The Society for engineering in agricultural, food, and biological systems

Paper Number: 037067
An ASAE Meeting Presentation

Acoustic trap for female Mediterranean fruit flies

Amos Mizrach, Amots Hetzroni, Timea Ignat, Joseph Grinshpun, Michal Mazor

Agricultural Research Organization, The Volcani Center, Israel.

Richard Mankin, Nancy Epsky, Dennis Shuman, Robert Heath

USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, USA.

Written for presentation at the
2003 ASAE Annual International Meeting
Sponsored by ASAE
Riviera Hotel and Convention Center
Las Vegas, Nevada, USA
27- 30 July 2003

Abstract. *Medflies are among the world's most economically harmful pests, and worldwide monitoring and control efforts are extremely expensive (about $800 million per year in Israel and the US alone). Efficient traps are vitally important tools for medfly quarantine and pest management activities; they are needed for control as well as early detection, for predicting dispersal patterns and for estimating medfly abundance within infested regions. Efficient tools to attract and catch the primary target, reproductively viable namely the female's are still in great need. The present research aimed to evaluate the attractiveness male song and synthetic sounds to mate-seeking female medflies, as a contribution to the design and testing of traps based on acoustic lures. The courtship behavior of female medflies in the presence and absence of calling male flies was observed, in order to evaluate the adequacy of the experimental setup. Male medfly calling song and synthetic tones were played at various intensities to laboratory-reared and wild female medflies during the morning and early afternoon periods of peak sexual activity. It was found in most bioassay experiments, in which sounds were played to laboratory and wild flies, that the female flies were attracted to sites near speakers more than to sites without sound. This study indicates that there is a possibility of using sound to enhance the attractiveness of acoustic traps to mate-seeking female medflies.*

Keywords. Mediterranean fruit fly, acoustic communication, digital signal processing, insect pest detection, entomology, behavior.

Introduction

Fruit flies are important agricultural pests in most parts of the world. The Mediterranean fruit fly *Ceratitis capitata* ('medfly') is polyphagous, attacking some 250 different types of fruit, including citrus as well as many deciduous and subtropical fruits (Fimiani 1989). The most common systems currently used to control the medfly are ultra-low-volume aerial spraying of poison-bait mixture (Rossler, 1989) or cover sprays, increasing public awareness of the environmental dangers from pesticides has generated interest in the development of more environment-friendly alternatives. Attractants, especially for females, are efficient tools for control, targeting infestations and reducing the environmental impact of medfly control measures. Attractants also are useful as 'early warning systems' against imminent invasions in unaffected regions (Cunningham 1989).

In general, two types of attractants are in common in for medfly monitoring and control. Trimedlure is a powerful attractant for males, and 'food lures', mostly protein hydrolysates, are used to attract females. The food lures are much less efficient than the trimedlure. Other female attractants based on emulated pheromones (Heath et al., 1991) and plant odors (Jang and Light, 1996) have been tested with limited success. Females, which cause direct damage to the fruit, are a main target for control. Much of the research on development of new insect detection and control techniques has been focused on mating behavior, because traps based on sexual communication signals tend to be efficient and highly selective. However, the medfly has a richly varied courtship, which complicates efforts to develop communication-based trapping systems.

Medfly males produce songs as part of their sexual communication system. Sivinsky (1989) identified three distinct sounds in medfly males' acoustical signals: 1. The calling song, produced simultaneously with pheromone emission and often in the absence of other nearby flies, 2. The courtship song, an intermittent series of buzzes made when other flies are nearby, and 3. The copula song, a brief sound produced by males when they mount a prospective mate. Of these acoustical signals, the calling song may be used as female-attractive element. The calling song was characterized by Webb et al (1983b) who described it as low-amplitude vibrations of the wings in low-amplitude vibrations at a frequency of ~350 Hz. Recording conducted by Mizrach et al (2002) confirmed Webb's measurements.

