

10



BARD

FINAL REPORT

PROJECT NO. IS-3137-99

Male Song as a Tool for Trapping Female Medflies

**A. Mizrach, R. Mankin, M. Mazor, A. Hetzroni, J. Grinshpun,
D. Shuman, N. Epsky, R. Heath**

2003

4531 5

**BARD Final Scientific Report
Cover Page**

Date of Submission of the report: December 1, 2002

BARD Project Number: IS-3137-99

PROJECT TITLE: Male song as a tool for trapping female medflies.

Investigators

Institutions

Principal Investigator (PI):

A. Mizrach

ARO. The Volcani Center, Bet Dagan.
Israel

Co-Principal Investigator (Co-PI):

R. Mankin

USDA-ARS Center, Gainesville, FL

Collaborating Investigators:

M. Mazor

ARO. The Volcani Center

A. Hetzroni

ARO. The Volcani Center

J. Grinshpun

ARO. The Volcani Center

D. Shuman

USDA-ARS Center

N. Epsky

USDA-ARS Center

R. Heath

USDA-ARS Center


Keywords: Mediterranean fruit fly, acoustic communication, digital signal processing, insect pest detection, Entomology, Behavior.

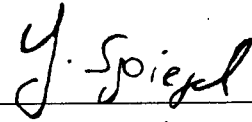
Abbreviations:

Budget: IS: \$ 50,000

US: \$ 50,000

Total: \$ 100,000


Signature
Principal Investigator


Signature
Authorizing Official, Principal Institution



Appendix G6a

1

BARD Final Scientific Report Cover Page

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)				
Submitted, in review, in preparation				
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)		1		

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

James Anderson 587-05-9076

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		2		2
Longer Visits (Sabbaticals)				

Description of Cooperation:

Dr. A. Mizrach from the ARO, Israel visited Drs. R. Mankin, D. Shuman and J. Anderson from the USDA-ARS Center, in Gainesville, FL during the month of July 2001. Dr. A Mizrach attended in the ASAE international meeting in Sacramento, CA during the month of August 2001. Dr. M. Mazor from the ARO visited Drs. R. Mankin, and D. Shuman from the USDA-ARS Center, in Gainesville, and Drs. N. Epsky and R. Heath from USDA/ARS, Subtropical Horticulture Research Station in Miami, FL, during the month of March 2002.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

BARD Final Scientific Report

Abstract

This interdisciplinary work combines expertise in engineering and entomology in Israel and the US, to develop an acoustic trap for mate-seeking female medflies. Medflies are among the world's most economically harmful pests, and monitoring and control efforts cost about \$800 million each year in Israel and the US. Efficient traps are vitally important tools for medfly quarantine and pest management activities; they are needed for early detection, for predicting dispersal patterns and for estimating medfly abundance within infested regions. Early detection facilitates rapid response to invasions, in order to contain them. Prediction of dispersal patterns facilitates preemptive action, and estimates of the pests' abundance lead to quantification of medfly infestations and control efforts. Although olfactory attractants and traps exist for capturing male and mated female medflies, there are still no satisfactorily efficient means to attract and trap virgin and remating females (a significant and dangerous segment of the population). We proposed to explore the largely ignored mechanism of female attraction to male song that the flies use in courtship. The potential of such an approach is indicated by studies under this project. Our research involved the identification, isolation, and augmentation of the most attractive components of male medfly songs and the use of these components in the design and testing of traps incorporating acoustic lures. The project combined expertise in acoustic engineering and instrumentation, fruit fly behavior, and integrated pest management. The BARD support was provided for 1 year to enable proof-of-concept studies, aimed to determine: 1) whether mate-seeking female medflies are attracted to male songs; and 2) over what distance such attraction works.

Male medfly calling song was recorded during courtship. Multiple acoustic components of male song were examined and tested for synergism with substrate vibrations produced by various surfaces, plates and loudspeakers, with natural and artificial sound playbacks. A speaker-funnel system was developed that focused the playback signal to reproduce as closely as possible the near-field spatial characteristics of the sounds produced by individual males. In initial studies, the system was tested by observing the behavior of females while the speaker system played songs at various intensities. Through morning and early afternoon periods of peak sexual activity, virgin female medflies landed on a sheet of filter paper at the funnel outlet and stayed longer during broadcasting than during the silent part of the cycle. In later studies, females were captured on sticky paper at the funnel outlet. The mean capture rates were 67 and 44%, respectively, during sound emission and silent control periods. The findings confirmed that female trapping was improved if a male calling song was played. The second stage of the research focused on estimating the trapping range. Initial results indicated that the range possibly extended to 70 cm, but additional, verification tests remain to be conducted. Further studies are planned also to consider effects of combining acoustic and pheromonal cues.

BARD Final Scientific Report

Achievements

Females attraction to recorded male calling song.

The BARD support was provided for 1 year to enable proof-of-concept studies of whether mate-seeking female medflies are attracted to male songs. "Proof-of-concept" bioassays were conducted to demonstrate attraction of females to recorded male calling song (Appendix No. 1, 2, 3 and 5). The calling songs for use in the bioassay were recorded from medfly males by means of an amplified microphone. Fundamental frequencies of the recorded calling songs taken from sterile males were not significantly different from the wild- and reared-sterile-male results obtained previously (Sivinski et al., 1989). Several different bioassays were conducted, to determine whether females could be attracted to an acoustic trap that broadcast recordings of the male calling song. The first bioassay system used a combination of a speaker and a funnel which broadcast a highly amplified calling song. The recorded signal was played off a personal computer in a continuous loop. A sheet of filter paper was placed near the outlet of the funnel for the female flies to land on. The behavior of virgin female medflies was observed in a net cage during the morning and early afternoon peaks of sexual activity. The total time spent by all flies on the filter paper "leaf" during a bioassay period was summed and divided by the number of flies present in the bioassay chamber to obtain a mean Fly Time on Leaf. Fly behavior was observed during several 10-minute periods with and without calling song, the latter being designated as controls, with ~70 dB background noise level. It was found that the Fly Time on Leaf was < 12 s for the controls, and near 100 s when the sound pressure level exceeded 105 dB, 10 cm from the outlet of the funnel. In a follow-up bioassay, testing was moved to a Plexiglas chamber equipped with net screens on the two narrow sides. A double-sided sticky paper-trapping surface was placed next to the funnel. The signal level was measured at 97-106 dB, at 10 cm from the outlet of the funnel. A second chamber was set up as a control bioassays; it was equipped with an identical funnel and sticky trap, but produced no sound. Twenty-two tests were run for 5-7 h beginning in mid-morning, and the Percentage Capture in each bioassay was measured. The sound treatment was rotated between the two chambers in different tests. The mean capture rates were 67 and 44%, respectively, in the experimental and control chambers. Higher percentages of female medflies were captured on the sticky paper adjacent to the male song. Efficacy evaluation of several pure tones playbacks in attracting female flies showed no significant differences in distributions patterns. Best results achieved with 250-Hz tone (Appendix 5).

BARD Final Scientific Report

Females attracted distance to recorded male song.

BARD support was provided to enable proof of concept studies of the distance over which the females would be attracted (descriptions of equipment, setups and measurements are presented in detail in Appendix No. 3). To estimate the distances over which the sound sources were attractive, we conducted a series of tests in which each cage contained an additional yellow sticky control trap in the middle of the chamber, 70 cm from the source. In 21 tests, both the trap next to the broadcasting source of the male calling song and the trap at the middle of the cage captured significantly greater percentages of females than the control trap at the far end (averages of 35, 24, and 12% for close to speaker, middle and far end, respectively).

Electronic trap design

The use of sticky paper has actually two advantages: it provides an estimate of the populations next to sound source and next to the control; and, at the same time, it serves as a trapping device. It is most unlikely that we would use this method for trapping medflies, and we probably need to eliminate the possibility of using toxic materials for future mass trapping, therefore the use of an electronic fly killer – a zapper – is suggested. After the UV bulbs had been removed, the electrical zapper's screen was installed inside the bioassay screen cage (description of equipment, setups and measurements are presented in detail in Appendix No. 4). Males were put in a small cage within and towards one side of the screen cage, and virgin females were released to the other side. Attracted females which flew toward the calling males, had to pass through the electric zapper screen. The zapping effect occurred when the fly was close as 1 cm from the screens; the electric shock acts quickly, with no burning effect and no sensible smell. Eighteen tests were conducted, 13 regular and five controls; in the latter the cage in male section was empty. Three tests were conducted every morning, around 8, 10 and 11 AM; they each lasted 1 h. The order of the tests was varied, so as to have controls during three difference periods. The activity of the females began when the light was switched on at 8 AM, and this was a time of very intensive female activity. When control tests were done at this time, the intensive activity caused zapping of relatively large number of flies on the control. However, around 11 AM, when female activity had decreased, there was a considerable difference between the numbers of zapped flies near the calling males and near the control. It was concluded that the electrical zapping screen was too big in area for the size of cage used, and an alternative system was installed. Two cylindrical metal screens, 6 cm in diameter and 10 cm long were placed in a screen cage, at opposite sides. A cotton-mesh cage was installed inside each of the two metal

BARD Final Scientific Report

screens, both of which were connected to the electronic zapper. Two calling males were placed in one of the cotton cages, the other of which stayed empty as a control. In this way we examined the attraction response of female by counting the numbers of zapped flies. Significant differences were recorded between the cage of male flies and the control: in 1 h an average of 14 flies were caught by the former, compared with an average of three flies in the latter. We also observed that the percentages of capture were higher in the early morning tests (tests 1 to 7) than in those performed later (tests 8 to 10). This can be accounted for by the increased activity of female fruit flies at dawn. However, 48% of female fruit flies were eliminated in the zapper around the male fly cage compared with 9% in the control, when tests were performed at dawn – 8:00 AM. Obviously, such a trap would be relatively more efficient early in the morning than later in the day. In further work, the live calling males in the cage were replaced with played-back male calling songs, broadcast through the loudspeaker and funnel. We are now analyzing the results obtained in those experiments.

Particle velocity and sound pressure measurements in anechoic and medfly bioassay chambers.

