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# Prospecting for Attractants for the Ten-lined June beetle

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**Introduction**. Although populations of the Ten-Lined June Beetle (TLJB) are patchy, this scarab beetle species is a chronic problem in orchards where it occurs. The immature insects, known as grubs, live in the soil and feed on the roots of several commercial tree species including almonds. Both incidence and populations of TLJB are believed to be increasing, and presently damage has been reported in orchards from San Joaquin Co. to as far south as Fresno Co. Control of larvae in the soil has proven largely difficult or even ineffective because the larvae are buried deep in the soil. Although adults cause no economic damage, this life stage is amenable to control strategies as they emerge out of the soil in summer. Pheromones and other attractants are important tools for monitoring and/or controlling insect populations. These chemicals are used to monitor populations and assist in treatment timing. Since TLJB females show very little mobility, it may be also feasible to use synthetic sex pheromone for mass trapping. The major goal of this research is to identify, synthesize, and develop new pheromone-based approaches for controlling TLJB populations.

**Results**. The ten-lined June beetle from Manteca was previously identified as *Polyphylla decemlineata* (Van Steenwyk et al., 1990), but the species collected by us during this project were examined by Dave Hawks, University of California-Riverside and identified as *Polyphylla sobrina* Casey 1914. This may explain the remarkably different in behavior described in the literature for Californian and other populations. For example, it has been reported that soon after emerging from the soil females of the ten-lined June beetle, *P. decemlineata*, in Lethbridge, Alberta, Canada flew directly to branches or leaves of trees at 8 to 18 ft above the soil where most mating took place (Lilly and Shorthouse, 1971), whereas no female flight was observed in California (Van Steenwyk et al., 1990). Over the last three years of this project we never observed a single female flying. We observed instead that females start attracting males while emerging from the soil and in innumerous occasions mating took place while the female's head was still buried (in the soil). We commonly observed a large number of males searching in a certain area from where a female emerged later. In fact, this male behavior guided us in our search for females in the field. Although both *P. decemlineata* and *P. sobrina* have the common name (TLJB), it is now known that they are two different species. Consequently, their differences in behavior are no surprises. Mating of *P. sobrina* took place at dusk for a very short period of time (<3 min) and soon after that females returned to the soil. If insecticides were to be applied, there is a very short window of mating activity (less than 90 min after dusk) when both males and females could be directly exposed to the treatment.

We have monitored populations of the TLJB for three years in Manteca, CA and accumulated a significant and accurate set of data to conclude that the flight season starts in the beginning of June and extends to the end of August, with a peak in the end of July. The capture data were obtained at different intervals of time because of the logistics (unexpected battery failure, trap/battery theft, rain, etc). To make the three-year capture data comparable, we have normalized the data so as to express catches in beetles/trap/night (Figures 1-3).

