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Prospecting for Attractants for the Ten-lined June beetle

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Introduction. The ten-lined June beetle (TLJB) is a chronic problem in orchards where it occurs. A member of the beetle family Scarabaeidae, its larvae, known as grubs, feed in the soil for a two-year period on the roots of several commercial tree species including almonds. TLJB incidence is believed to be increasing, and damage is reported in orchards from San Joaquin Co. to as far south as Fresno Co. When adult beetles emerge in summer they may be most susceptible to control measures (as control of larvae in the soil has proven difficult or even ineffective). To control adult populations, attractants (pheromones and other semiochemicals) are highly desired. The major goal of this research is to develop new attractants for monitoring populations of the TLJB to assist in adult treatment timing. In addition, the feasibility of using the pheromone for mass trapping of TLJB will be also explored.

Results. To improve GC-EAD performance and facilitate the identification of the female-produced pheromone constituent(s), we have studied the morphology of male and female antennae. We observed that the second lamella of the male antennae is markedly different from female lamella (Fig. 1) and entirely covered by olfactory plates (pheromone detectors). A highly sensitive GC-EAD system was made possible by using the second lamella as the sensing element. From a viewpoint of practical application, the exquisite olfactory system in the TLJB has advantaged and disadvantages. While insects having extremely sensitive pheromone systems are prone to manipulation (control) with synthetic chemicals (pheromones), isolation and identification of the active compounds may be challenging. To identify a pheromone, it is essential to detect an active peak so as to get structural information (mass spectral data, infrared spectroscopy information, chemical derivatization, etc), particularly when the pheromone is

novel. TLJB males are so sensitive to the female-produced pheromone that they can detect amounts below the detection limits of analytical instruments.

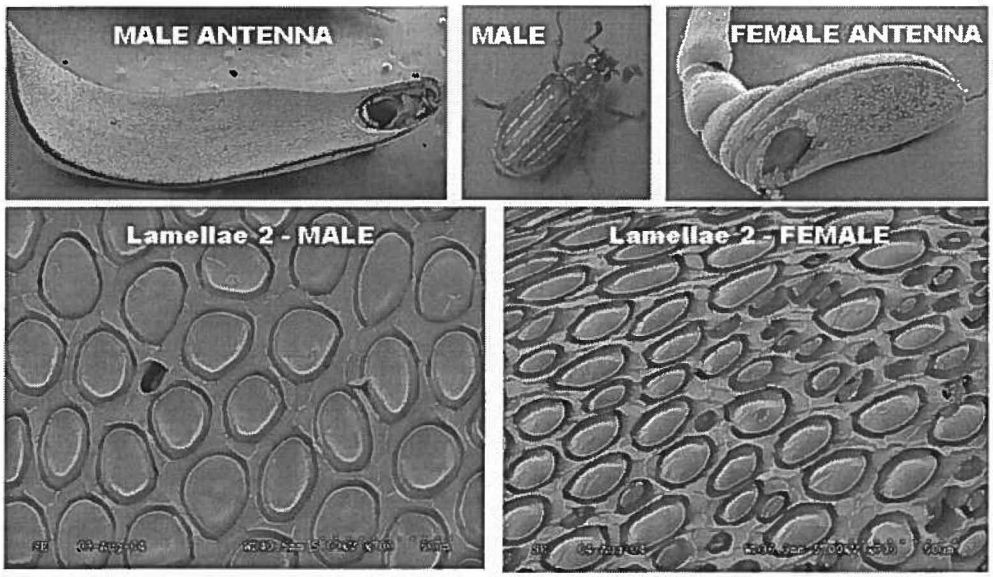

Figure 1.Scanning electronic micrographs of the TLJB male and female antennae.

We have been able to identify the retention time of the TLJB pheromone while monitoring the activity by GC-EAD, but we can not "see" the active peak (Fig. 2) by a conventional GC detector (flame ionization detector, FID).

