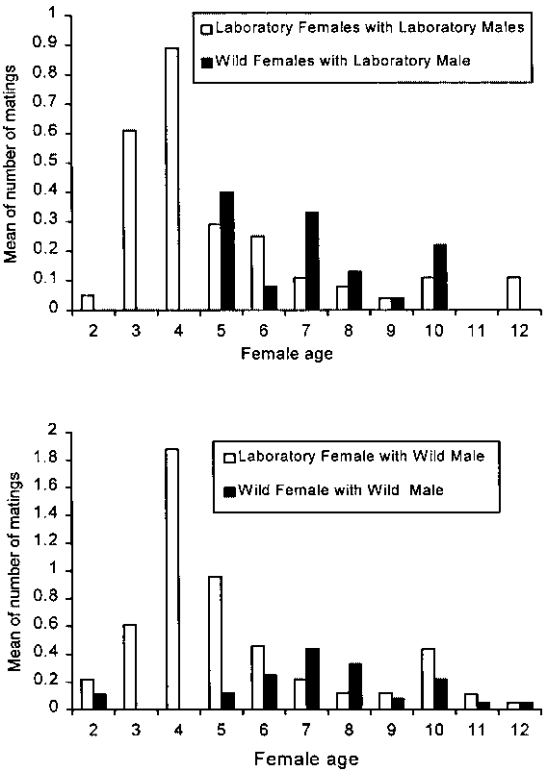

Fig. 3. Age-specific mean number of matings by wild and laboratory-reared females under field cage conditions.

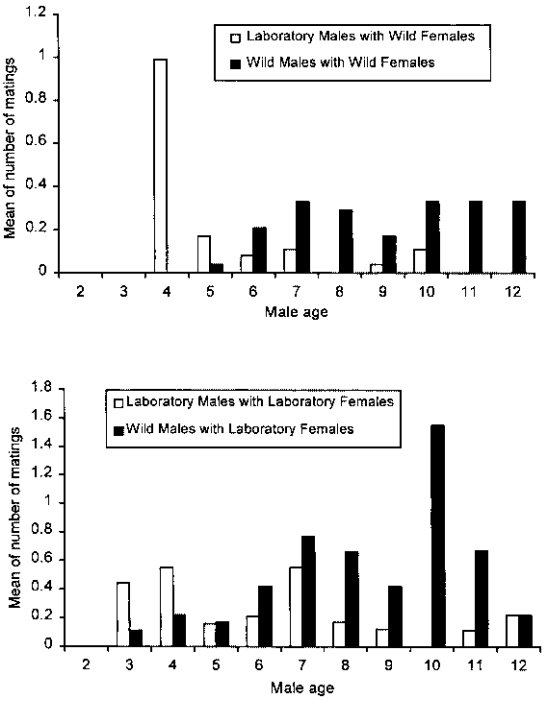

Fig. 4. Age-specific mean number of matings by wild and laboratory-reared males under field cage conditions.

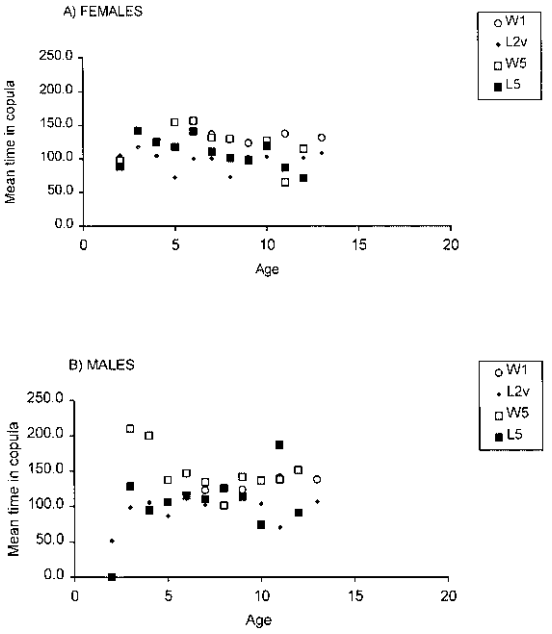

Fig. 5. Mean time in copula of wild and laboratory-reared flies as a function of age. W1 represents data from experiment 1 in which only wild flies were tested. L2v represents data from virgin laboratory-reared flies tested in experiment 2. W5 represents data from wild flies in experiment 5 in which wild and laboratory-reared flies were tested together. L5 represents data from laboratory-reared flies in experiment 5.

for wild and laboratory reared, respectively. Copulas that last less than one minute were discarded for the analysis. The maximum time in copula was 308 minutes (13-day-old wild male with 11-day-old wild female). The mating status of the flies (virgin or mixed) showed no significant effect on the duration of copula ($F = 1.25, P = 0.25$).

Location of Matings

When only wild flies were observed, 52.5% of the matings took place on the bottom part of the leaves, 3.5% occurred on the top part of the leaves, and the rest were observed on the cage screen (44.0%). When both, wild and laboratory reared flies were tested, the same pattern was observed, with 78.3 and 6.3% of the matings located at the bottom and top part of the leaves, respectively. The rest (15.4%) occurred on the screen cage.