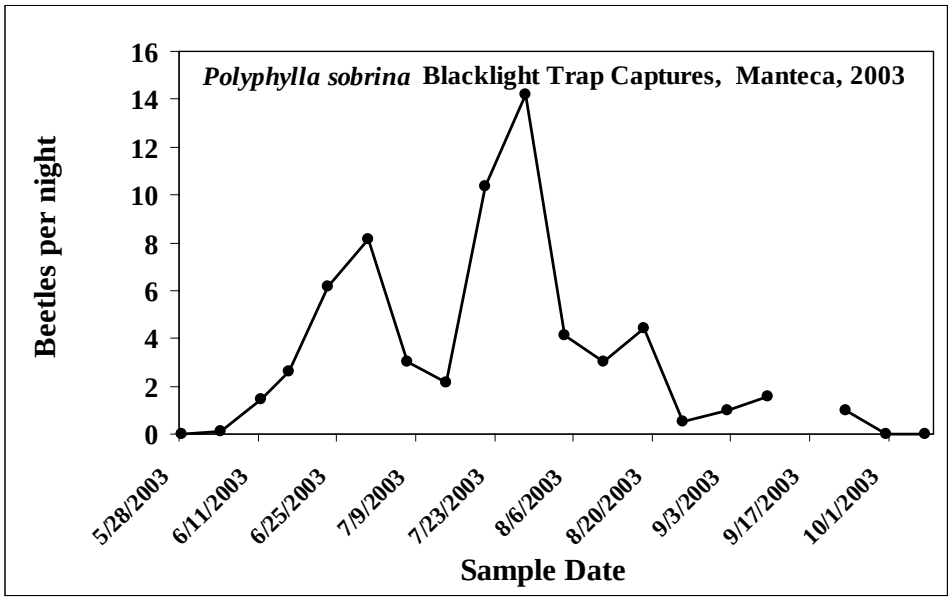


**Figure 1. Seasonal activity of the TLJB in Manteca (2003).**

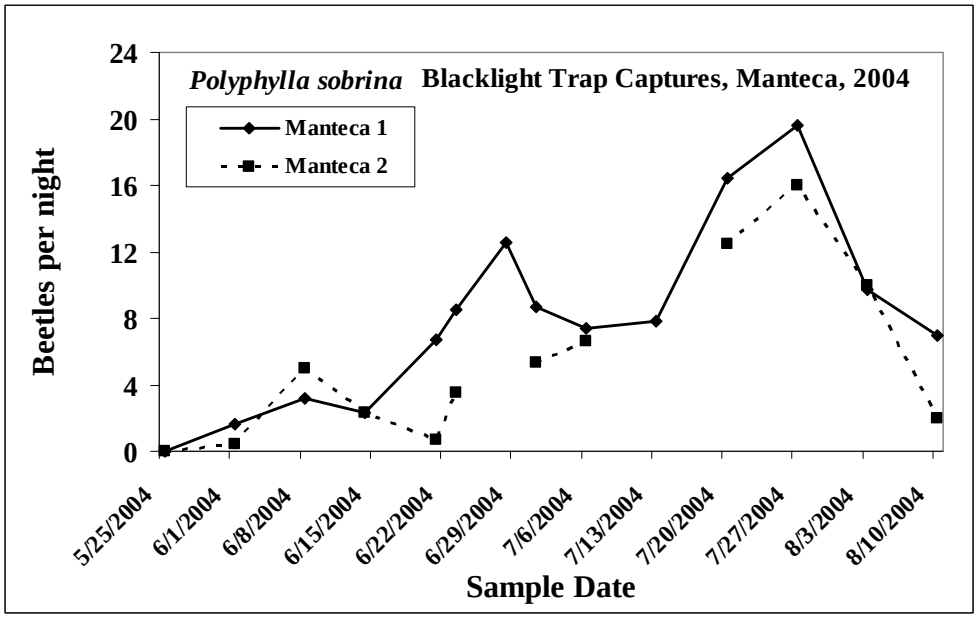


**Figure 2. Seasonal activity of the TLJB in Manteca (2004).**

Contrary to capture data for 2003 (Figure 1), data for 2004 (Figure 2) and 2005 (Figure 3) were obtained at the same location in the same orchard. Comparison of the capture

data for 2004 (Figure 2) and 2005 (Figure 2005) clearly indicates that populations of TLJB in the area increased dramatically. These field data support the notion that, in addition to increase in incidence, populations of TLJB are also increasing. Our trapping data also suggest that occurrence is patchy as we can observe large number of beetles flying in one part of an orchard, but very limited flight activities in other areas (of the same orchard).