Many insects without tympanal ears do not perceive the pressure component of sound, but instead have movement receptors (usually small hairs on the body or antennae) that are sensitive to sound particle velocity – oscillations of air particles in the sound field. In our laboratory, efforts to develop an acoustic trap for mate-seeking female medflies have centered on utilizing the particle velocity component of the male acoustic calling signal.

Bioassays in 61 x 61 x 152-cm chambers have demonstrated that traps which reproduce male fruit fly calling songs capture virgin female fruit flies. In order better to understand the spatial dynamics of sound particle velocity in these bioassay chambers, simultaneous measurements/recordings of sound pressure and particle velocity were made and compared with recordings made inside an anechoic room. We also recorded particle velocities in the vicinity of a multilure fruit fly trap. The results are discussed in relation to bioassay effectiveness (descriptions of equipment, setups and measurements, and detailed results are presented in detail in Appendix No. 6).

References

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.

BARD Final Scientific Report

Appendix - Table of contents

Appendix 1 - Laboratory establishment in the ARO.	8
Appendix 2 - Females' attraction to recorded male calling song.	9
Appendix 3 - Attraction distance of females to recorded male song.	15
Appendix 4 - Electronic trap design.	17
Appendix 5 - Attraction of females to male courtship song and pure-tone playback.	21
Appendix 6 - Comparison of particle velocity and sound pressure measurements in anechoic and medfly bioassay chambers.	24 24
Appendix 7 - The response of the Female medfly to playback of the male mating call during bioassay experiment – PowerPoint presentation.	33
Acknowledgements -	35

Appendix No. 1

Laboratory establishment in the ARO.

During the first six months of our experiments we established our bioassay setup and constructed the experimental laboratory. Three chambers were built. A Plexiglas chamber measuring 60 x 60 x 150 cm in dimensions (Fig. 1) was used to run the initial entomological experiments, and a screen chamber measuring 30 x 30 x 60 cm (Fig. 2) was used to run initial trapping activities. Another screen chamber, measuring 30 x 60 x 60 cm was built for initial playback experiments.

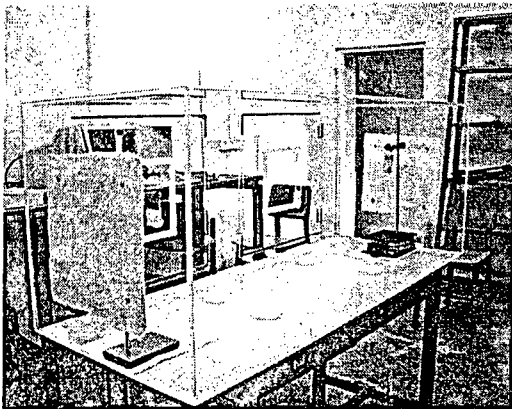


Fig. 1 - Plexiglas chamber.

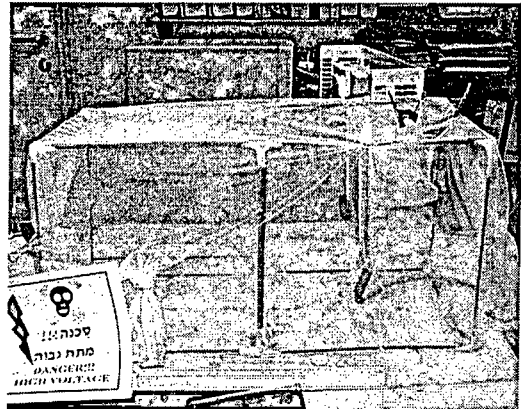


Fig. 2 - screen chamber.

Because of the failure of existing equipment, i.e., an out-of-date amplifier, in our laboratory we obtained a low signal-to-noise ratio in our recordings of male fruit flies. Our attempts at off-line signal cleaning, with the Matlab® software failed. Therefore, existing recordings of male medfly courtship songs, <http://cmave.usda.ufl.edu/~rmankin/medfly11.wav>, produced by the US team, were used. We evaluated the efficacy of several loudspeakers installed in a 30 x 60 x 60-cm chamber on laboratory-reared female medflies. Several off-the-shelf were tested; bare loudspeakers equipped with front-mounted flat membranes, 4-inch car door speaker, embedded box loudspeaker, embedded box loudspeaker with sound concentrator and a subwoofer speaker installed in its original box equipped with bass reflex outlet and produced a high sound pressure field. Most of the followed experimental achieved by using a system of 100-W sub-mid-range 5" loudspeaker originally installed in a wooden box and equipped with a sound concentration funnel.

BARD Final Scientific Report

Appendix No. 2

Females' attraction to recorded male calling song.

"Proof-of-concept" bioassays were conducted, that demonstrated the attraction of females to recorded male calling song.

The calling songs for the bioassay were recorded from sterile males obtained as pupae from the Moscamed Modular Mediterranean Fruit Fly Rearing Facility, El Pino Guatemala (standard Medfly strain, sterilized by a 14.5-kR radiation dose). Three to five 3-day-old male medflies were isolated in a 10 x 10 x 7-cm screened enclosure. A Bruel and Kjaer (B&K) (Naerum, Denmark) model 4145 microphone was positioned 0.5 cm above the male being recorded. The microphone was connected to a B&K measuring amplifier (model 2610; input gain = 20; output gain = 20), and a Krohn-Hite (Avon, MA) model 3100 bandpass filter (0.2-6 kHz). The output was stored on a digital audio tape recorder (DAT) (TEAC model DA-P1, Montebello, CA; input level = 5). Recorded signals were subsequently digitized at 25 kHz, and analyzed by means of the DAVIS insect sound analysis program (Mankin 1994, Mankin *et al.* 2000a, 2000b). The fundamental frequencies of the recorded calling songs were not significantly different from those of the wild- and reared-sterile males, presented in Table 1 from Sivinski *et al.* (1989).

A sample of recorded song is available at <http://cmave.usda.ufl.edu/~rmankin/medfly11.wav>.

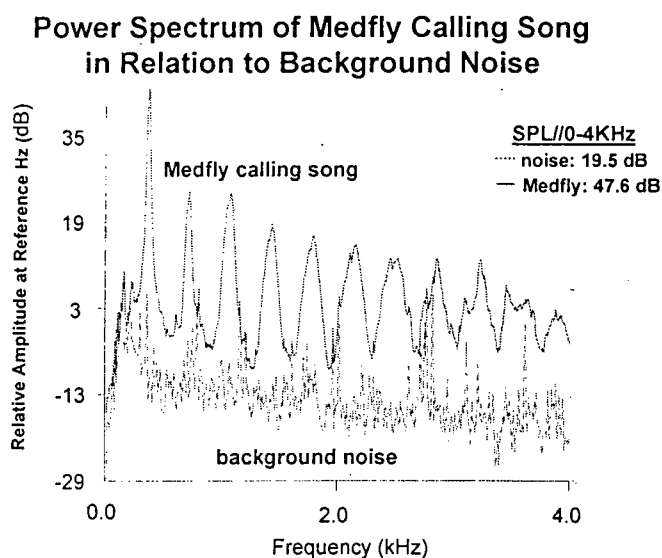


Fig. 3. - Mean power spectrum of a 10-s segment of male medfly calling song: Sound Pressure Level (SPL) and Signal Amplitude are in relative dB scale.

BARD Final Scientific Report

Fig. 3 shows a 520-point mean spectrum of a 10-s sample of song from one male. The peak at 350 Hz is the fundamental frequency, and the subsequent peaks at multiples of 350 Hz are lower-intensity harmonics. In Fig. 4, the sound pressure levels (SPLs) and signal amplitudes are shown on relative scales. Precise measurements of absolute amplitude and SPL have not yet been completed (see, e.g., Mankin *et al.* 2000a).

Two different bioassays were conducted to determine whether females could be attracted to an acoustic trap that broadcast the recorded male calling song. The first bioassay system used a combination of a 20-cm-diameter, 250-W speaker and a 15-cm-long funnel (Fig. 4) that broadcast the calling song at signal levels up to 105 dB, as measured by the B&K model 4145 microphone placed 10 cm in front of the funnel outlet.

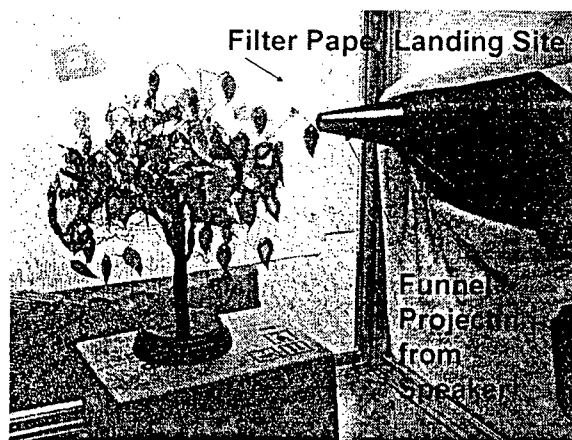


Fig. 4. - Medfly acoustic response observation system, including a speaker-funnel combination producing sound attractive to virgin and remating medflies, and a landing site for attracted flies.

To obtain high sound levels, the signal was amplified with a B&K model 2610 signal amplifier and a Realistic Model SA-102 (Radio Shack, Ft. Worth, TX) speaker amplifier. The recorded signal was played from a personal computer in a continuous loop, by means of Creative Wave Studio software (Creative Technology, Ltd, Singapore). The outlet of the funnel projected near a ~5-cm diameter sheet of filter paper on which the females could land and be observed. (Note: the artificial "tree" at the left, was tested as a visual stimulus in several experiments, but was not particularly effective in attracting flies).

In individual bioassays, the behavior of 20 virgin (3- to 10-day-old) female (sterile) medflies was observed in a 38 x 38 x 46-cm screened cage during the morning and early afternoon periods of peak sexual activity (Arita and Kaneshiro 1989). The total time spent by all flies on the filter

BARD Final Scientific Report

paper "leaf" during a bioassay period was summed and divided by the number of flies present in the bioassay chamber, to obtain a mean *Fly Time on Leaf*. Bioassays were conducted for 10-minute periods, with and without the calling song. The results are summarized in Fig. 5.