Regarding orientation, most matings occurred in the East and Southeast part of the cages (45.9%), which was the sunny part when most mating activities took place (08:00-11:00 h).

Mating activity was strongly associated with temperature conditions. A multiple regression analysis was done with the number of matings and the temperature, for the four field cage tests with laboratory-reared flies (Fig. 6). Mating activity occurred within a range from 23 to 31°C. The greatest number of matings occurred when the temperature was 26°C.

Discussion

Considering the two tests with wild flies (experiments 1 and 5), we conclude that the age range for greater mating activity (optimal range) in wild flies is between 7 and 13 days. The non significant difference among ages in wild flies (Table 1), could be attributed to the range tested (7-13) that was too small for resolution. However, in the experiment with wild and laboratory-reared flies (WL5), we find no matings by 3 and 4-day-old wild females and 2-day-old wild males (Figs. 3 and 4). This is consistent to what was reported by other authors following similar methods, regardless of the geographic origin of the flies. In Reunión Island, Quilici & Franck (1996) recorded the maximum number of matings by 9-day-old flies (optimal range: 7-9 days old), the range they tested was from 3 to 9-day-old flies. In Argentina, Calcagno et al. (1996), tested a range from 3 to 17 days old and they recorded the maximum number of matings from 13-day-old flies (optimal range: 11-17 days old). In Greece, Economopoulos & Mavrikakis (1996) recorded the maximum number of matings by 14-day-old flies (optimal range: 8-14 days old), the range they tested was 2 to 14-day-old flies. There were two common features in all these tests, 1) males mature one or two days earlier than females, and 2) in most cases, the age with the maximum number of matings, was the oldest age tested. The only exception was in Argentina, where also was the only

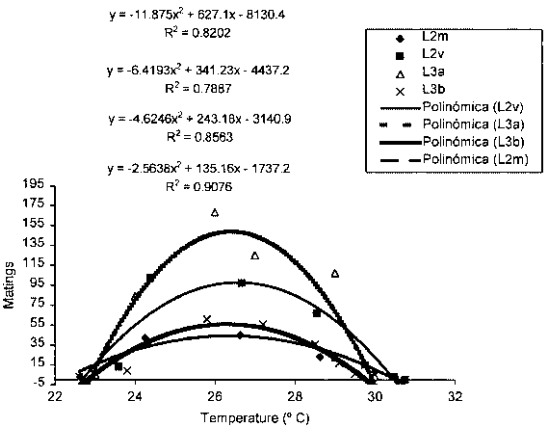

Fig. 6. Multiple regression analysis between number of matings and temperature. L2m and L2v represent data from experiment 2 with mixed and virgin laboratory-reared flies, respectively. L3a and L3b represent data from laboratory-reared flies used in experiment 3 with and without replacement, respectively.

case where flies older than 13 days old were tested. We believe that this characteristic might be due, at least in part, to the fact that flies were kept virgin before the test (see below).

The optimal age range for laboratory-reared flies was between 3 and 5 days old. This is consistent with demographic data that shows that mass-rearing conditions select for early maturation, and flies adapted to this conditions start laying eggs much earlier than wild flies normally do (see Liedo & Carey 1996 and references therein).

Another characteristic of laboratory reared flies, was that females were more prone to mate than wild females. This was particularly clear in the test in which wild and laboratory-reared flies were compared (WL5). Despite that the particular strain we tested was only two years under mass-rearing conditions, 72.0% of all matings were by laboratory-reared females. Again, this is a common characteristic of mass-reared flies (Calkins 1984, Harris et al. 1986, Hendrichs 1986).

The mating status of the flies showed a strong effect on their mating propensity. Virgin flies were more prone to mate, as we were expecting. This might explain the increase in mating propensity with age (i.e., this increase could be an artifact of the experimental design, using virgin flies) and can be attributed to physiological changes that happen in the females after mating. If the females have not mated, they will still be responding to male signals. However, once mated, females will be more interested in finding a host to laid their eggs. The reduced number of matings by mixed males (L2), could be attributed to the low mating propensity of the mixed females.

The mating status could also affect female choice. Virgin females were more selective with respect to male age than mixed females (Table 2).

We believe there are three important applied implications of these results. For quality control purposes, we recommend use of virgin flies at their optimal age for mating propensity (7 to 13 for wild and 3 to 5 for laboratory-reared flies) in field cage tests. This will increase selectivity by females and will increase the mating probability, producing more robust data for statistical analysis and will make quality control efforts more efficient. Also, we believe that our laboratory test (L4) represents an alternative for quality control programs, particularly when the availability of wild flies is a limiting factor and/or when field cage tests represent a risk for fruit fly free zones or areas under eradication or suppression.

Regarding the release of sterile flies, our results indicate that releases when sterile flies are 1 or 2 days old, is the optimal condition. This will reduce the chance of mating before release, and therefore, these flies will be virgin and with a high propensity to mate. Keeping the flies for longer time before release would reduce their mating propensity, could result in high mortality due to crowding conditions, and will increase unnecessarily program costs because of the space required.

Finally, our results support the concept that releasing males only will result in more effective sterile fly release programs, since this will avoid matings between sterile flies, and this will result in males more motivated to court females and to mate.

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