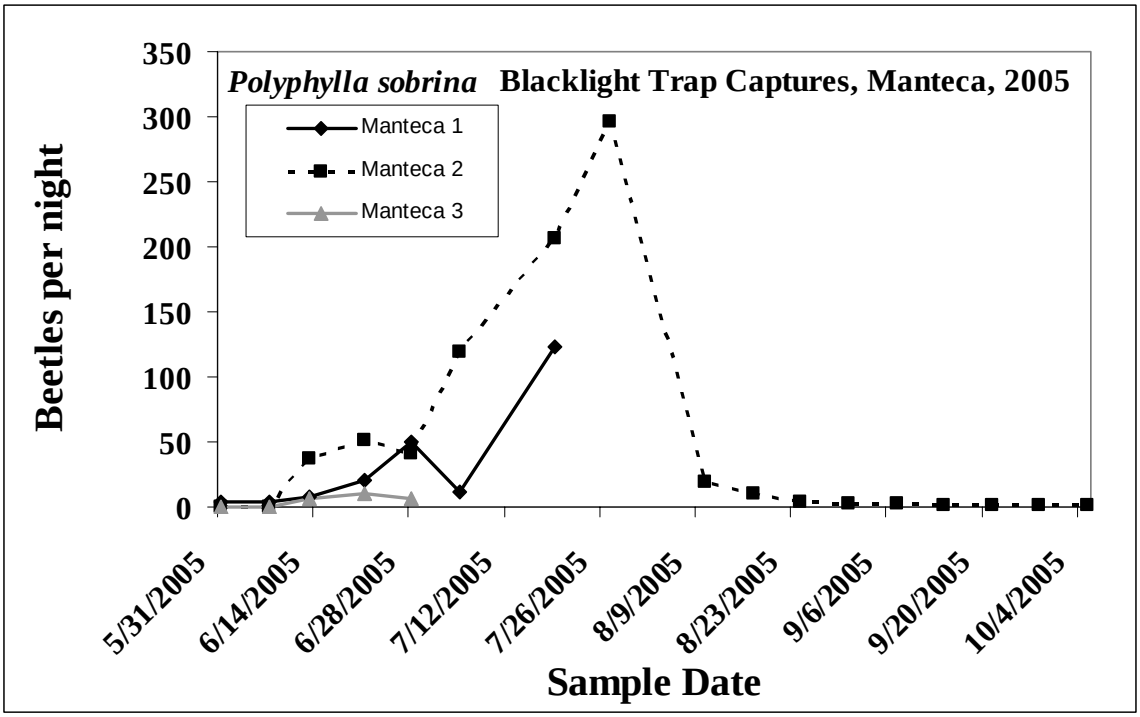


**Figure 3. Seasonal activity of the TLJB in Manteca (2005).**

During the 2005 flight season we organized over 40 field night trips to Manteca to collect females, in addition to a number of day time trips to fix traps, exchange batteries, etc. We were able to catch 754 females, which were brought to the lab and extracted in the following night at the time of the peak of pheromone production and mating activity. The flight season of 2006 seems to be a little bit delayed as few adults were captured up to the end of the month. We have collected quite a few grubs by digging the soil around infested trees in Fresno.

In an attempt to optimize pheromone extraction, we tested solvents of different polarities. As indicated by GC-EAD experiments, there was no significant difference in the amounts of pheromone extracted with non-polar (hexane) and polar (ether) solvents. We, therefore, decided to proceed with hexane extractions given that this solvent is easier to handle and can be generated in high purity in the lab by distillation with all-glass apparatus. We compared pheromone extraction by airborne volatile collections and solvent washing of the whole body, with the latter generating more EAD-active material than aeration. The amount of pheromone produced per female is extremely low (in the order of 1 pg/female) making isolation and identification a challenge even with the state-of-the-art analytical instrumentation. Initially, we suspected that pheromone collection could be improved with virgin females. We obtained virgin females from pupae kindly provided by Marshall Johnson, which were raised in his lab from field-collected grubs. Surprisingly, amounts of pheromone

extracted from virgin were not significantly higher than the amounts obtained from field-collected females. This might be due to the fact that most females were captured soon or even before they starting copulation. Thus, field-collected females may not have accumulated enough sperm to block pheromone production due to interruption of mating activity. Alternatively, females of the TLJB mate multiple times and mating does not inhibit pheromone production.

In an attempt to improve pheromone collection, we have explored two additional avenues. We extracted pheromone from field-collected and laboratory raised virgin females by solid-phase microextraction (SPME). GC-EAD analysis showed that enough pheromone amounts were collected for electroantennographic response, but there was not enough material to generate an FID peak. We obtained whole-body extracts of first-, second-, and third-instar grubs but observed no EAD response, thus, indicating that contrary to what has been observed in *Cyclocephalla* species, the pheromone is not produced by immature insects, but only by adult females.