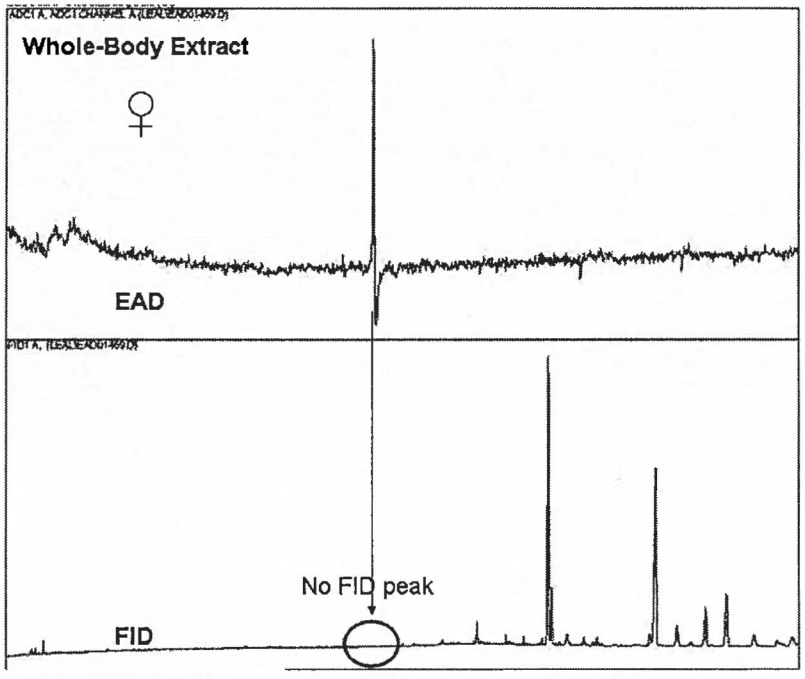

Figure 2. GC-EAD experiments obtained with whole body (hexane) extract of field-collected TLJB females. A strong EAD-active peak indicates the occurrence of pheromone in the extract; the amount is so low that it could not be detected by FID.

We tried to circumvent this barrier by comparing the retention time of the pheromone with the retention times of all known scarab beetle pheromones. Unfortunately, none of the previously identified scarab pheromones has a retention time that matches that of the TLJB. Therefore, we were left with the task of identifying a novel pheromone from a species that produces only scant amounts of the active compound.

Initially, we concentrated our efforts on the isolation of the pheromone by airborne volatile collections. Chemicals in the air circulating through a glass apparatus housing field-collected females were trapped on a polymer (Super Q), washed out with an organic solvent (hexane), and analyzed by GC-EAD. These "aerations" collect not only pheromones, but also compounds excreted by insects, metabolites, etc. While the extracts generated by aerations of female TLJB were behaviorally and EAD active, the amounts of collected pheromones were extremely low. Next, we explored the possibility of increasing the yields by whole-body extractions. As shown above (Fig. 2), the female-produced pheromone can be extracted with hexane or ether. Similar extracts from male beetles (Fig. 3) indicated that the EAD-active peak (as seen in Fig. 2) is indeed due to a female-produced sex pheromone. The amounts obtained by whole-body extractions are, however, below the detection limit of state-of-the-art analytical instruments. Thus, no FID peaks were detectable.