Until now, almost all research on medfly attractants has focused on chemical lures, or shape and color cues, and there have been few attempts to attract the female fruit flies to the male calling song only. In an early study, Webb et al. (1983a) found that traps for female Caribbean fruit fly flies baited only with song were more effective than silent controls. Recently, Mankin et al. (2000b) elaborated on this finding, and demonstrated that pre-exposure to male pheromone enhanced the response of female caribflies to male song. However, despite years of research, there is still no reliable and efficient trap for monitoring the presence and abundance of mate-seeking female fruit flies.

The objective of the present study is to assess the attraction of mate-seeking female medflies to recordings of male song and synthetic sounds.

Materials and Methods

Preparation of flies

The sources of the medflies were laboratory mass-reared flies (Lab flies) and wild flies (Wild flies). The Lab flies were obtained as pupae from the Citrus Marketing Board of Israel from the genetic stock, “Vienna”, a stock maintained in the laboratory since 1964. Wild flies emerged from parasitized fruits. The pupae were placed in 30 x 40 x 20-cm plastic cages with screened ventilation openings and glass lid and were kept on a 12-h L:D light cycle. Males and females were sexed two days after emergence and thereafter were kept in separate cages at temperatures of 24-26 ºC and on a 12-h L:D light cycle. The flies were fed water, sugar and a mixture of sugar and yeast hydrozylate. Virgin females and males were used in bioassays 3 to 7 and 14 to 18 days old for Lab and Wild flies, respectively.

Source of recording and playbacks of male medfly sounds

The recordings of male medfly calling songs were made in an anechoic chamber (Mankin et al. 1996) at the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology and were adapted for the present study. Since the fundamental frequencies of recorded calling songs taken from sterile males were not significantly different from those of wild and laboratory-reared sterile males obtained previously (Sivinski et al. 1989), the existing recordings of male medfly calling songs were used. Songs produced by 3-day-old male medflies during the first 4 h of photophase were monitored with a Model 4145 microphone (Brüel and Kjær (B&K), Naerum, Denmark), positioned about 0.5 cm above a 10 x 10 x 7-cm screened cage containing three to five males. The microphone was connected to a B&K measuring amplifier (model 2610; input gain = 20, output gain = 20). The output was stored on a digital audio tape recorder (Mankin et al. 1996).

A representative song chosen for playback in this study was taken from a recorded collection (Mankin et al. 1996b) (e.g.: http://cmave.usda.ufl.edu/~rmankin/medfly11.wav). A 25-s segment of calling song recorded from an individual male was selected from the collection for broadcasting in bioassays. The song segment was played in a continuous loop from an Ulead MediaStudio Pro 5.0 (Ulead Systems, Inc, USA) program operating on a personal computer. The output was amplified with a 100-W (Sansui, Japan) speaker amplifier.

Sound Production Systems

Evaluation of the efficacy of several playback devices, installed in a 30 x 60 x 60-cm chamber was performed on laboratory-reared female medflies. Several off-the-shelf loudspeakers were tested; bare loudspeakers equipped with front-mounted flat membranes, a 4-inch 40-W generic car door speaker, an embedded box loudspeaker, an embedded box loudspeaker with sound concentrator funnel and a 100-W subwoofer speaker (Cambridge SoundWorks, Malaysia) installed in its original box equipped with bass reflex outlet and producing a high-sound-pressure field. Most of the experimental described below were obtained by using a 100-W sub-mid-range 5” loudspeaker (Moral, Israel) installed in its original wooden box and equipped with a sound concentration funnel.

Laboratory bioassays.

The behavior of female medflies was observed in bioassays which involved several different setups, chambers, sound sources, and counting methods. The experiments were performed in anechoic room (400 x 300 x 250 cm) with a controlled atmosphere (30ºC, 57% RH). Bioassay chambers were placed in the center and along of the anechoic room. Laboratory tests were performed with three different sound sources: live calling song, played-back calling song, and pure tones.

Live calling song bioassay

The distribution of female med flies inside a test chamber in response to a live male calling song was examined to evaluate the environmental setup. Two techniques were employed: visual counting and capture of landing flies.