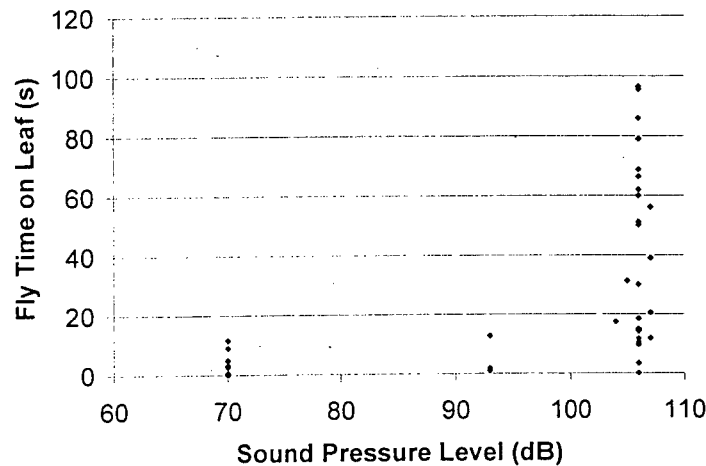


Fig. 5. - Mean Fly Time on Leaf for virgin female medflies exposed to male song at various sound levels.

The mean *Fly Time on Leaf* per bioassay is shown on the vertical axis and the *Sound Pressure Level* (10 cm in front of speaker) on the horizontal axis. The control is at the ~70-dB background noise level. The *Fly Time on Leaf* was < 12 s for the controls with no signal, and it increased to near 100 s when the sound pressure level exceeded 105 dB.

In a follow-up bioassay, testing was transferred to larger Plexiglas chambers (61 x 61 x 152 cm) which were screened on the two narrow sides. A 15 x 15-cm trapping surface was constructed from the double-sided yellow sticky paper (Atlantic Paste and Glue Company, Brooklyn, NY) that is used in dry chemical-attractant medfly traps (Epsky *et al.* 1996). The signal level was measured at 97-106 dB, 10 cm from the outlet of the funnel.

For control bioassays, a second chamber was set up with an identical funnel and sticky trap but no sound. Tests were run for 5-7 h beginning in mid-morning. The *Percentage Capture* in each bioassay was measured as $100 \times (\text{number captured} / \text{number placed in cage})$. Results for 21 tests are shown in Fig. 6.

BARD Final Scientific Report

Results of bioassays carried out in large plexiglass chambers (61x61x152 cm). N (below columns) = total females in each chamber. Male song was played back continuously in the experimental chamber (near the sticky trap) but not in the control chamber.

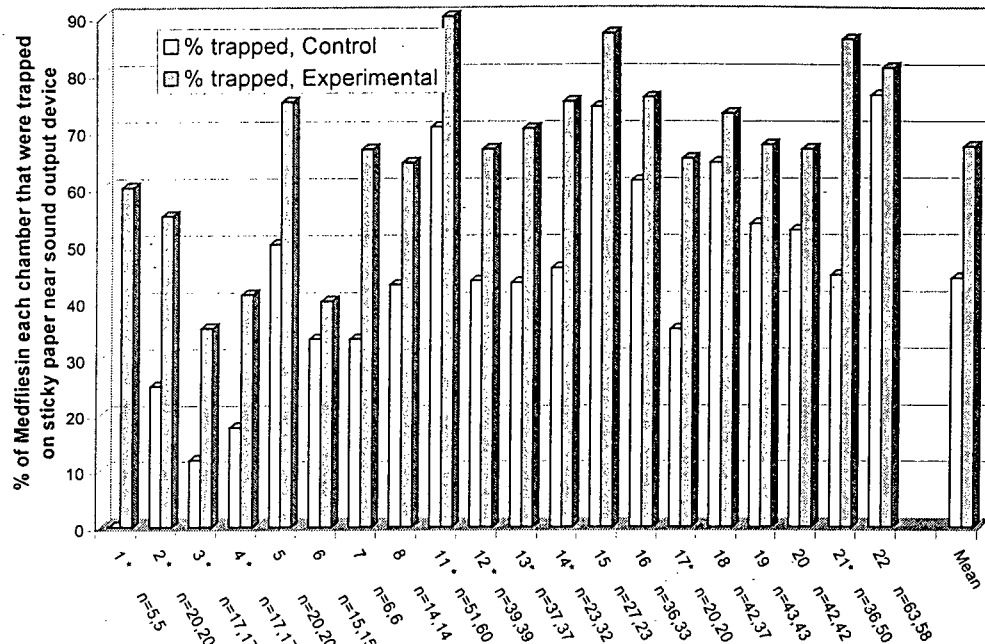


Fig. 6. - Comparison between *Percentage Capture* of virgin female medflies on sticky paper in the presence and absence of broadcast male calling song.

The sound treatment was alternated between the two chambers in different tests. Tests marked with asterisks were conducted with sound broadcast in one of the two chambers; those without asterisks had sound broadcast in the second chamber. The mean capture rates were 67 and 44% in the experimental and control chamber, respectively. Higher percentages of medflies were captured on the sticky paper adjacent to the male song chamber than on that near the control chamber ($t = 4.08$, 19 df, $P < 0.001$ for H_0 : the null hypothesis of no difference between daily captures in the control and experimental chambers).

In a follow-up, bioassay experiments were conducted in a Plexiglas chamber measuring 60 x 60 x 150 cm (Fig. 1), with laboratory flies, calling males and virgin females. Traps were set up in pairs at opposite ends of the chamber. One of each pair contained two laboratory males and the other was empty, as control. Five tests, lasting 1 hour each, were performed with 50 virgin females. Landings of females on the trap containing the males were compared with those on the control. The results are summarized in Fig. 7.

BARD Final Scientific Report

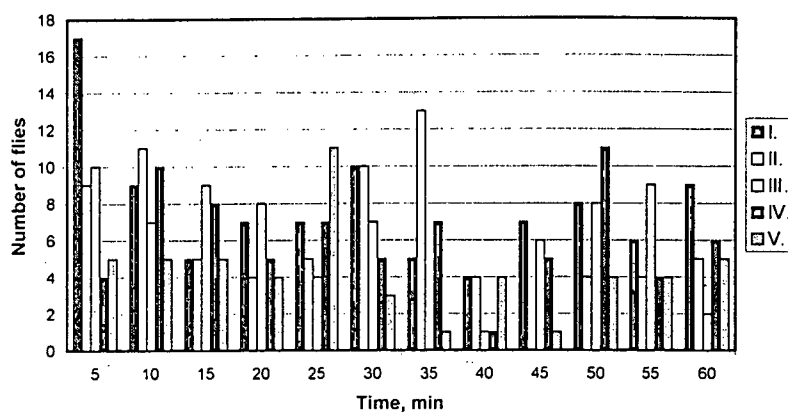


Fig. 7 - One-hour female landing experiments (50 females, two males).

The total numbers of females landing on the cage containing calling males, in each experiment are summarized in Fig. 8.

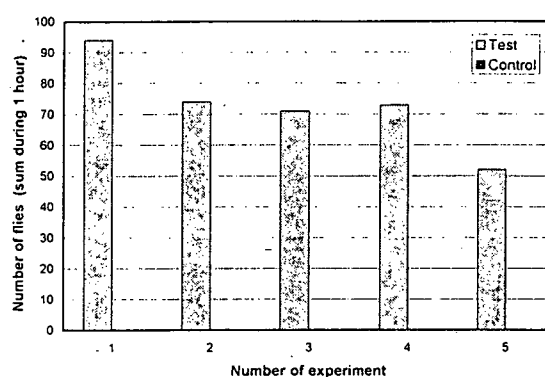


Fig. 8 - Total numbers of females landing on the cage containing the males and on the control for each 1-h experiment (50 females, two males).

The landing count on the control was found to be zero in all tests, but we think that this result means that the landing area was relatively small for the size of cage and the number of flies. We also did some tests with 20 x 30-cm size sticky paper, and caught relatively large quantities of flies on it, but it was impossible to distinguish between landings on the control and those on the calling-males cage. We assume that in this case the area of sticky paper was too large for the cage size, and additional tests have been performed. The results are now being processed and will be summarized soon.

BARD Final Scientific Report

References:

- Arita, L. H., and K. Y. Kaneshiro (1989). Sexual selection and lek behavior in the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *Pacific Science* 43: 135-143.
- Mankin, R. W. (1994). Acoustical detection of *Aedes taeniorhynchus* swarms and emergence exoduses in remote salt marshes. *J. Am. Mosq. Cont. Assoc.* 10: 302-308
- Mankin, R. W., J. Brandhorst-Hubbard, K. L. Flanders, M. Zhang, R. L. Crocker, S. L. Lapointe, C. W. McCoy, J. R. Fisher, and D. K. Weaver. (2000a) Eavesdropping on insects hidden in soil and interior structures of plants. *J. Econ. Entomol.* 93: 1173-1182
- 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.
- 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.

Appendix No. 3**Attraction distance of females to recorded male song.**

In a confirmatory (two-trap) bioassay with virgin sterile female medflies, 15 x 15-cm yellow sticky paper (Atlantic Paste and Glue Company, Brooklyn, NY) traps were set up in pairs at opposite ends of a 61 x 61 x 152-cm screened cage. Three comparisons were performed each day: speaker apparatus broadcasting male calling song (SndApp) at trap vs. control (Cont) trap, speaker apparatus without sound (App) at trap vs. control trap, and control trap vs. control trap. Forty-five tests were performed. The traps next to speakers broadcasting the male calling song (Fig. 9) captured significantly greater percentages of females than the corresponding control traps. Without sound, the percentages captured near the speakers did not differ from those near the paired controls, and there were no differences in percentages captured in two control traps without a speaker apparatus.

