CRB-FUNDED FINAL RESEARCH REPORT

Standard yellow sticky trap.

NOVEL ATTRACTANT AND TRAP FOR MORE SENSITIVE ACP MONITORING AND DETECTION

Darek Czokajlo, Lukasz Stelinski, Kris Godfrey and Mamoudou Setamou

The Asian citrus psyllid (ACP), *Diaphorina citri*, vectors three phloem-restricted bacteria in the genus *Candidatus* Liberibacter, which have been associated with huanglongbing (HLB), otherwise known as citrus greening disease. Citrus trees infected by HLB produce small, misshapen fruit characterized by bitter taste, rendering the juice and related products unmarketable. Infected trees gradually decline, drop much of their fruit load and ultimately die.

ACP was first reported in Florida in 1998, but has invaded many more regions, which include all citrus growing areas of the continental U.S., Puerto Rico and Hawaii. HLB was first discovered in Florida in 2005 and is now well-established. It has been confirmed in several commercial groves in Texas and in only one residential tree in California. Whereas the psyllid has been detected in several areas in Arizona, neither psyllids nor plant material has tested positive for Liberibacter.

Current sampling protocols for adult ACP rely on passive sticky traps, which capture ACP by incidental or random encounters of flying adults with these sticky surfaces or by tap sampling in which tree branches are shaken to dislodge psyllids onto a sticky surface held below the branch. This renders adult psyllid monitoring for forecasting or evaluating insecticide applications inaccurate. In 2008-09, a new ACP trap was developed through the joint research of Mamoudou Setamou, Ph.D., of Texas A&M University in Kingsville and Darek Czokajlo, Ph.D., of Alpha Scents, Inc. (unpublished data) **(Figure 1)**. We have found that this lime green trap captures significantly more ACP than the standard yellow traps commonly used. The un-baited, yellow sticky traps currently used for monitoring ACP adult populations are only marginally effective without an attractant.

Currently, a semiochemical-based lure (chemical used for communication between individuals) to attract ACP is not commercially available. ACP exhibits strong preference for citrus volatiles and aggregate and lays eggs exclusively on young unexpanded leaves. Thus, plant-related chemicals are crucial signals used by adults for plant selection. In addition, there is evidence documenting that mate location in ACP is mediated by a volatile sex pheromone and hydrocarbons emitted from the cuticle or outer surface of the insect. In this research, we evaluated the potential of using individual and