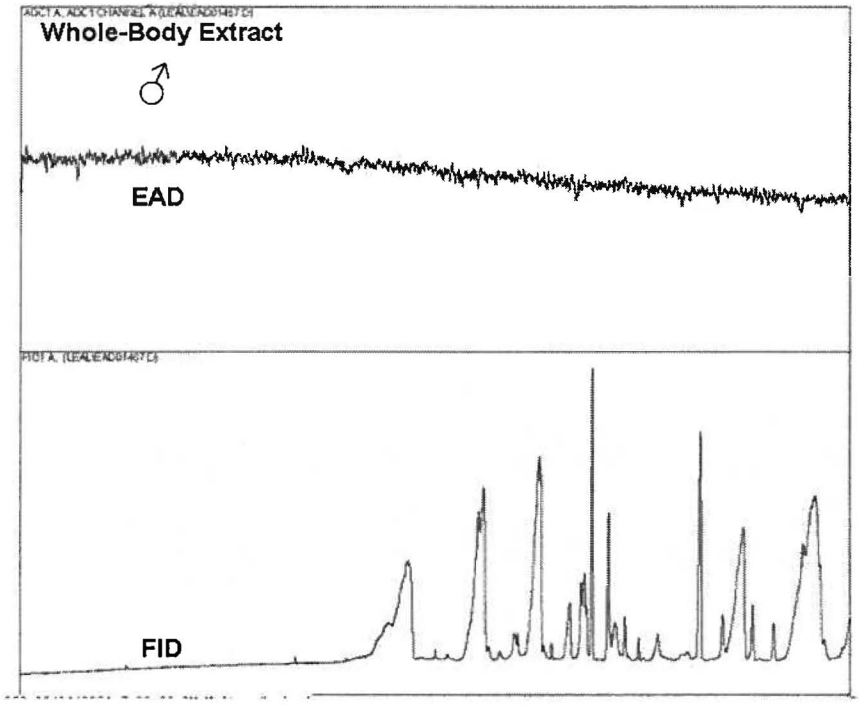

Figure 3. GC-EAD of the whole-body extract of TLJB males.

As in 2003, we ran black light traps in 2 commercial almond orchards (Manteca and Escalon) from May (before adult beetles emerge) through the end of September to establish seasonal activity of adults. Contrary to previous report, male flight activity was observed from late May to early September, with a peak of TLJB population in the end of July (Fig. 4). As in 2003, all of the beetles captured in the light traps were males. We also collected males and females in the orchard on a number of occasions at sunset and early evening for the laboratory studies, but also made a number of interesting and useful behavioral observations. Our field observations this season confirmed that females of the TLJB spent most of their adult life time buried in the soil. Soon after sunset, females emerge, mate, and immediately return to the soil to lay eggs. They remain buried until the following night. It is, therefore, logistically difficult to collect a large number of live females for pheromone extraction because they come out of the soil for a very short period of time.