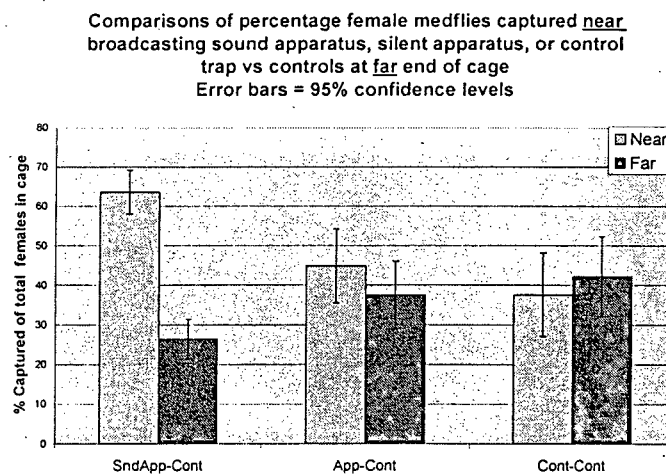


Fig. 9. - Percentages of total numbers of females in cage captured near broadcasting sound apparatus, silent sound apparatus, or a control trap, all at one end of cage, vs. those in a control trap at the far end.

To estimate the distances over which the sound sources were attractive, we conducted a third series of tests, in which each cage contained an additional yellow sticky paper control trap in the middle of the chamber, 70 cm from the source. In 21 tests (Fig. 10), both the trap next to the source broadcasting the male calling song and the one in the middle of the cage captured significantly greater percentages of females than the control trap at the far end.

BARD Final Scientific Report

Comparisons of percentage female medflies captured near broadcasting sound apparatus (Near SndApp) or silent apparatus (Near App) vs. control traps at the middle (Mid) and far (Far) end of cage
Error bars = 95% confidence levels

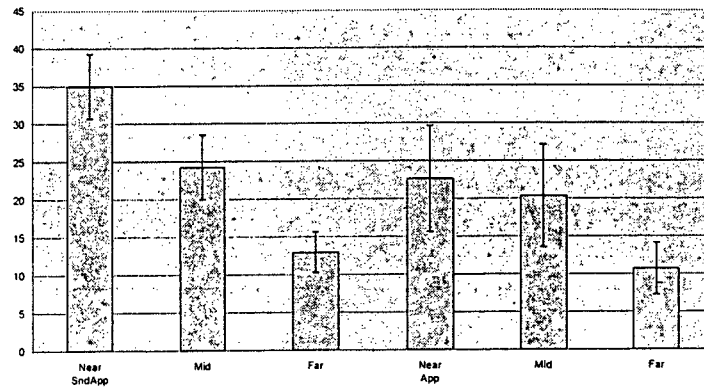


Fig. 10. - Comparisons of the percentages of female medflies captured in two cages with (SndApp) or without (App) male calling song broadcast from a speaker apparatus, and on yellow sticky traps set near the apparatus, in the middle, or at the far end of the cage.

However, the percentage capture of females in the middle trap was not significantly greater than those in traps where a sound apparatus was present but silent at the near end of the cage. Because these tests were somewhat inconclusive, we will need to consider alternative bioassays that estimate the attraction distance more directly.

BARD Final Scientific Report

Appendix No. 4

Electronic trap design.

Another potentially useful trap was constructed by taking apart a TITAN 30 device made by PestWest (<http://www.pestwest.com/>). The bulbs were removed as well. The electronic zapper screen was installed inside a screen cage (Fig. 11).

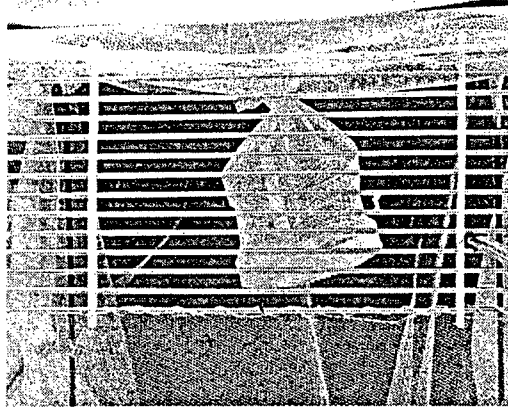


Fig. 11 – Electronic zapping screen in front of calling males cage.
(Flies can be seen for reference of size).

Five males were put in a small cage within and at one side of the screen cage, and 20 virgin females were released into the other side. A curtain was placed between the electrical zapper screen and the female section to avoid initial panic in the screen cage when the flies were loaded into it. Fifteen minutes after loading, the curtain was opened for experiments, each of which lasted 1 hour. Attracted females, which flew toward the calling males section had to pass through the electric zapper screen. The zapping effect occurred within a 1-cm spacing between screen and fly. The electric shock was fast with no burn effect and no smell.

Eighteen tests were conducted, 13 regular and five controls; in the controls, the cage in the male section remained empty. Three tests were conducted every morning, at 8:00, 10:00 and 11:00 AM, and each lasted one hour. The order of the tests was varied so as to have control during each of the three time periods. Average changes of zapped flies and control during 1 hour of experimentation are summarized in Fig. 12.

BARD Final Scientific Report

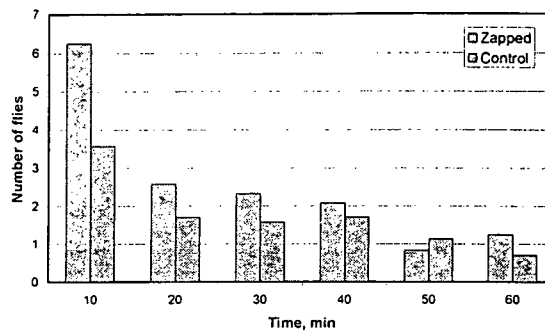


Fig. 12– Average changes of zapped flies during a 1- h experiment.

Figure 13 presents the averages of all zapping and control tests together. The activity of the females started immediately when the light was switched on at 8:00 AM, at which time there appears to have been very intensive female activity.

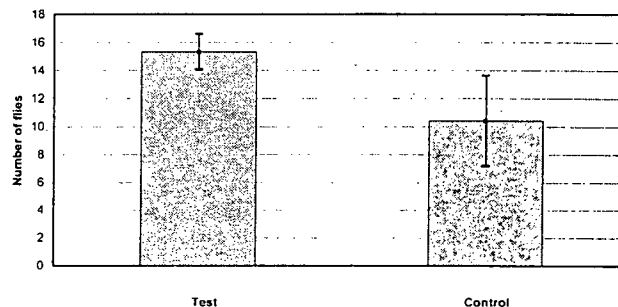


Fig. 13 - Averages of female captures in 1-hour experiments (20 females and five males) Error bars represent 95% confidence levels.

When control tests were done at this hour, the intensive activity caused zapping of relatively high number of flies, as could be seen in the relatively large error bars of the control measurements. We could not distinguish between control and zapped flies at that time. However, at 11:00 AM, when female activity and excitement had decreased, we could see a large difference between the numbers of zapped flies at the calling males cage and those at the control cage. We concluded that the electrical zapping screen was too big in area for the size of cage used, therefore, we installed two cylindrical metal screens, 6 cm in diameter and 10 cm long, one in each of the opposite sides of a new and bigger screen cage (60 x 60 x 150 cm). A cotton-mesh cage was installed inside each of the two metal screens, both of which were connected to the

BARD Final Scientific Report

electronic zapper. Two calling males were placed in one of the cotton cages, the other of which remained empty, as a control. The results of 10 experiments are present in Fig. 14.

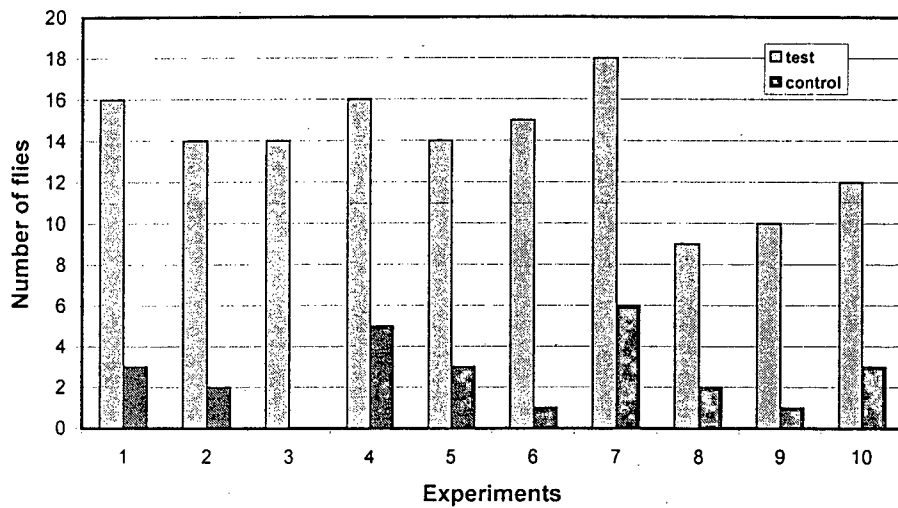


Fig. 14 - Averages of 1-h experiments (20 females and five males); Error bars indicate 95 % confidence levels.
9:00 AM for tests 1 to 7; 11:00 AM for tests 8 to 10.

Significant differences were recorded between the male flies' cage and the control: an average of 14 flies were caught at the former, compared with an average of three in the latter, during 1 h of experiment (Fig. 15). We observed that in the early morning tests (1 to 7) the capture percentages were higher than in the later tests (8 to 10), but we attribute this to the known increase in activity of female fruit flies at dawn.

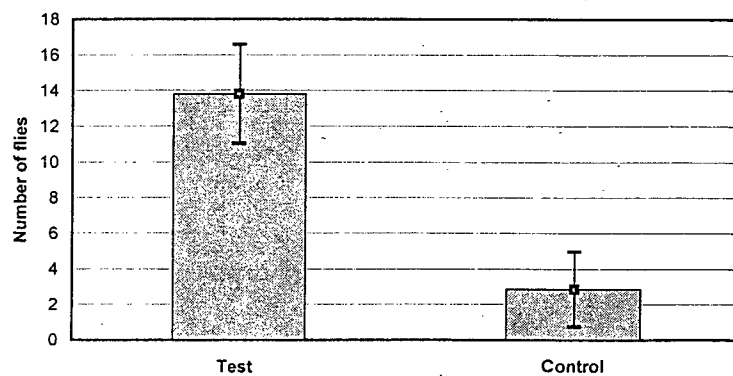


Fig. 15 - Averages of 1-h experiments (20 females and five males) Error bars indicate 95 % confidence levels.
Tests 1 to 10.

