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Source: Florida Entomologist, 90(1) : 175-179

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2007)90[175:MCBICC]2.0.CO;2

MALE COURTSHIP BEHAVIOR IN *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) THAT HAVE RECEIVED AROMATHERAPY WITH GINGER ROOT OIL

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ABSTRACT

The results of previous studies that showed that exposing mass-reared male Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) to ginger root oil ("aromatherapy") increases the likelihood of mating with wild females were confirmed. The increased male success could be due to female responses to changes in male behavior or male pheromones. There were no significant differences in the types of courtship movements executed by males with and without aromatherapy. The durations of movements also did not differ when mass-reared males were paired with mass-reared females; however, when they were paired with wild females, there were a few, small differences. Previous studies indicated that the effectiveness of the male long-distance attractant pheromone is not affected by aromatherapy, but these studies did not consider pheromones released at close range during courtship, which behavioral analyses suggest may be different. We propose the following possible explanation for the different effects of aromatherapy with different females. Selection on males under mass rearing may have altered their close-range pheromones in ways that can be remedied by aromatherapy; and only wild females respond because the pheromonal responsiveness of mass-reared females has also changed. We propose observations that could test these ideas.

Key Words: aromatherapy, *Ceratitis capitata*, close range pheromones, mating behavior, Mediterranean fruit fly, sexual selection

RESUMEN

Los resultados de estudios previos que muestran que al exponer machos criados en masa de la mosca mediterránea de la fruta *Ceratitis capitata* (Wiedemann) al aceite de la raíz del jengibre ("aromaterapia") aumentó la probabilidad del apareamiento con hembras naturales fueron confirmados. El aumento en el éxito de los machos puede ser debido a las respuestas de las hembras a los cambios en el comportamiento o feromonas de los machos. No hubo una diferencia significativa en la clase de los movimientos del cortejo ejecutados por los machos con y sin la aromaterapia. La duración de los movimientos tampoco fue diferente cuando los machos criados en masa fueron apareados con hembras criadas en masa; sin embargo, cuando ellos fueron apareados con hembras naturales, resultaron unas pequeñas diferencias. Los estudios previos indicaron que la eficacia de la feromona atrayente de machos de larga distancia no esta afectada por la aromaterapia, pero estos estudios no consideran las feromonas sueltas en un rango corto durante el cortejo, cuando el análisis de comportamiento sugiere que puede ser diferente. Nosotros proponemos la explicación siguiente para los efectos diferentes de la aromaterapia con las diferentes hembras. La selección de machos bajo condiciones de cria en masa puede haber alterado las feromonas de rango corto de manera que puede ser remediada por la aromaterapia; y solamente las hembras naturales responden por que también ha cambiado la respuesta de las hembras criadas en masa a la feromona. Nosotros indicamos observaciones que pueden probar estas ideas.

The Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) is a serious agricultural pest, and much effort is expended in attempting to control populations in the wild. One important technique involves releasing large numbers of mass-reared sterile males to mate with wild females, thus rendering their eggs inviable. The success of this technique depends on the ability of mass-reared males to successfully mate with wild females. Unfortunately, males from mass-rearing strains are often inferior to wild males, apparently because their courtships are less effective (Lance et al. 2000). Although several aspects of male courtship behavior are known to have changed in at least some mass-reared strains (Limantainen et al. 1997; Briceño & Eberhard 1998;

Calcagno et al. 1999; Briceño et al. 2002b), it is not clear whether these or other male traits are more important in producing this inferiority (Eberhard 2000). Further understanding of the causes of male inferiority would be useful in designing better systems for mass rearing, and in testing the quality of mass-reared males.

The recent discovery that exposing mass-reared males to certain male pheromone analogues or precursors of pheromone production ("aromatherapy") greatly increases their ability to compete with wild males for females (Shelly et al. 1996; Shelly 1999, 2001; Shelly & McInnis 2001), may help alleviate the problem of reduced competitiveness. Male ability to copulate with wild females was improved by exposing them to these compounds the day before mating. The mechanism by which this effect occurs is not known, other than that it can be obtained by exposing the male to only the aroma of the substance; feeding is not required. There are several non-exclusive possible explanations for the greater success of treated males, including that they are more motivated and insistent, that their production of attractant substances is somehow altered, or that their courtship behavior is altered. This study focused on possible changes in courtship behavior.

Materials and Methods

All observations were made in the Tephritid Fruit Fly Laboratory of the University of Hawaii at Manoa, Oahu, Hawaii. The mass-reared flies used were from a small laboratory colony that was derived in 1996 from the old "HiLab" mass-reared strain (44 years or about 748 generations), and since maintained by D. McInnis. Wild flies were collected from coffee on the island of Kauai. Larvae and pupae were reared in the laboratory, and adults were separated by sex within 24 h of emergence. Flies were held in 5-L buckets (maximum number: 150 flies) topped with screen, with *ad libitum* access to water and food (hydrolyzed yeast and sugar, 1:3).