The landing behavior of females in response to the presence of live med fly males calling song was examined in a 60 × 60 × 150-cm Plexiglas chamber equipped with widely opening doors (Fig. 1). Two cylindrical net cages (6 cm long × 5 cm dia.) were placed at mid-height on two opposite sides of the chamber. Sheets of filter paper (5-cm dia.) were placed next to each net cage, with their planes parallel to the sidewall of the chamber. Five live male medflies were put in one of the net cages, the other cage was left empty as a control, and 40 virgin laboratory females were released in the central part of the chamber. Landings of female flies were observed during the morning and early afternoon periods of peak sexual activity. The number of landings on the filter papers was recorded every 5 min for 1 h. The experiment was repeated five times with a fresh group of females each time.

Female flies were captured with a modified electronic fly killer (TITAN 30, PestWest Electronics Ltd., Ossett, West Yorkshire, UK). The UV bulbs and the zapping screen were removed from the fly killer case, and a helical metal zapper comprising two double helices (3 mm dia wire, helical diameter 82 mm, Helical space 7 mm, Fig. 2) replaced the original screen. The two coils that formed each a double helix, were fixed and electrically insulated from each other, and each was connected to one outlet of the high-voltage generator of the fly killer. A cylindrical net cage (6 cm long × 5 cm in dia.) was installed inside each helix and placed at mid-height inside the chamber on opposite sides. To avoid disturbance when flies were first placed into the chamber, a dividing curtain was installed to separate the release section from the zappers. Five live male medflies were put into the cylindrical net cage, and 20 virgin females were released in the center of the chamber. The curtain was lifted 15 min after the flies were introduced. Attracted females, which flew toward the calling males had to pass through the zapper wires, and were zapped within 1 cm from the screen.

Calling song playback and pure tone attraction bioassay

The efficacy of playing male calling song and pure sine-wave tones in attracting wild and laboratory female flies was evaluated. All tests were conducted in a net chamber, 60 x 60 x 150 cm (Fig. 3) equipped with two loudspeakers (one active, the other as control) installed one at each end of the cage (long dimension). Sheets of filter paper (5 cm dia.) were placed perpendicularly in front of the speakers. Each experiment lasted 2 h and involved 20 female flies, which were released in the middle of the chamber. The sound was turned on and off at 10-min intervals at two intensities: 67 and 110 dB (measured 100 mm from loudspeaker). The total time spent by all flies on the filter paper during each 10-min period was summed and divided by the number of flies in the cage as a measure of mean resting time. The experiments were repeated five times per day, and the flies were replaced daily. The length of the cage was virtually divided into three equal sections (referred to as ‘near’ - the section closest to the active speaker; ‘middle’, and ‘far’). Prior to each experiment, the distribution of the flies in the cage was sampled and recorded. The “songs” were played at the two sound intensities; they comprised various pure tones (sine-wave signals at 150, 250, 350, and 450 Hz) and a male calling song (‘male song’, available at http://cmave.usda.ufl.edu/~rmankin/medfly11.wav). During the playback of the tones/song, the number of flies in each virtual section of the cage was recorded every 5 min.

Fig. 1 - Plexiglas chamber equipped with speaker attachments (calling medfly males and control cage in both sides of the chamber).

Fig. 2 - Helical metal zapper screen cages (dia. 50 mm by 80 mm)..

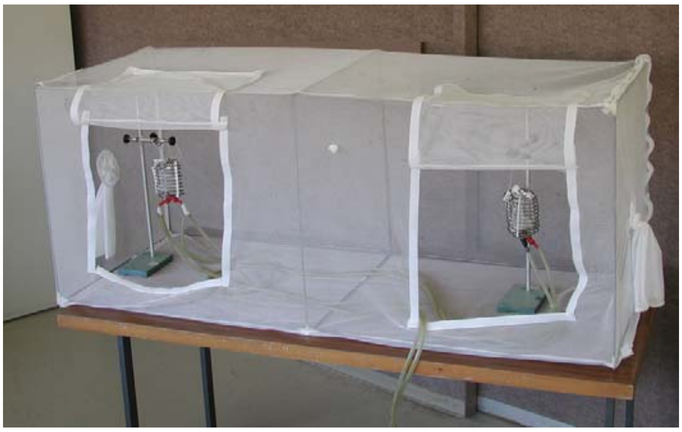

Fig. 3 – Net chamber equipped with speaker attachments.