BARD Final Scientific Report

However, 48% of female fruit flies were eliminated in the zapper around the male fly cage compared with 9% in the control, when tests were performed at dawn, 8:00 AM (Fig. 16).

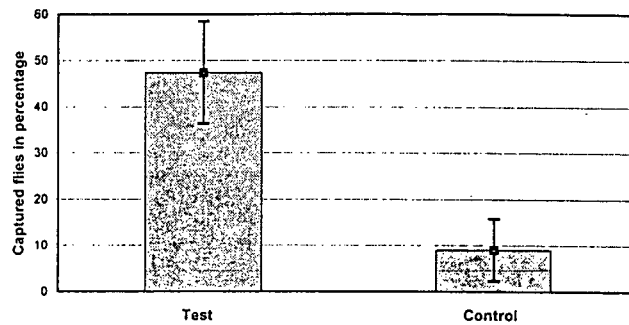


Fig. 16 - Averages of 1-h experiments (20 females and five males) Error bars indicate 95 % confidence levels.
Tests 1 to 8 only.

Obviously, such a trap would be relatively more efficient early in the morning and less during the day. In further work, the live calling males in a cage were replaced with male calling song broadcast through a loudspeaker and funnel. We are working now on the results obtained in those experiments.

Appendix No. 5

Attraction of females to male courtship song and pure-tone playback.

These experiments were intended to evaluate the efficacy of playing male courtship song and several pure tones in attracting female flies. Laboratory female flies (flies reared in the laboratory) and wild female flies (flies that emerged from collected fruits) were used in the experiment. Each experiment involved 20 female flies, which were released in a net cage (30 x 30 x 90 cm). The experiments were repeated five times a day with a new batch of flies each day. Loudspeakers were installed at both ends of the cage (longer dimension), and either one of them was active in any of the various experiments. The length of the cage was virtually divided into three equal sections. Prior to each experiment, the distribution of the flies in the cage was sampled and recorded. The “songs” were played at two sound intensities (67 and 110 dB as measured 100 mm from loudspeaker) and comprised various pure tones which were sine-wave signals at 150, 250, 350, and 450 Hz. (fixed frequencies) and a male courtship song (“US song” is 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. The sections were referred to as ‘loudspeaker’ (the section closest to the active speaker), ‘second part’, and ‘third part’ (the section furthest from the speaker).

Tests with laboratory flies

Average results of attraction of female laboratory flies by male courtship song and by pure tone playback, played at sound intensity levels of 67 and 110 dB are summarized in Figures 17 and 18, respectively.

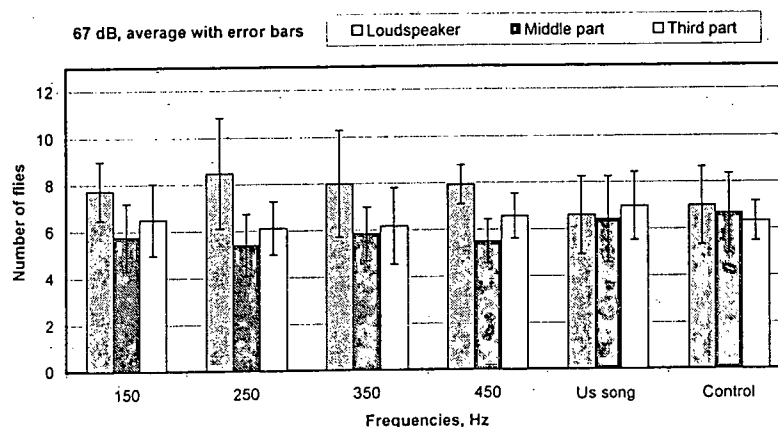


Fig. 17 –Average response of laboratory females to male courtship song and to several pure tones played at 67 dB sound intensity ($\alpha = 0.001$).

BARD Final Scientific Report

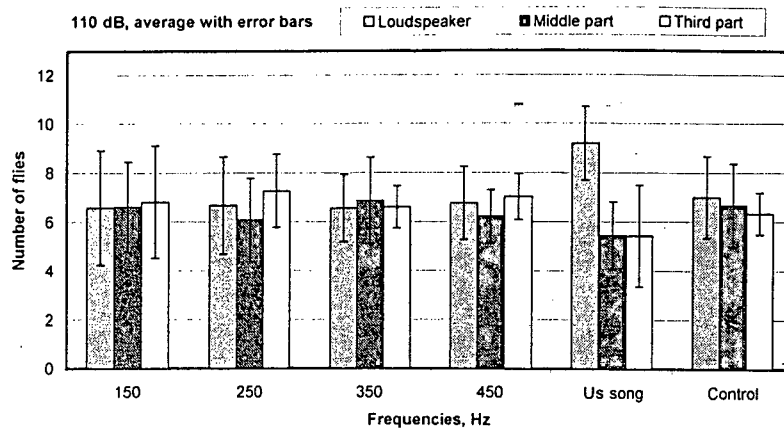


Fig. 18 –Average response of laboratory females to male courtship song and to several pure tones played at 110 dB sound intensity ($\alpha = 0.001$).

Significant differences in fly distribution were observed when “US song” was played at 110 dB: the female flies preferred to stay near the loudspeaker. In the control setup, the flies were evenly distributed at both sound intensity levels. No significant differences in distributions patterns were found in any of the other experiments, although the tones played at 65 dB were marginally attractive to females, with best results for the 250-Hz tone.

Tests with wild flies

Daily results of attraction of wild female flies to playback of pure sine tones and of the male courtship song, played at sound intensity levels of 67 and 110 dB, are summarized in Figures 19 and 20. The left and right sides of each figure present the 67 and 110 dB experiments, respectively. The results with wild female flies are presented here as summaries of the daily raw data. The final analysis has not yet been completed; therefore, no sound contributions can be derived yet.

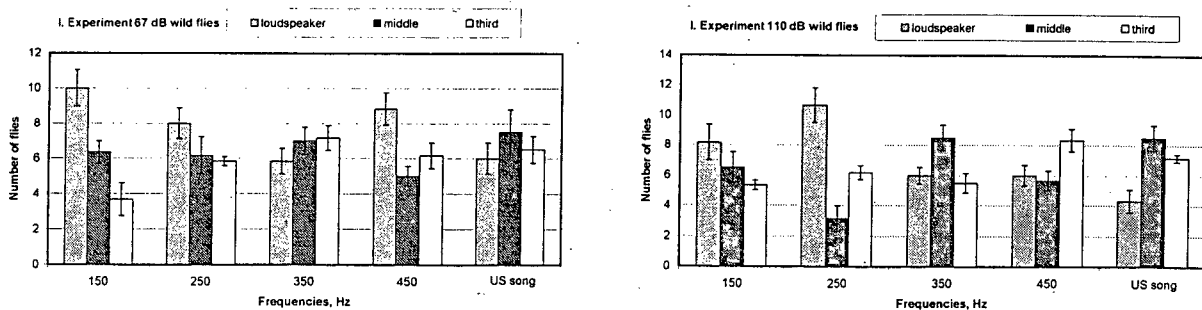


Fig. 19 – First day response of wild females to male courtship song and to several pure tones played at 67 and 110 dB sound intensity ($\alpha = 0.001$).

BARD Final Scientific Report

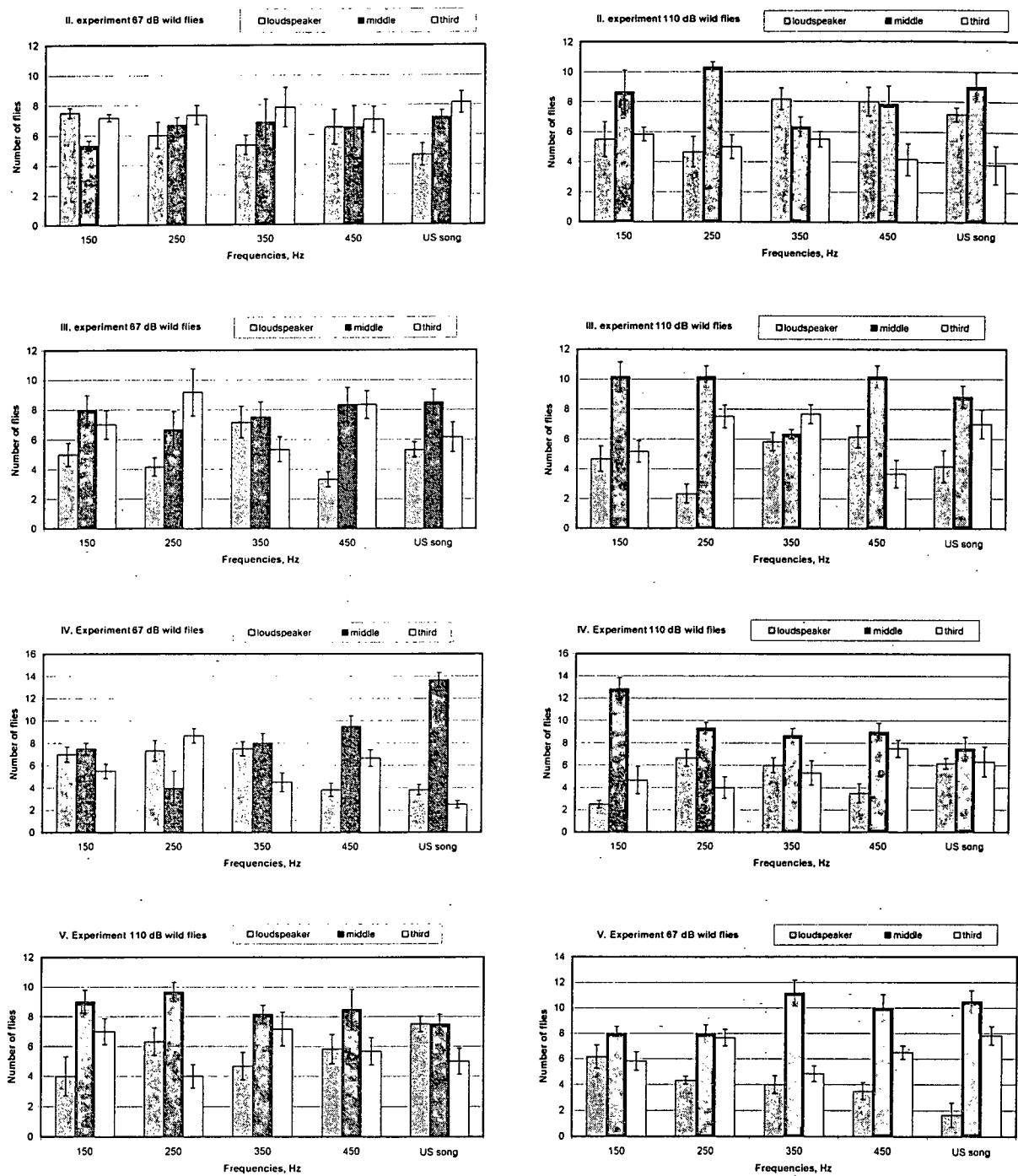


Fig. 20 Second to fifth days' responses of wild females to male courtship song and to several pure tones played at 67 and 110 dB sound intensity ($\alpha = 0.001$).