Aromatherapy consisted of placing a small vial containing 20 micro liters of ginger root oil ("Oil Ginger Chinese FCC", Citrus and Allied Essences Ltd. of Lake Success, New York, U.S.A.), on blotting paper (placed in a foil-lined Petri dish) for 7 h in each bucket containing 50 mass-reared males the day before the mating trial. The males could not touch the oil. Mating trials were performed in plastic Petri dishes 13.7 cm in diameter and 1.8 cm deep, and were videotaped from below through a glass table (Briceño & Eberhard 1998) with a Sony Hi8 camcorder equipped with +6 closeup lenses. Flies in mating trials were 5-10 d old, and each fly was used only once.

Durations of different components of courtship behavior in interactions that led to a mounting attempt were determined to the nearest 0.03 s with frame by frame analyses of the videotapes. Only a single mounting was analyzed for each male to avoid pseudoreplication. Male behaviors analyzed were continuous vibration (wings directed postero-laterally and vibrated rapidly dorsoventrally), intermittent buzzing (wings moved periodically from being directed dorsally over to the body to anteriorly while also being vibrated) (for more detailed descriptions see Briceño & Eberhard 2002b), and initial head rock (rotating and lateral movements of the head that occurred just before intermittent buzzing began). Because contact with the male's sexually dimorphic aristae during buzzing appears to be part of medfly courtship (Briceño & Eberhard 2002a), intermittent buzzing was divided into two parts: prior to full arista contact (the male contacted the female, usually her aristae, with one of his aristae), and after full arista contact (both male aristae contacted those of the female). Also noted was the total time the female had remained immobile before the male launched his mounting attempt, the total number of buzzes, and whether the mount was successful (resulted in copulation). All means are followed by ± 1 standard deviation. Statistical tests were Mann-Whitney *U* Tests performed by the statistical package "Statistica" unless otherwise specified.

Results

The same behavior patterns (continuous vibration, intermittent buzzing, head rocking) were executed with qualitatively similar movements by both control and experimental males. Aromatherapy did not produce consistent differences in the measured aspects of male courtship behavior (Table 1). There were no significant differences in durations and numbers of repetitions between control and experimental males when courting mass-reared females. There were 2 differences in the small sample of 9 experimental males courting wild females; these differences were not evident, however, in the larger sample of experimental males courting mass-reared females (in fact, the 2 trends involving durations of arista contact were reversed with mass-reared females). Combined data from courtships of wild and mass-reared females showed no significant effects of aromatherapy on male behavior (see discussion of combining data below). Comparisons between courtships preceding successful and unsuccessful mounts also failed to reveal consistent differences (Table 2).

As in previous studies, aromatherapy increased the probability of copulation with wild females (78% of 9 vs. 29% of 34) ($\chi^2 = 6.96$, $df = 1$, $P = 0.008$), but did not increase success with mass-reared females (22% of 36 vs. 25% of 40) ($\chi^2 = 0.08$, $df = 1$, $P = 0.78$). Aromatherapy resulted in greater likelihood that mounting would be suc-

TABLE 1. COURTSHIP BEHAVIOR OF MASS-REARED MEDFLY MALES WITH AND WITHOUT AROMATHERAPY, WHEN THEY COURTED WILD AND MASS-REARED FEMALES (SUCCESSFUL AND UNSUCCESSFUL COURTSHIPS ARE COMBINED). COMPARISONS ARE BETWEEN CONTROL AND AROMATHERAPY MALES (**$P < 0.01$, *$P < 0.05$).