Results and Discussions

Live calling song bioassay

The mean landing rate of female medflies on the filter paper next to the male net in response to the live male calling song was 72.8 ± 13.9% of the population (α = 0.05, N = 5). No flies landed on the control filter paper in the same period. This relatively high landing percentage was expected and confirms similar results reported in the literature. Pheromones emitted by medfly males are obviously part of the courtship phenomenon and probably contributed to the female response.

The mean capture rate of female medflies by the zapper next to the male cage was 47.4 ± 7.9% (α = 0.05, N = 10), and that by the control zapper (no males) was 9.1 ± 4.5% (α = 0.05, N = 10). The results indicated the expected significant preferences of the female Medfly for the side of the chamber where the male flies were placed.

Calling song playback and pure tone attraction bioassay

Average landing results of female flies attracted by the male calling song and by continuous sine-tone playback at low and high pressure level of 67 and 110 dB, respectively, are summarized in Table 1.

Table 1 Count of flies landing in test chamber that was virtually divided into three sections (close, middle, far). The control treatment included no external audio intervention. The other treatments involved the playing of: the male calling song ('male Song'); and a continuous sine wave at various frequencies (150, 250, 350, 450 Hz). The sound intervention was repeated at 67 and 110dB sound intensity.

Origin	Treatment	Sound intensity	Close to the loudspeaker		Middle part of the cage		Far end of the cage	
			Mean	STD	Mean	STD	Mean	STD
Laboratory flies	Control	-	7.00 a*	1.41	6.25 a	1.50	6.75 a	0.96
	150 Hz	67 dB	7.83	1.92	5.58	1.93	6.58	1.92
	250 Hz	67 dB	8.75	2.78	5.25	1.90	6.00	1.71
	350 Hz	67 dB	8.17	2.74	5.69	1.91	6.14	2.11
	450 Hz	67 dB	7.97	1.68	5.53	1.65	6.50	1.84
	Male Song	67 dB	6.69	1.86	6.25	2.01	7.06	1.94
	150 Hz	110 dB	6.92	2.71	6.50	2.26	6.58	2.72
	250 Hz	110 dB	6.83	2.31	6.14	2.00	7.03	1.99
	350 Hz	110 dB	6.58	1.83	6.69	2.21	6.72	1.16
	450 Hz	110 dB	6.92	2.25	6.03	1.84	7.06	1.71
	Male Song	110 dB	9.03	2.01	5.47	2.08	5.58	2.60
Wild flies	Control	-	3.86 c	2.04	9.29 a	2.56	6.86 b	1.46
	150 Hz	67 dB	7.06	2.00	7.11	1.39	5.83	1.71
	250 Hz	67 dB	5.89	1.91	6.37	2.00	7.77	1.68
	350 Hz	67 dB	5.94	1.78	8.03	2.37	6.03	2.01
	450 Hz	67 dB	5.26	2.39	7.91	2.36	6.83	1.36
	Male Song	67 dB	4.37	1.99	9.43	2.73	6.20	2.27
	150 Hz	110 dB	5.06	2.36	9.34	2.59	5.60	1.35
	250 Hz	110 dB	6.09	2.92	8.69	2.93	5.23	1.83
	350 Hz	110 dB	6.23	1.59	7.74	1.60	6.00	1.68
	450 Hz	110 dB	6.09	1.93	8.00	2.11	5.91	2.27
	Male Song	110 dB	5.80	1.75	8.34	1.39	5.86	1.87

* Mean values in the same row followed by the same letters are not significantly different by Duncan's multiple range test ($p < 0.05$). (SAS Institute, 1992)

We expected that the distribution of fly landings in the control chamber would be uniform, and, in fact, the mean values in the control count of the Lab flies followed such a pattern (Table 1). However, the landings of wild flies showed a non-uniform spatial distribution in the control chamber (Table 1); they showed a significant preference for the central section of the chamber. We are not able to explain this bias, therefore, we have used this spatial distribution as our reference (baseline). Thus, we normalized the control data, and all further findings were accordingly adjusted.