Appendix No. 6

Comparison of particle velocity and sound pressure measurements in anechoic and medfly bioassay chambers.

J. B. Anderson and R. W. Mankin

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

(Proceedings of the Fourth Symposium on Agroacoustics 8)

ABSTRACT

Many insects without tympanal ears do not perceive the pressure component of sound, but instead have movement receptors (usually small hairs on body or antennae) that are sensitive to sound particle velocity -- oscillations of air particles in the sound field. In our laboratory, efforts to develop an acoustic trap for mate-seeking female medflies have centered on utilizing the particle velocity component of the male acoustic calling signal.

Bioassays in 61 x 61 x 152-cm chambers have demonstrated that traps reproducing male fruit fly calling songs capture virgin female fruit flies. In order to better understand the spatial dynamics of sound particle velocity in these bioassay chambers, simultaneous measurements/recordings of sound pressure and particle velocity were made and compared with recordings made inside an anechoic room. We also recorded particle velocities in the vicinity of a Multilure fruit fly trap. The results are discussed in relation to bioassay effectiveness.

INTRODUCTION

Particle velocity and sound pressure are both quantitative attributes of a sound wave. As the wave moves out and away from its source, air molecules bounce back and forth, jostled by others nearer the sound source, and small variations in ambient air pressure are produced. Most large animals have evolved specialized pressure transducers (a.k.a. ears or tympannae) by which they detect and extract information from these pressure fluctuations. Many small animals, such as ants or fruit flies, without tympanal ears, apparently do not perceive the pressure component of sound, but instead have specialized movement receptors (usually small hairs on body or antennae) that detect sound particle velocity -- the oscillations of air molecules in a sound field (Ewing, 1978; Fletcher, 1978; Tautz, 1979; Towne and Kirchner, 1989; Kamper and Kleindienst, 1990; Humphrey, et al., 1993; Shaw, 1994; Tschuch and Brothers, 1999; Gopfert, 1999; Hickling

BARD Final Scientific Report

and Brown, 2000). Although particle velocity and sound pressure are both attributes of the same sound wave, it is possible that each of these different sensory modalities or 'perspectives' on sound might convey somewhat different subsets of information or possess unique qualities, particularly near the sound source.

In our laboratory, efforts to develop an acoustic trap for mate-seeking female Mediterranean fruit flies have centered on utilizing the particle velocity component of the male acoustic calling signal. The mating system of this major pest insect is complex, involving the formation of male leks, or non-resource-based mating aggregations (Yuval and Hendrichs, 2000) and the use of chemical, acoustic and visual signals to allure and seduce females. Typically, small leks (3-5 males) form in mid-morning on the sunny side of particular trees (Whittier et al., 1992; Shelly et al., 1994). Each male positions himself on the underside of a separate leaf, and everts his balloon-like rectal epithelial sac to release sex pheromone (Prokopy and Hendrichs, 1979; Arita and Kaneshiro, 1989). Males also generate a long, droning, wing vibration or 'calling song' at a mean fundamental frequency of ~ 350 Hz (Webb et al., 1983; Sivinski et al., 1989). When a female approaches by landing on the top of his leaf, the male orients to her shadow, and continues calling until she comes underneath. He then switches to a pulsing 'approach song' (lower in pitch: fundamental frequency ~ 195 Hz.) consisting of short bursts of 'wing fanning', during which gusts of pheromone are directed toward the female (Arita and Kaneshiro, 1989), and moves toward the female, displaying visual signals such as his white 'capitata' (orbital bristles) and 'head rocking' (Holbrook et al., 1970; Landolt et al., 1992). If the female remains receptive, the male leaps onto her back and attempts copulation (which, to be successful in transferring sperm, must last 1-2 hours) (Seo et al., 1990). Female choice is evidently always possible; females can and do walk away at any stage (Shelly, 2000). Courtship in this species has been studied intensively, as the entire success of the SIT (Sterile Insect Technique) program rests on the sexual prowess of the released (sterile) males.

Although some studies have found released males less successful at mating than wild males, the authors could identify no single element of behavior to account for this difference (Liimatainen et al., 1997). They concluded that the timing and correct sequence of behaviors was less organized in mass released males, but some of their findings could be interpreted differently. For example, they found that females did not respond to calling by (unsuccessful) mass released males by approaching (as they did for successful wild males) but only by standing where they

BARD Final Scientific Report

happened to be (Liimatainen et al., 1997). The quality of the male's calling sound could very well be critical in this matter.

We made recordings of male calling songs, and discovered that unmated female medflies were attracted when these were played back through a plastic funnel that was free to vibrate. We have done extensive bioassays in 61 x 61 x 152-cm bioassay chambers to demonstrate that virgin females are captured by fruit fly traps that reproduce male calling song in this manner. In order to better understand the particle velocity patterns to which the females were exposed in this context, we made several simultaneous measurements /recordings of sound pressure levels (SPL) and particle velocity levels (PVL) inside an anechoic room and, within the Plexiglas bioassay chambers. We also made both types of measurements around a Multi-Lure fruit fly Trap (Better World Manufacturing, Fresno, CA).

METHODS

We conducted three types of comparative measurements of both particle velocity and sound pressure: 1. Sound level vs. distance within an anechoic room 2. Sound level at different locations in the medfly bioassay chambers, and, 3. Measurements 1 cm from the top opening of a Multi-Lure fruit fly Trap (MLT) in the anechoic room, positioned over a meter from the sound source.

As a sound source, we used a simple tone at ~ 350 Hz. generated with either a function generator (Leader Model LFG1300S) or an "acoustic laser" (small thermoacoustic engine) (Garrett and Chen, 2000; Garrett and Backhaus, 2000, or we used a recording of the male calling song (e, g., <http://cmave.usda.ufl.edu/~rmankin/soundlibrary.html>). Sound pressure levels (SPL) were measured with a B&K model 4145 microphone, and particle velocity levels (PVL) were measured with a Microflown half-inch ICP® probe (Microflown Technologies, Eisenstraat, The Netherlands). These signals were recorded with a B&K Model 2639 preamplifier, and a digital audio tape recorder (DAT) ((TEAC model DA-P1, Montebello, CA; input level = 5), subsequently digitized at 25 kHz and analyzed using the DAVIS insect sound analysis program (Mankin, et al., 2000a, b), as well as a speech analysis system (CSL Model 4300B) (Kay Elemetrics Corp., Lincoln Park, NJ).

RESULTS AND DISCUSSION

Anechoic room: attenuation with distance

We found that, under simple conditions (i.e., far field, no echoes), simultaneous measurements of sound pressure levels (SPL) and particle velocity levels (PVL) are very equivalent. The two types of measurements in the anechoic room matched almost exactly except at near field distances, where the PVL component, as expected, showed increasingly higher values closer to the sound source (Figure 21). Outside the near field, both measurements showed attenuation proportional to $(\text{distance})^2$ that was expected.

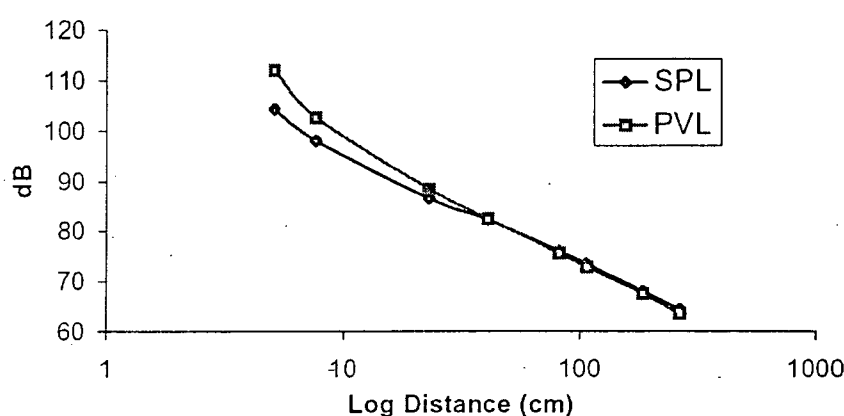


Fig. 21. - Measurements of sound pressure levels (SPL) and particle velocity levels (PVL) at different distances from a single sound source (350 Hz. tone) in an anechoic room.

The fact that PVL attenuates more rapidly than SPL in the near field has led to the common assumption that, as an effective sensory modality, particle velocity is limited to short distances. However, as our results clarify, outside the near field, PVL attenuates at the same rate as SPL and is, at least potentially, discernable by insects with movement receptors specialized to detect it. The addition of the funnel to the speaker amplified the sound of a 350 Hz. tone about 10 dB (at a sound level of ~65 dB) for both SPL and PVL.

Inside the Plexiglas bioassay chambers, neither of the types of measurement showed much attenuation with distance (Figure 22) Even within chamber #1 containing the sound apparatus, both measurements were relatively uniform across the chamber, especially in the case of PVL, with SPL showing a pattern of lower values in the center of each chamber. The room length was

BARD Final Scientific Report

2.44 m, almost exactly 2.5 times the wavelength of the 350 Hz. signal, (0.984m) which could lead to a strong standing wave resonance effect within each room, and account for the uniformity of the sound level measurements. Because of the spatial uniformity of the signal, the females did not have good spatial cues to identify the location of the sound except where the sound was most intense, within a few cm of the source.