Male behavior duration (s)	Wild females		Mass-reared females		Totals	
	Control	Aromatherapy	Control	Aromatherapy	Control	Aromatherapy
Continuous vibration	6.20 ± 7.6	3.50 ± 2.1	5.60 ± 6.7	4.80 ± 4.7	5.90 ± 7.1	4.60 ± 4.2
Intermittent buzzing	12.20 ± 7.9	7.80 ± 2.1	10.50 ± 6.0	8.90 ± 6.2	11.30 ± 6.9	8.60 ± 5.5
Initial head rocking	1.10 ± 1.0	0.70 ± 0.4	1.50 ± 1.9	1.30 ± 0.9	1.30 ± 1.5	1.20 ± 0.9
Single arista contact	6.10 ± 4.7	1.00 ± 1.6	3.90 ± 2.5	8.80 ± 7.5	5.40 ± 4.1	5.40 ± 6.2
Full arista contact	8.50 ± 5.3**	3.60 ± 2.0	6.90 ± 4.5	6.80 ± 5.3	7.60 ± 4.9	5.90 ± 4.8
Total arista contact	9.30 ± 8.0*	4.30 ± 1.9	6.90 ± 4.8	7.50 ± 5.4	8.00 ± 6.5	6.70 ± 4.9
Total courtship	17.50 ± 12.7*	9.40 ± 2.1	15.10 ± 10.2	12.70 ± 8.6	16.20 ± 11.4	11.80 ± 7.6
Number of buzzes	49.00 ± 30.5	34.40 ± 10.5	39.60 ± 21.7	47.40 ± 23.7	43.90 ± 26.4	44.20 ± 21.8
Frequency of buzzes (number/s)	3.80 ± 0.8	4.35 ± 0.5	3.95 ± 0.9	4.02 ± 1.42	3.90 ± 0.9	4.10 ± 1.3
Female Behavior						
Female immobile	13.20 ± 9.2*	6.30 ± 3.3	8.60 ± 5.3	7.50 ± 4.2	10.70 ± 7.7	7.20 ± 3.9
n	34	9	40	30	74	39

cessful with wild females than with mass-reared females (78% of 9 vs. 22% of 36) ($\chi^2 = 10.0$, $df = 1$, $P < 0.0016$).

DISCUSSION

It is not obvious how to interpret some of these results. Aromatherapy resulted in courtships that were more likely to induce wild females to copulate, but did not affect the receptivity of mass-reared females. What aspects of courtship changed? On the one hand, the durations of nearly all aspects of male courtship of wild-type females were reduced when males had received aromatherapy. Only two aspects (durations of arista contact and total courtship) were statistically significant. However, the number of pairs was small, and most traits were quite variable. On the other hand, there were no indications of corresponding differences in the larger samples of males courting mass-reared females, or in the totals combining wild and mass-reared females. It is not clear, however, whether combining the data in this way is justified. If the origin of female does not affect male courtship behavior, it is reasonable to combine the samples; but if it does alter male behavior, pooling the data is inadvisable. One previous study showed that, in another pair of strains, the male's courtship did not change when the strain of the female varied (Briceño & Eberhard 1998), suggesting that combining data from different strains in the present study may be reasonable. However, differences in the cues that affect female behavior in different strains could determine whether or not it is appropriate to combine data. It is possible, for instance, that male pheromones did not vary between strains in the previous study (Briceño & Eberhard 1998) (thus arguing in favor of combining data), but that they did vary in the present study (thus arguing against combination) (see below).

One possible explanation for increased acceptance by wild females of males that received aromatherapy is that some aspect of the complex mix of male sexual pheromones (Millar 1995) is changed by aromatherapy. There are indications that the effectiveness of the male long-distance attraction pheromone mix (Millar 1995) is not affected by aromatherapy (Shelly 2001), but there are no data testing the possibility that it affects the male pheromone(s) released at close range during courtship, after the female has approached the male to within approximately one to five body lengths. Assuming that the male rectal epithelium and his abdominal pleura do not emit the same blend of 90+ components found in the pheromones of this species (Millar 1995), the fact that different male structures are everted at different stages during interactions with females suggests that males release different pheromone mixes at close-range and long-range (Eberhard 2000).

It should be kept in mind that the durations of most of the aspects of male medfly courtship behavior that we measured may be influenced not only by the male's own tendency to pace his courtship, but also by the female's responses to his behavior. Courting male medflies are more or less immobile, and movements by the female are usually responsible for bringing the pair of flies close together (Feron 1962), and in orienting the female to face directly toward the male so he can touch her aristae with his and attempt to mount (Briceño & Eberhard 2002a, 2002b). If males use female proximity and orientation to trigger

TABLE 2. MALE COURTSHIP BEHAVIOR IN SUCCESSFUL AND UNSUCCESSFUL COURTSHIPS OF WILD AND MASS-REARED MEDFLY FEMALES. COMPARISONS WERE BETWEEN SUCCESSFUL AND UNSUCCESSFUL COURTSHIPS (**$P < 0.01$, *$P < 0.05$), FOR BOTH CONTROL AND AROMATHERAPY MALES.