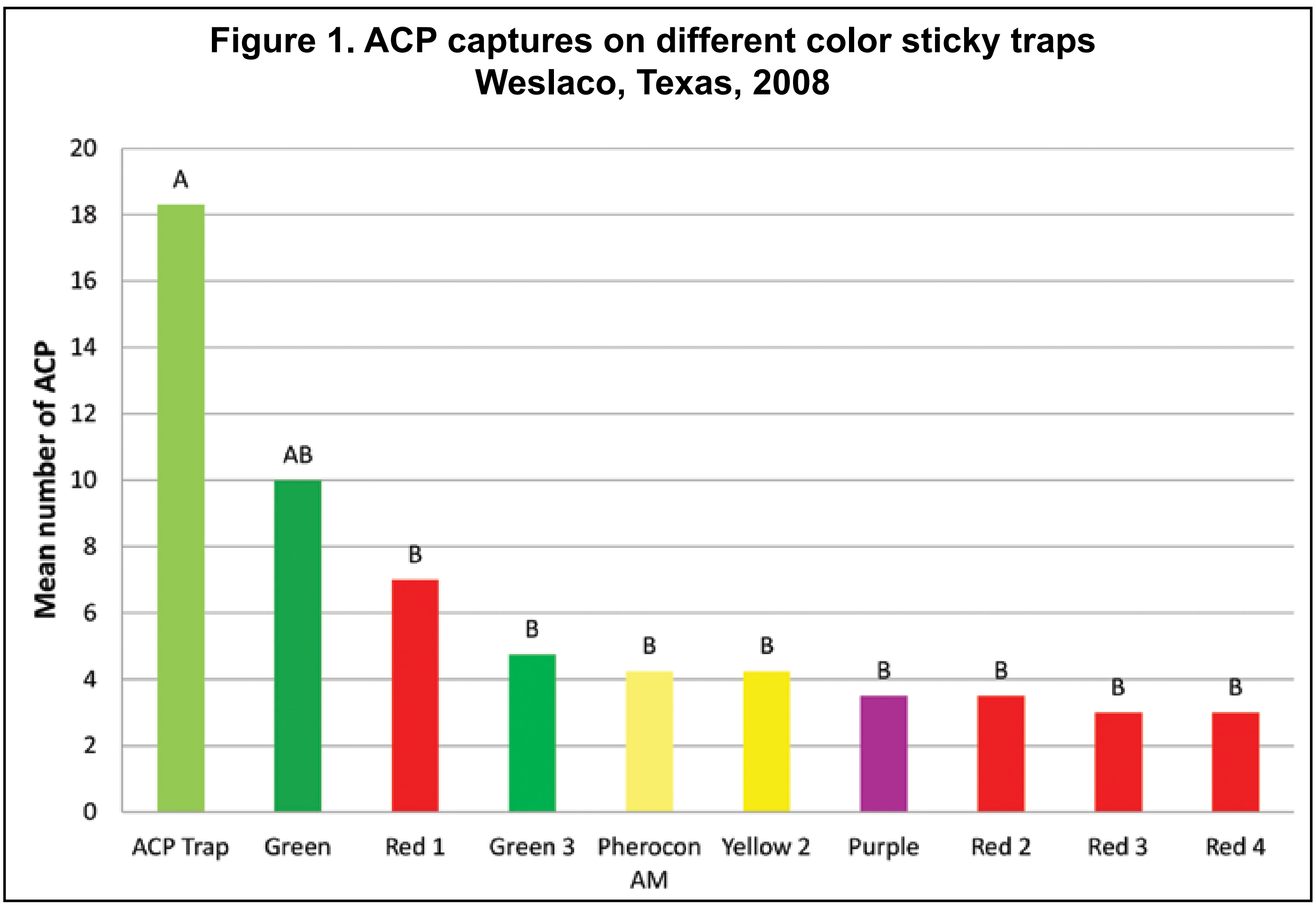

Figure 1. Monitoring low populations of ACP with different color sticky traps, Texas A&M University-Kingsville, Weslaco, Texas, October 25-November 6, 2008.

Figure 2. ACP lure on Alpha Scents green ACP trap

blends of plant volatile and pheromone compounds as field attractants to improve monitoring. The attraction of adult ACP to traps baited with synthetic compounds was compared to captures on un-baited (control) ACP traps.

To test potential semiochemicals for their ability to attract adult ACP, the number of ACP captured on baited green traps was compared to those captured on non-baited green traps. Traps were placed on the outer canopy of citrus trees at five feet above the ground along the edge of all four sides of groves. Trees were spaced about 24 feet apart; only one trap per tree. Formulations of active compounds were placed directly on traps in a controlled release device **(Figure 2)**. All treatments were replicated eight times at three different sites in Florida, Texas and California. In Florida, the lures were tested in Valencia and Hamlin orange groves. In Texas, evaluations were conducted in lemon groves. In California, trapping was conducted in residential trees.

There were two experiments. In Experiment One, lures were tested in June and July 2014; and in Experiment Two, they were conducted in September and October 2014. Traps were checked and replaced weekly, and the number of ACP captured on traps was counted.

For Experiment One, Alpha Scents formulated a total of eight different lures composed of host plant volatiles and ACP-produced compounds. Five lures were blends of host-plant volatiles only, and three lures were blends of plant volatiles and ACP-made compounds. All eight blends were tested in Texas, and seven blends were tested in Florida and California.

Based on results from Experiment One, we made another seven blends of plant host volatiles for Experiment Two. Three lures were blends of host-plant volatiles only, and four lures were blends of plant volatiles and ACP compounds. All blends were tested in Texas, and five blends were tested in Florida and California. The composition of the blends is not revealed to protect proprietary information. Data were subjected to analysis of covariance and a permutation test to determine statistical differences between means, although this data is not presented here.

Several of the blends tested resulted in a significant increase in the number of psyllids trapped as compared to un-baited control traps **(Figures 3-6)**. Specifically, in Florida the experimental blends MS1, MS1L, MS1AL and MS1HC appeared most promising and promoted greater capture of ACP on traps as compared with un-baited traps. Despite the lower numbers of ACP populations in Texas during June and July of 2014, significant differences in the effectiveness of blends in luring adult ACP were observed. Consistent with data obtained in Florida, traps baited with the experimental blends MS1A and MS1L captured significantly more ACP than the un-baited ones. During the September-October 2014 study period in Texas, higher ACP densities were recorded. Consequently, the number of ACP captured dramatically increased, and marked differences were recorded in the performance of experimental blends tested. Traps baited