Bioassay chambers: spatial variation in sound levels

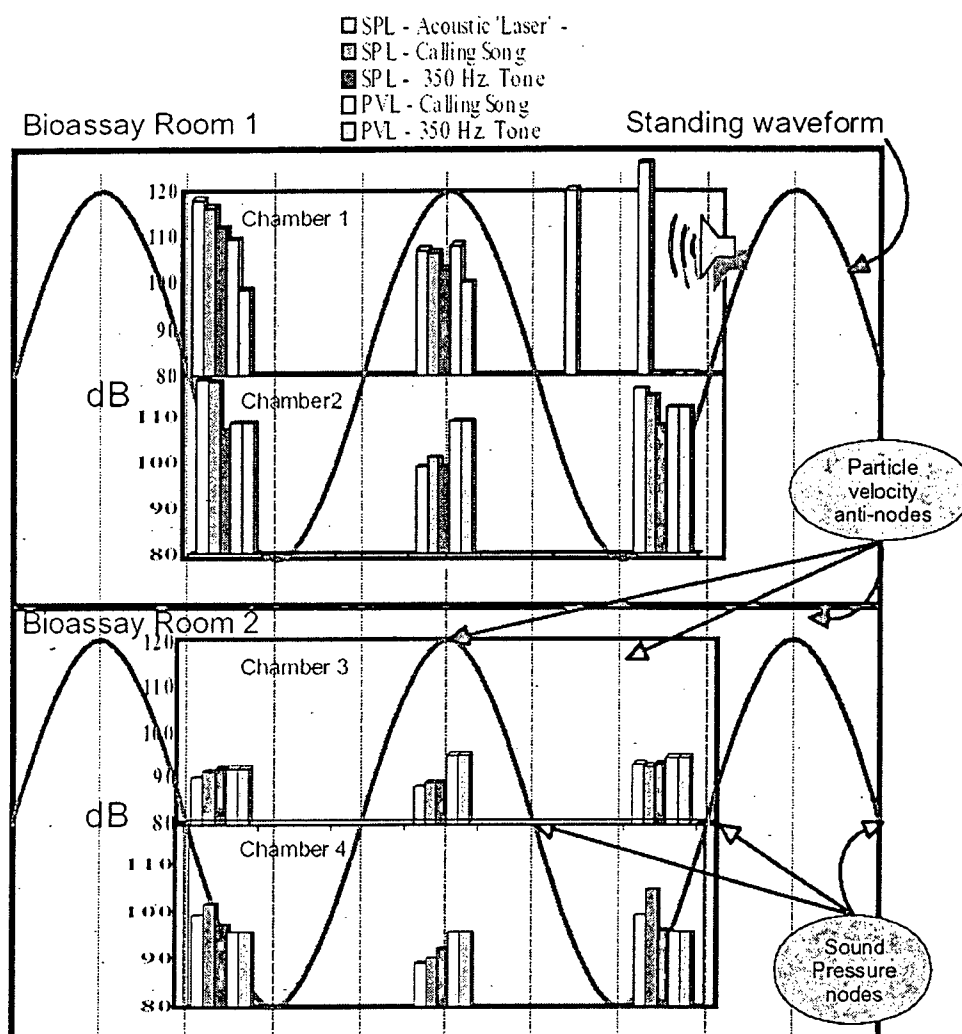


Fig. 22. - Sound Pressure Levels (SPL) and Particle Velocity Levels (PVL) in Medfly bioassay arena with two chambers stacked on each other in two adjacent rooms. The 350 Hz standing wave is drawn to scale and predicted zones of high and low SPL and PVL are identified.

The sound pressure levels and the pressure velocities had different spatial distributions in the chambers. This difference derives partly from the fact that sound pressure and particle velocity

are 90degrees out of phase, and under resonant conditions the spatial variations in each of these parameters caused by wave interference would be segregated by a quarter wavelength (with a 350 Hz. tone, this is 24.57 cm).

Multi-lure fruit fly trap: resonance near upper opening

The largest, divergence in our comparisons of sound pressure levels (SPL) and particle velocity levels (PVL) occurred at the small, upper opening of the Multi-Lure Trap (MLT). Both parameters were measured 1cm from this opening when either a 350 Hz. tone or Medfly song was played from a speaker over a meter away. Figure 23 shows the surprising results: a large increases in PVL but almost no change in SPL. Figure 23, A & B shows recordings made while the spherical plastic trap, hanging from a string, was made to rotate so that the upper opening at each revolution passed 1 cm from the B & K microphone (SPL) and the Microflown (PVL). The SPL hardly changed as the opening rotated past, whereas the PVL increased more than 2-fold. Figure 23D shows a frequency scan (from 280 - 380 Hz. over ten seconds) with the trap stationary and both sensors positioned 0.5 cm from the opening. The frequency of highest PVL resonance is around 300 Hz., but somewhat lower with respect to SPL (~ 290 Hz.).

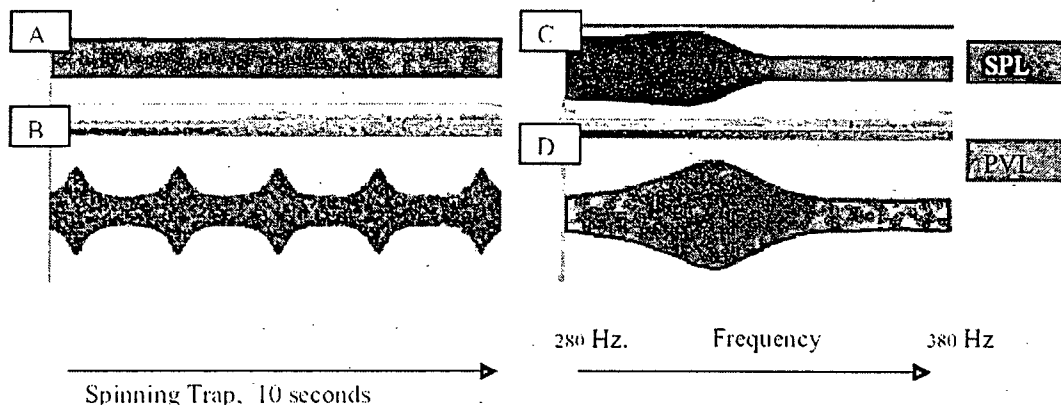


Fig. 23. - Measurement of SPL (3.A&C) and PVL (3.B&D) recorded near the upper opening of a MultiLure fruit fly trap in an anechoic room 107 cm from a sound source (350 Hz. tone). 3A and B were measured 1 cm from a spinning trap during a 10-s interval. 3C and D were recorded 0.5 cm from the opening of a stationary trap, during a frequency scan (sound source changing from 280 to 380 Hz. over 10 seconds).

This is another example of the spatial segregation of sound pressure (nodes) and particle velocity (anti-nodes) under standing wave conditions. In the presence of an ambient 350 Hz.

BARD Final Scientific Report

tone, the area around the trap opening showed a high standing PVL, with the air oscillating back and forth out of the opening. Zones of high SPL should exist inside (with denser accumulations of molecules and not much air movement), where maximum air compression occurs at each cycle of vibration. These sound pressure nodes, locations of standing sound pressure maxima, like those mapped out in the bioassay room (Figure 22), will be loud to animals with eardrums. On the other hand, to insects having movement receptors for 'hearing' sound particle velocity, the opening of a resonator (a PVL anti-node) must also have a 'loud' or unusual sound.

REFERENCES

- Arita, L. H., and K. Y. Kaneshiro (1989). Sexual selection and lek behavior in the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *Pac. Sc.* 43: 135-143.
- Ewing, A. W. (1978). The antenna of *Drosophila* as a 'love song' receptor. *Physiol. Entomol.* 3: 33-36.
- Fletcher, N. H., (1978). Acoustical response of hair receptors in insects. *J. Comp. Physiol. A.* 127:185-189.
- Fletcher, B.S. (1989). Movements of tephritid fruit flies. In A.S. Robinson and G. Hooper (eds): *Fruit flies; their biology, natural enemies and control*. Amsterdam: Elsevier, pp. 209-219.
- Garrett, S.L., and R. Chen. (2000). Build an "Acoustic Laser" *Echoes.* 10, 3. p.4-5.
- Garrett, S.L., and S. Backhaus. (2000). The Power of Sound. *American Scientist.* 88, 516-525.
- Gopfert, M.C. H. Briegel, and D. Robert (1999). Mosquito hearing: Sound-induced antennal vibrations in male and female *Aedes aegypti*. *J. Exp. Biol.* 202: 2727-2738.
- Hendrichs, J. (1996) Action programs against fruit flies of economic importance: session overview. In B.A. McPherson and G.J. Steck (eds): *Fruit fly pests; a world assessment of their biology and management*. Delray Beach: St. Lucie Press, pp. 513-519.
- Hickling, R., and R.L. Brown (2000). Analysis of acoustic communication by ants, with particular application to *Solenopsis richteri*. *J. Acoust. Soc. Amer.* 108: 1920-1929.
- Holbrook, F. R., L. F. Steiner, and M. S. Fujimoto. (1970). Mating competitiveness of Mediterranean fruit flies marked with fluorescent powers. *J. Econ. Entomol.* 63: 454-455
- Humphrey, J.A.C., R. Devarakonda, I. Iglesias, and F.G. Barth. (1993). Dynamics of arthropod filiform hairs. I. Mathematical modeling of the hair and air motions. *Phil. Trans. R. Soc. Lond. B.* 340: 423-444.
- Kamper, G., and H.-U Kleindienst (1990). Oscillation of cricket sensory hairs in a low frequency sound field. *J. Comp. Physiol.* 167: 193-200.
- Landolt, P.J., R.R. Heath, and D.L. Chambers. (1992) Oriented flight responses of female Mediterranean fruit flies to calling males, odor of calling males, and a synthetic pheromone blend. *Entomol. Exp. Appl.* 65: 259-266.