The adjustment used the following model:

$$\overline{A_i} = \frac{\overline{a_i}}{3\overline{c_i}} \qquad [1]$$

where $\overline{A}_i$ is the adjusted count of flies; $\overline{a}_i$ is the observed count of flies; and $\overline{c}_i$ is the average of the observed counts of flies in the control treatment. The variance of the adjusted counts was estimated by means of the first-order Taylor approximation:

$$S^2 = \sum_i S_i^2 \left(\frac{\partial f}{\partial x_i} \right)^2 \quad [2]$$

where S^2 is the estimated variance of the adjusted count of flies ($\overline{A}_i$); $f(x_1, x_2)$ is the adjustment model (Cochran, 1977).

The average adjusted ratios of landing results of female lab flies attracted by male calling song and by continuous sine tone playback at low and high intensities of 67 and 110 dB are summarized in Figures 4 and 5, respectively.

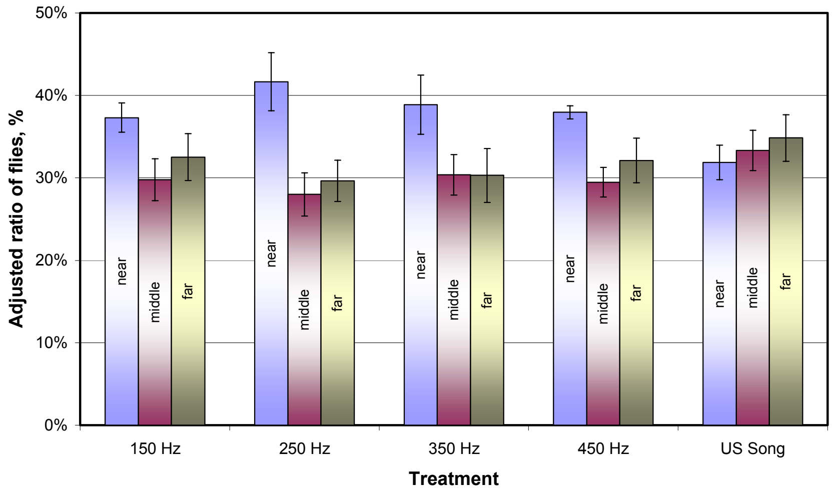

Fig. 4 – Average landing results of laboratory female flies attracted by male calling song and by continuous sine tone playback at 67 dB sound intensity; vertical lines delineate confidence limits of the mean value (α = 0.05).

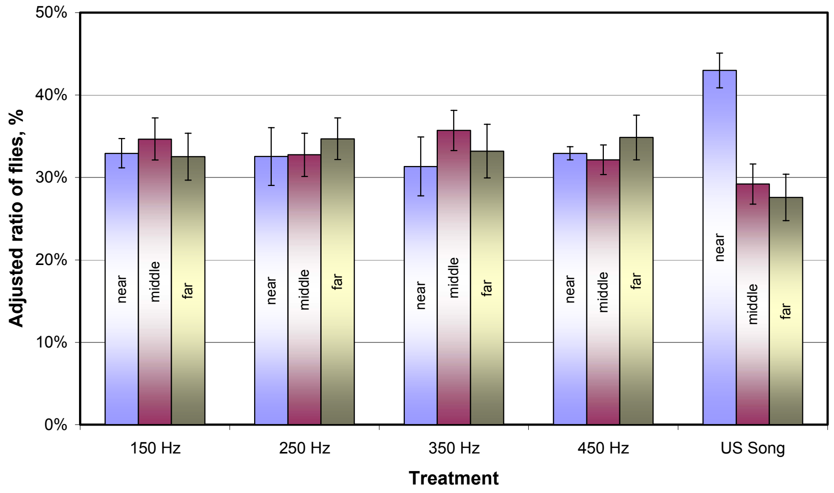

Fig. 5 – Average landing results of laboratory female flies attracted by male calling song and by continuous sine tone playback at 110 dB sound intensity; vertical lines delineate confidence limits of the mean value (α = 0.05).

When sounds were played at the intensity of 67 dB the female flies preferred to land near the speaker in response to all tones, except for the 'male song' which elicited no significant preference.