Each batch of a whole-body extract was fractionated by flash column chromatography on silica gel by successive elution with hexane and ether mixtures. After concentration, activity of each fraction was monitored by GC-EAD. In the first attempt to isolate the active fraction(s), we recovered EAD activity in a hexane plus 10% ether fraction (hereafter 10% fraction), with some of the material start eluting in the previous fraction (5%). Further column chromatography with a smaller increase in polarity (0, 1, 2, 3, 4, 5, 7.5, 10, 20, and 50% ether) led to the isolation of EAD-active peak in the 4% fraction (Figure 4), suggesting that the pheromone is a compound with low polarity.

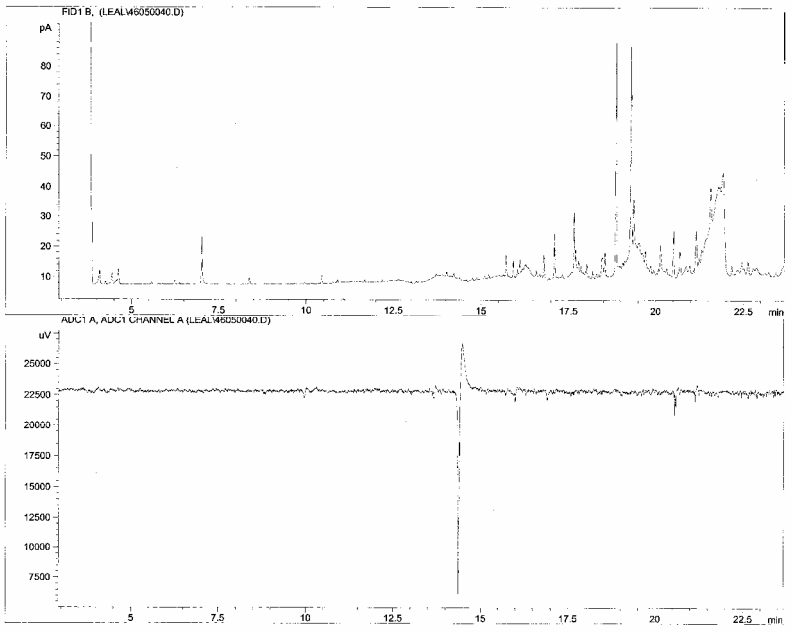


**Figure 4. Simultaneous GC (upper) and EAD (lower trace) recording from a 4% fraction derived from female extract and flash chromatographic separation. A male TLJB antenna was used as the sensing element in EAD.**

Pheromone identification is simplified when the active compound(s) has (have) been previously identified (e.g, pheromones from other species, plant-derived compounds, etc).

We have already compared the retention times and EAD activities of all scarab beetles pheromones identified to date. Unfortunately, the TLJB pheromone chemistry is new at least for the group of scarab beetles. Characterization of novel compounds are normally challenging due to sample limitation. The TLJB is an extreme case because females produce (what seems to be) the lowest amounts of insect pheromone. Sample is limited also because females are hard to collect/raise in the lab. With 754 females collected in this flight season (2005), we have accumulated enough material possibly to run a single GC-MS analysis. If the pheromone chemistry is already known, GC-MS and chemical derivatization may generate enough information to determine the structure. Once we get enough information to propose a chemical structure, we will synthesize the compound for chemical, physiological, and behavioral comparisons with the natural product. Ultra low scale chemical derivatizations (hydrogenation, hydrolysis, etc) are underway to generate additional structural information before the isolated pheromone sample (4% fraction) is pooled for GC-MS analysis and probably exhausted.

**Conclusions and practical applications**. Blacklight trap data indicate that TLJB populations are increasing. Our field observations and trap data identified a peak of flight activity and a window of opportunity to target both males and females in chemical and/or biological (e.g.: fungi, nematode, etc) treatments. Chemical characterization of the pheromone is underway. An EAD-active peak has been isolated in one active fraction, but chemical identification is yet to be completed. While the fact that females produce unusually low amounts of pheromone hampers pheromone identification, it is also encouraging for future practical applications as such an active pheromone has a tremendous potential for mass trapping. Behavioral and field observations support this notion given that population is patchy and females have limited mobility.

## References

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Van Steenwyk, R.A., Rough, D. and Verdegaal, P.S. (1990). *Biology and control of the tenlined June beetle in almonds*. California Agriculture 44, 25-27.