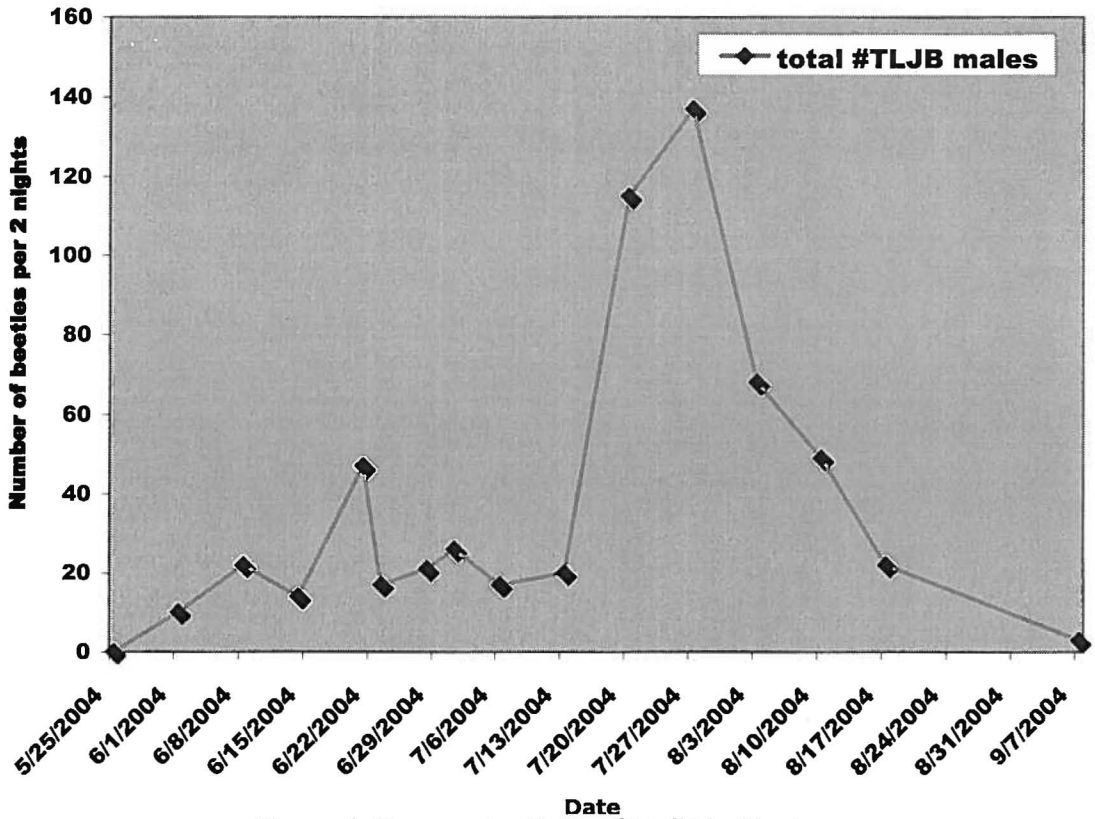

Figure 4. Seasonal activity of TLJB in Manteca

Surprisingly, we observed males in the field trying to copulate with debris of dead females. For example, we observed in many instances males being attracted to elytra of dead females (which were the only body parts left after the beetles were scavenged by ants). Interestingly, the behavior of the approaching males was similar to that of males approaching calling females. This observation led us to

explore the possibility of extracting pheromone from dead females. Indeed, hexane extracts from dead females showed that the active compound remains (and can be extracted) from dead females (Fig. 5). This unexpected source of pheromone is now being pooled with pheromone extracted from live females so as to allow accumulation of enough material for structural elucidation (chemical identification of the TLJB sex pheromone.

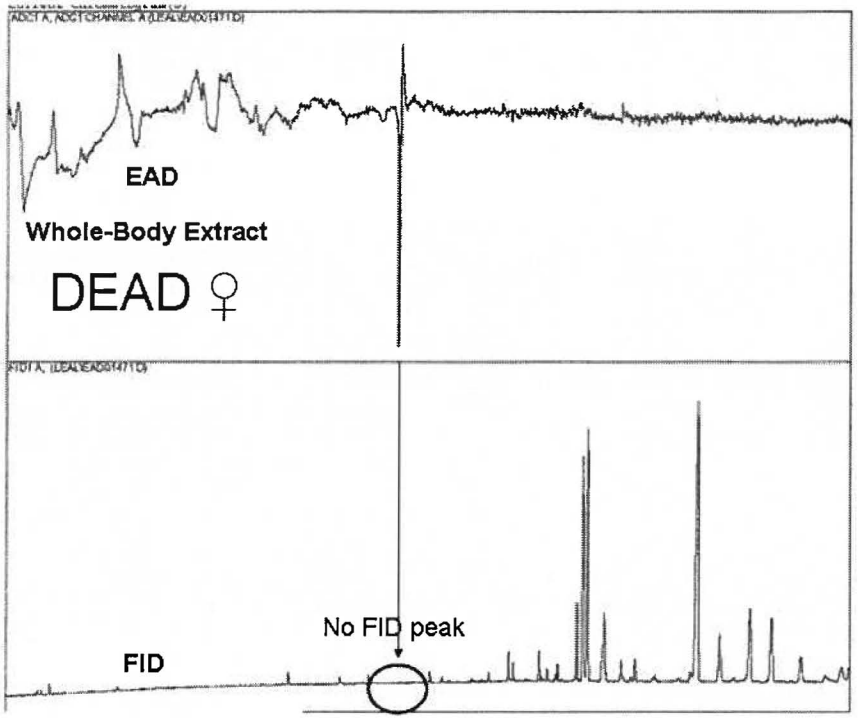

Figure 5. GC-EAD obtained with hexane extract of dead females.

Conclusions and practical applications. As indicated by captures in light traps in Manteca, flight activity of TLJB males starts in late May and extends to early September, with a peak at the end of July. Using highly sensitive gas chromatography with electroantennographic detection (GC-EAD), we have identified a female-specific compound (sex pheromone) from airborne volatile collections and whole-body extracts from female TLJB. The active compound generated EAD and showed behavioral activity in males. The material extracted from both live and dead females is now being pooled for chemical identification of the sex pheromone. Our observations in the field suggest that the sex pheromone of the TLJB has tremendous potential not only for monitoring populations, but also for control strategies, such as mating disruption and attract-and-kill. Although challenging, the identification of the pheromone may pave the way for novel, environmentally-sound, and effective strategy for controlling populations of the TLJB.