When the same sounds were played at an intensity of 110 dB, no significant preferences were observed in response to the pure tones, but when the 'male song' was played, the laboratory female flies significant preferred to land by the 'near' speaker.

Adjusted average landing counts of wild female flies attracted by male calling song and by continuous sine tone playback at low and high intensities of 67 and 110 dB are summarized in Figures 6 and 7, respectively.

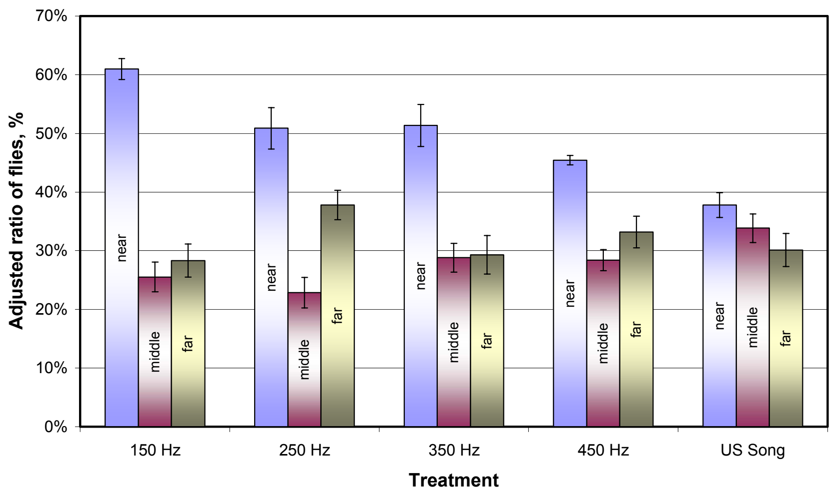

Fig. 6 – Average landing results of wild female flies attracted by male calling song and by continuous sine tone playback at 67 dB sound intensity; vertical lines delineate confidence limits of the mean value (α = 0.05).

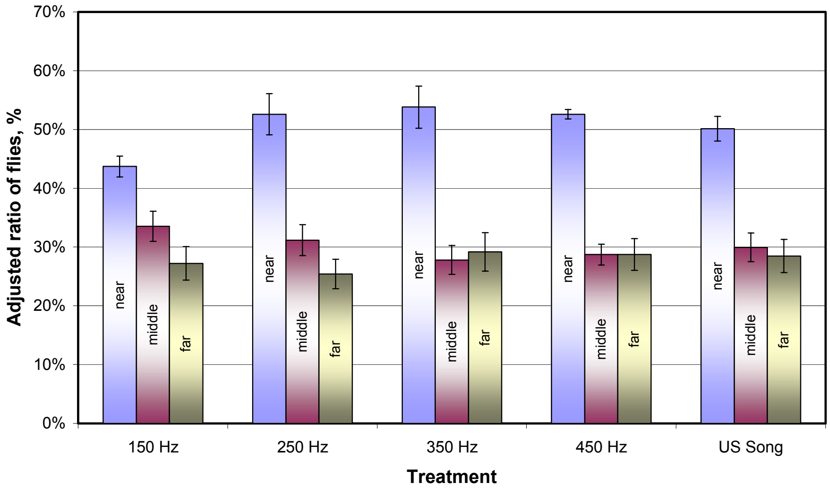

Fig. 7 – Average landing results of wild female flies attracted by male calling song and by continuous sine tone playback at 110 dB sound intensity; vertical lines delineate confidence limits of the mean value ($\alpha = 0.05$).

In almost all experiments the wild female flies significantly preferred to land in the vicinity of the speaker ('near') with the exception of the 'male song' played at 67 dB which elicited only marginal preference for the 'near' landing site.

Conclusion

To consider the feasibility of developing a female-targeted acoustic trap for medflies, we first observed females in the presence and absence of a broadcast calling song to identify responses that showed potential to increase trap captures. The findings of the bioassay test of the attraction of females to the presence of male flies agreed with the known and expected behavior. Thus, it can be conclude that the experimental setup employed in this study had no influence on the expected behavior of flies.