BARD Final Scientific Report

- Liimatainen, J., A. Hoikkala, and T. Shelly. (1997). Courtship behavior in *Ceratitis capitata* (Diptera, Tephritidae) Comparison of Wild and Mass-Reared Males. *Ann. Entomol. Soc. Am.* 90: 836-843.
- Mankin, R. W., J. Brandhorst-Hubbard, K. L. Flanders, M. Zhang, R. L. Crocker, S. L. Lapointe, C. W. McCoy, J. R. Fisher, and D. K. Weaver. (2000a) Eavesdropping on insects hidden in soil and interior structures of plants. *J. Econ. Entomol.* 93: 1173-1182.
- 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.
- Penrose, R. (1993) The 1989/1990 Mediterranean fruit fly eradication program in California. In M. Aluja and P. Liedo (eds): Fruit flies; biology and management. New York Berlin Heidelberg London Paris Tokyo Hong-Kong Barcelona Budapest: Springer-Verlag, pp. 401-406.
- Penrose, D. (1996) California's 1993/1994 Mediterranean fruit fly eradication program. In B.A. McPherson and G.J. Steck (eds): Fruit fly pests; a world assessment of their biology and management. Delray Beach: St. Lucie Press, pp. 551-554.
- Prokopy, R. J. and J. Hendrichs (1979). Mating behavior of *Ceratitis capitata* on a field-caged tree. *Ann. Entomol. Soc. Amer.* 72: 642-648.
- 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.). Elsevier Sci. Publishers B.V., Amsterdam. 329-336.
- Seo, S. T., R. I. Vargas, J. E. Gilmore, R. Kurashima, and M. S. Fugimoto. (1990). Sperm transfer in normal and gamma-irradiated laboratory-reared Mediterranean fruit flies (Diptera: Tephritidae). *J. Econ. Entom.* 83: 1949-1953.
- Shaw, S.R. (1994) Detection of airborne sound by a cockroach 'vibration detector': A possible missing link in insect auditory evolution. *J. Exp. Biol.* 193: 13-47.
- Shelly, T. E., T. S. Whittier, and K. Y. Kaneshiro. (1994). Sterile insect release and the natural mating system of the Mediterranean fruit fly *Ceratitis capitata* (Diptera, Tephritidae). *Ann. Entom. Soc. Amer.*, 87: 470-481.
- Shelly, T. E.. (2000). Male signaling and lek attractiveness in the Mediterranean fruit fly. *Anim. Behav.* 60: 245-251.
- 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.
- Tautz, J. (1979) Reception of particle oscillation in a medium -- An unorthodox sensory capacity. *Naturwissenschaften* 66: 452-461.
- Towne, W.F., and W.H. Kirchner (1989) Hearing in honey bees: Detection of air-particle oscillations. *Science* 244: 686-688.
- Tschuch, G., and D. J. Brothers (1999) Modeling vibration and sound production in insects with nonstridulatory organs. *J. Acoust. Soc. Amer.* 106: 3706-3710.


BARD Final Scientific Report

- Webb, J.C., C.O. Calkins, D.L. Chambers, W. Schwienbacher, and K. Russ (1983) Acoustical aspects of behavior of Mediterranean fruit fly, *Ceratitis capitata*: Analysis and identification of courtship sounds. *Entomol. Exp. & appl.* 33: 1-8.
- Whitier, T. S., F. Y. Nam, T. E. Shelly, and N. Y. Kaneshiro. (1994). Male courtship success and female discrimination in the Mediterranean fruit fly (Diptera: Tephritidae). *J. Insect Behav.*, 7: 159-170.
- Yuval, B., and J. Hendrichs, 2000. Behavior of flies in the genus *Ceratitis* (Dacinae: Ceratitidini) In: Fruit flies in the genus *Anastrepha* (Tephritidae): phylogeny and evolution of behavior, M. Aluja and A.L. Norrbom [eds], CRC Press, Boca Raton, FL. Pp. 459-489.

Appendix No. 8

The response of the Female medfly to playback of the male mating call during bioassay experiment – PowerPoint presentation

The Response of the Female Medfly, *Ceratitis capitata*, to the Playback of the Male Mating Call during Bioassay Experiments



Janet Lane
In collaboration with Dr.
Richard Mankin and Dr. Jim
Anderson
USDA-ARS, CMAVE
June – August 2002
Gainesville, Florida

Methods: Bioassay Chambers

Plexiglass chambers = 61x61x154 cm

2 Rooms, with a Top and Bottom Chamber in Each Room

Sticky Paper on Far Ends to Catch Flies

Rooms Face East and West

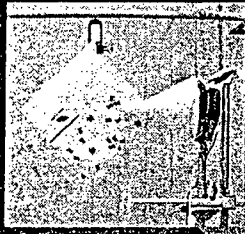
Food and Water Provided

Chambers were modified from previous experiments to add air ducts for pheromone experiments.

Possible Solutions under Study

- Play a recorded male medfly call of $F_0 = 350$ Hz to attract virgin or re-mating females to a sticky paper trap
- Combine the sound with the male sex pheromone

= Artificially simulating a male lek may attract females to traps



Bioassay Chamber Modifications

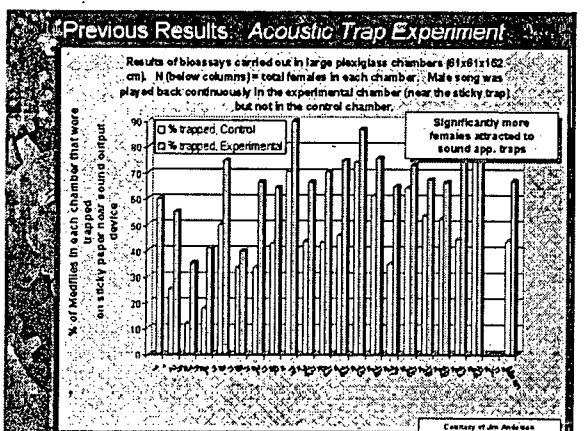
Chambers No Longer Directly Contored under Fluorescent Lights

Exhaust Tube for Pheromone Experiment

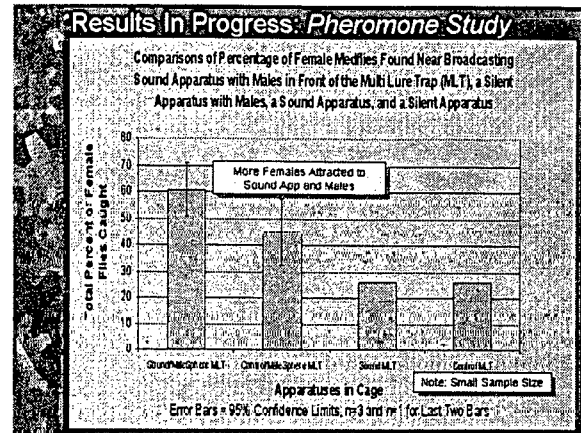
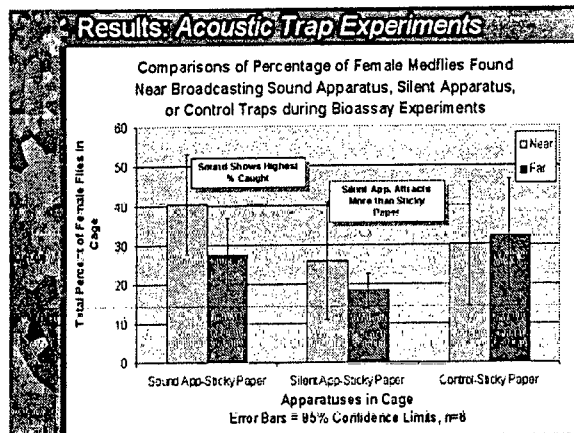
Plexiglass End Closes off Cage End

Hypotheses

- Female medflies will be attracted more to traps that have the recorded male sound than without sound.
- Female medflies will be attracted to traps at a higher rate if male medflies, that release sex pheromones, are added to the traps with sound.

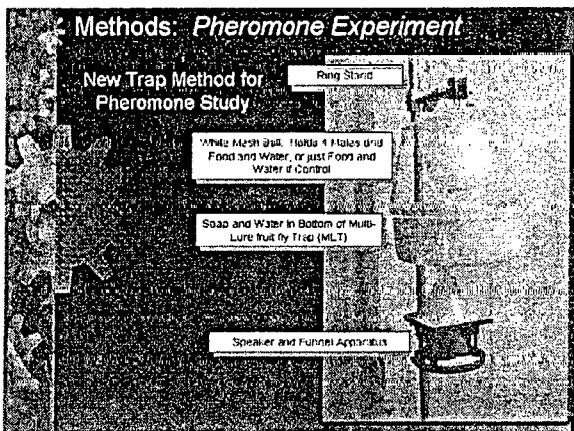


BARD Final Scientific Report



- ### Conclusions: Acoustic Trap Experiments
- When the sound was off, the females' location in the cages was = not statistically significant.
 - Sound apparatus collected the most flies, in comparison to the silent apparatus, or the sticky paper itself = not statistically significant.
 - The silent apparatus trap collected more flies than the end away from the trap, or by the sticky paper itself = flies attracted to the apparatus?
- Larger Sample Sizes are Needed

- ### Conclusions: Pheromone Experiment
- More flies attracted to the sound app. + males, than without sound = not statistically significant
 - More flies attracted to the sound app. + males, than to apparatuses without males = statistically significant
 - More flies attracted to silent app. + males than apparatuses without males = not statistically significant
 - Same # flies attracted to sound app. and the MLT trap itself
- Larger Sample Sizes are Needed



- ### Future Studies
- ✓ Resolve possible confounding variables:
 - light
 - temperature
 - airflow from exhaust fan
 - cage location/end effect
 - ✓ Determine at what distance the sound effectively attracts females.
 - ✓ Substitute live males with a visual stimuli of a male to see if females attracted.

BARD Final Scientific Report

Acknowledgements

This study was funded in part by a BARD Research Grant (No. IS-3137-99), to Amos Mizrach, Principal Investigator, Israel, and Richard Mankin, USA).

Special thanks to Timea Ignat, visiting research associate, for her significant contribution to the project.

We appreciate the expert technical assistance of Alexander Piesachis and Victor Poltorak at the department of Entomology, ARO, Israel and of Everett Foreman, from the Center for Medical and Veterinary Entomology (CMAVE) Gainesville, FL. We thank Stephen Garrett for his custom built, 'acoustic laser'.