	Control males		Aromatherapy males	
Male behavior duration (s)	Successful	Unsuccessful	Successful	Unsuccessful
A. Mass-reared males × wild females				
Continuous vibration	7.9 ±8.6	5.3 ± 7.0	5.00 ± 1.2	1.4 ± 1.6
Intermittent buzzing	15.9 ± 10.2	10.7 ± 6.4	8.30 ± 2.1	5.8 ± 2.1
Initial head rocking	0.9 ± 0.5	1.2 ± 1.1	1.10 ± 0.1	0.3 ± 0.2
Single arista contact	10.1 ± 10.1	4.9 ± 4.7	1.90 ± 1.6	—
Full arista contact	11.1 ± 4.3**	7.3 ± 5.4	3.30 ± 2.2	4.1 ± 4.2
Total arista contact	13.1 ± 8.4**	7.8 ± 7.5	4.40 ± 2.2	4.1 ± 3.9
Total courtship	23.8 ± 12.4*	14.9 ± 12.1	9.90 ± 2.0	7.1 ± 7.3
Number of buzzes	68.8 ± 27.7	40.7 ± 28.2	37.10 ± 10.4	25.0 ± 12.7
Frequency of buzzes (number/s)	3.9 ± 0.4	3.8 ± 0.96	4.36 ± 0.64	4.3 ± 0.6
Female Behavior				
Female immobile	19.2 ± 12.7	10.6 ± 5.9	7.30 ± 2.8	1.9 ± 1.1
n	10	24	7	2
B. Mass-reared males × mass-reared medly females				
Continuous vibration	4.3 ± 4.3	6.0 ± 7.4	2.90 ± 2.7	5.6 ± 5.1
Intermittent buzzing	10.1 ± 6.0	10.7 ± 6.1	10.00 ± 10.3	8.6 ± 4.3
Initial head rocking	1.3 ± 1.2	1.5 ± 2.1	0.90 ± 0.5	1.4 ± 1.0
Single arista contact	—	3.9 ± 2.5	—	8.8 ± 7.5
Full arista contact	3.8 ± 2.8**	8.0 ± 4.6	7.40 ± 8.4	6.6 ± 3.6
Total arista contact	3.8 ± 2.8**	8.0 ± 4.9	7.40 ± 7.6	8.4 ± 4.0
Total courtship	13.5 ± 8.1	15.7 ± 11.0	12.20 ± 9.1	12.9 ± 8.7
Number of buzzes	32.1 ± 16.2	42.2 ± 23.0	60.50 ± 39.6	42.1 ± 10.7
Frequency of buzzes (number/s)	3.5 ± 0.8	4.1 ± 0.9	4.40 ± 1.7	5.0 ± 4.7
Female Behavior				
Female immobile	7.7 ± 5.4	9.0 ± 5.4	2.80 ± 1.4	9.3 ± 3.5
n	10	30	8	28

mounting attempts (Briceño & Eberhard 2002b), then quicker female approaches and alignments could result in shorter durations of several components of male courtship. The shorter durations (if they exist) of the courtship of wild females by males that received aromatherapy could result from more rapid female approaches, perhaps due to improved male short-range pheromone.

The limitation of this effect to interactions with wild but not mass-reared females could be explained if female chemical criteria for accepting males have changed under mass-rearing. Changes in the criteria of mass-reared females for another male courtship trait have been documented in this species (Briceño & Eberhard 2000a, 2002b). It seems undeniable that the pheromonal mileu in mass-rearing cages must be quite different from that under natural conditions, so selection on both male pheromone production and female responsiveness to pheromones is likely to be different.

Whatever the correct interpretation of these aspects of our data, they emphasize the central role of females in determining the effect of aromatherapy on the ability of males to obtain copulations, and focus attention on possible mechanisms responsible for this effect. If aromatherapy causes changes in the durations of different aspects of male courtship behavior, this change occurs only when the male courts wild females, not mass-reared females. If aromatherapy does not directly cause such changes in male courtship behavior, then increased acceptance by wild females is presumably due to responses to other cues from the treated males (probably chemical) that induce the females to accept copulation when mounted.

Future studies could test the possible importance of male and female roles in increased acceptance and courtship duration by concentrating on male and female movements prior to mounting. If changes in duration of male behavior are due to greater female attraction, courtships by males that have received aromatherapy should produce more rapid and extensive female turning to orient toward the male, more frequent female walking movements toward him, and approaches that occur with

less delay. If, in contrast, aromatherapy changes the male and makes him attempt to court more quickly, then perhaps male orientation and movements toward the female will be more frequent.

A technically more difficult, but more direct test would involve checking whether pheromones differ from courting males that have, and have not, received aromatherapy. Still another test would be to determine both female response behavior and male copulation success for males with aromatherapy when paired with females whose ability to sense pheromone was experimentally altered. This alteration would also be technically challenging, as the chemical sensors on the female antennae would have to be modified without changing their sensitivity to the tactile stimuli that also influence female acceptance (Briceño & Eberhard 2002a).

Acknowledgments

A BARD grant (project No IS-2689-SGR) awarded to B. Yuval and T. E. Shelly financed the trip of RDB to Hawaii to film the flies. Additional support was provided by the International Atomic Energy Agency, and the Vicerrectoría de Investigación of the Universidad de Costa Rica.

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