In most bioassay experiments, where sounds were played to laboratory and to wild flies, the female flies were attracted to sites near speakers in preference to sites without sound. Therefore, it can be concluded that the technique employed here can be used to attract female flies, in order to monitor and control the fruit fly pest.

Acknowledgements

This research was supported in part by Grant No. IS-3137-99 from BARD, the United States-Israel Binational Agricultural Research and Development Fund. Contribution from the Institute of Agricultural Engineering, the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel, No. 709/03, 2003 series.

References

Cochran, W.G. 1977. Sampling techniques. John Wiley & Sons Ltd.

Cunningham, R.T. (1989) Population detection. In A.S. Robinson and G. Hooper (eds): Fruit flies; their biology, natural enemies and control. Amsterdam: Elsevier, pp. 169-173.

Economopoulos, A.P. (1989). Use of traps based on color and/or shape. In A.S. Robinson and G. Hooper (eds): Fruit flies; their biology, natural enemies and control.: Amsterdam: Elsevier, pp. 315-327.

Fimiani, P. (1989) Mediterranean region. In A.S. Robinson and G. Hooper (eds): Fruit flies; their biology, natural enemies and control. Amsterdam: Elsevier, pp. 39-50.

Heath, R. R., P. J. Landolt, J. H. Tumlinson, D. L. Chambers, R. E. Murphy, R. E. Doolittle, B. D. Dueben, J. Sivinski, and C. O. Calkins (1991). Analysis, synthesis, formulation, and field testing of three major components of male Mediterranean fruit fly pheromone. J. Chem. Ecol. 17: 1925-1940.

Jang, E. B., and D. M. Light. (1996). Olfactory semiochemicals of Tephritids, pp. 73-90. In Fruit Fly Pests A World Assessment of Their Biology and Management, B. A. McPheron and G. J. Steck, [eds]. St. Lucie Press, Delray Beach, FL.

Mankin, R. W., A. Malavasi, and C. Aquino (1996). Acoustical comparisons of calling songs from *Anastrepha* species in Brazil. In B. A. McPheron and G. J. Steck, (eds): Fruit Fly Pests A World Assessment of Their Biology and Management,. St. Lucia Press, Delray Beach, FL. pp. 37-42.

Mankin, R. W., E. Petersson, N. D. Epsky, R. R. Heath, and J. Sivinski. (2000b) Exposure to male pheromones enhances *Anastrepha suspensa* (Diptera: Tephritidae) female response to male calling song. Fla. Entomol. 83: 411-421.

Mizrach, A., R. W. Mankin, M. Mazor, A. Hetzroni, D. Shuman, N. Epsky, R. Heath, and J. Grinshpun. (2002). Male song as a tool for trapping female medflies. Binational (US - Israel) Agricultural Research and Development Funds (BARD) Grant No. IS-3137-99. Final Report.

Rossler, Y. (1989). Insecticidal bait and cover sprays. Reprinted from: World Crop Pests, Volume 3B, Fruit Flies, their Biology, Natural Enemies and Control, Robinson and Hooper (eds.). Amsterdam: Elsevier. 329-336.

SAS. 1992. Users guide: Statistics version 6.08. Cary, NC: SAS Institute.

Sivinski, J., C.O. Calkins, and J.C. Webb (1989). Comparisons of acoustic courtship signals in wild and laboratory reared Mediterranean fruit fly *Ceratitis capitata*. Fla. Entomol. 72: 212-214.

Webb, J.C., T. Burk, and J. Sivinski (1983a) Attraction of female Caribbean fruit flies, *Anastrepha suspensa* (Diptera: Tephritidae), to the presence of males and male-produced stimuli in field cages. Ann. Entomol. Soc. Am. 76:996-998.

Webb, J.C., C.O. Calkins, D.L. Chambers, W. Schwienbacher, and K. Russ (1983b) Acoustical aspects of behavior of Mediterranean fruit fly, *Ceratitis capitata*: Analysis and identification of courtship sounds. Entomol. Exp. Appl. 33: 1-8.

SAS. 1992. Users guide: Statistics version 6.08. Cary, NC: SAS Institute.