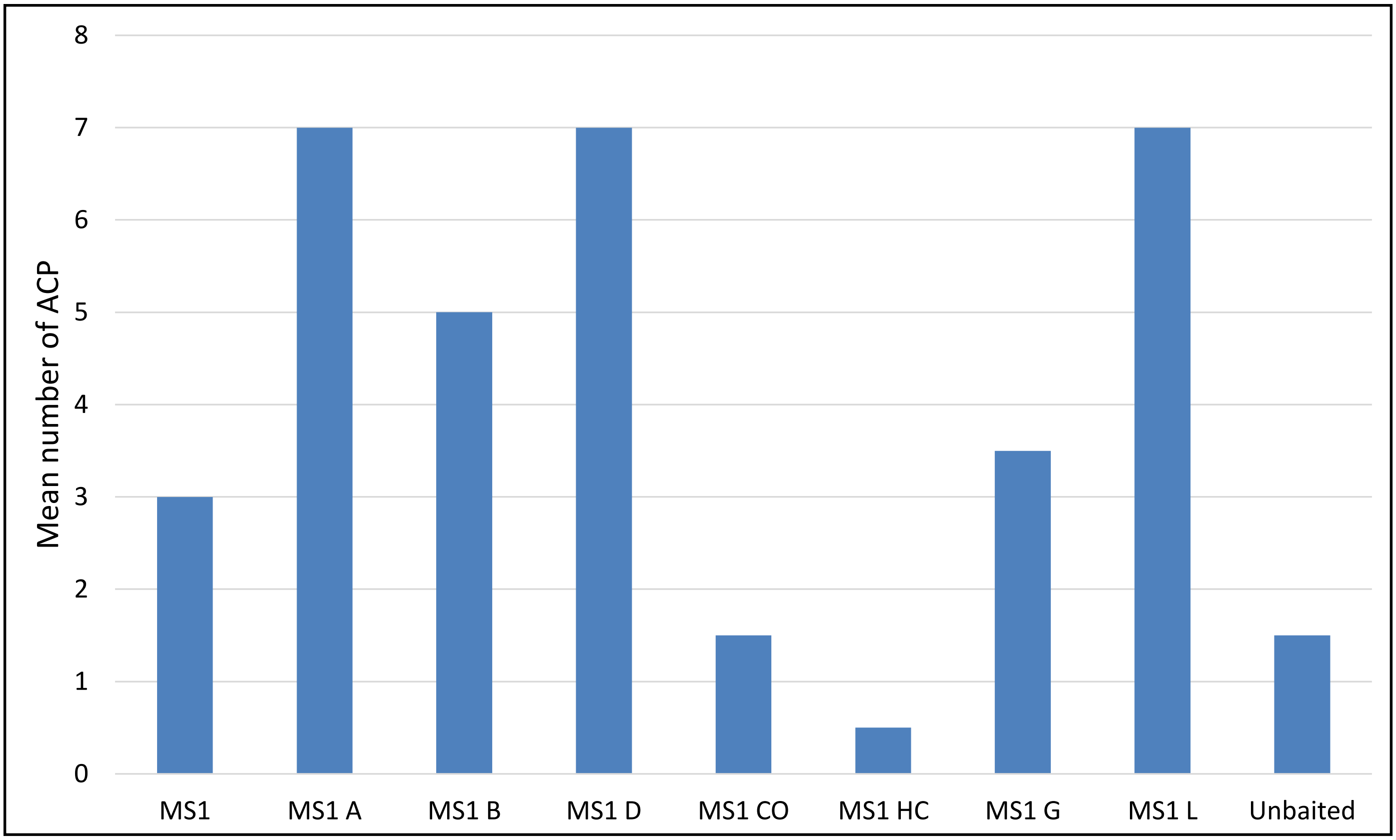

Figure 3. ACP captures, Weslaco, Texas, July 2014.

with the MS1A, , MS1L and MS1AL lures captured significantly more ACP adults. In California, most of the baited traps captured more ACP than un-baited traps, but the data were inconclusive (data not shown). Because traps were deployed in residential trees rather than commercial groves, problems were encountered during this trial including an unequal number of traps recovered. Therefore, the California data could not be analyzed to obtain a meaningful interpretation. Although our results indicate that these new lures increase the capture of ACP as compared with un-baited controls, the data were not consistent between states. Specifically, the data from California did not indicate efficacy as clearly as in Florida and Texas. These field trials need to be repeated in order to clarify this discrepancy. One possible explanation is the differences in ACP population density between the three states. Methods used to conduct trials will be the same at each location in the future to avoid inconsistencies in results.

An effective trapping system is paramount for quarantine programs, such as those currently in place in California and Texas. Quarantine programs rely heavily on the detection of pests at very low population levels in order to successfully eradicate targeted pest with insecticides. Given that visual color-based traps are only attractive to ACP from a short distance, an attractive lure that would capture ACP more effectively from longer distances and when psyllid populations are low would increase the confidence level in survey results. Pesticides used for eradication purposes could be used more judiciously.

An effective lure for monitoring ACP would also be useful in areas of the country where ACP are endemic and eradication is no longer possible, such as in Florida. Growers rely heavily on insecticides to reduce ACP populations in order to reduce the spread of the pathogen that causes HLB. Monitoring for the pest is difficult, time consuming and expensive. However, the increased need for insecticide sprays to control ACP is also very expensive, and the potential for the development of insecticide resistance in ACP populations already has been proven scientifically in Florida. Therefore, a more effective trap and lure system would be useful to more accurately estimate the number of psyllids in a given area. Pesticide applications could be based on ACP population density rather than on a calendar basis, and, therefore, greatly reduce the number of applications.

In summary, several promising blends for the capture of ACP were determined in this investigation. These blends hold potential as possible commercial lures for improved monitoring of ACP. Three to four times higher trap captures were achieved using various blends as compared to un-baited traps. These blends need to be further tested in larger field studies for confirmation of their effectiveness and possible refinement of their final formulations. All experimental lures

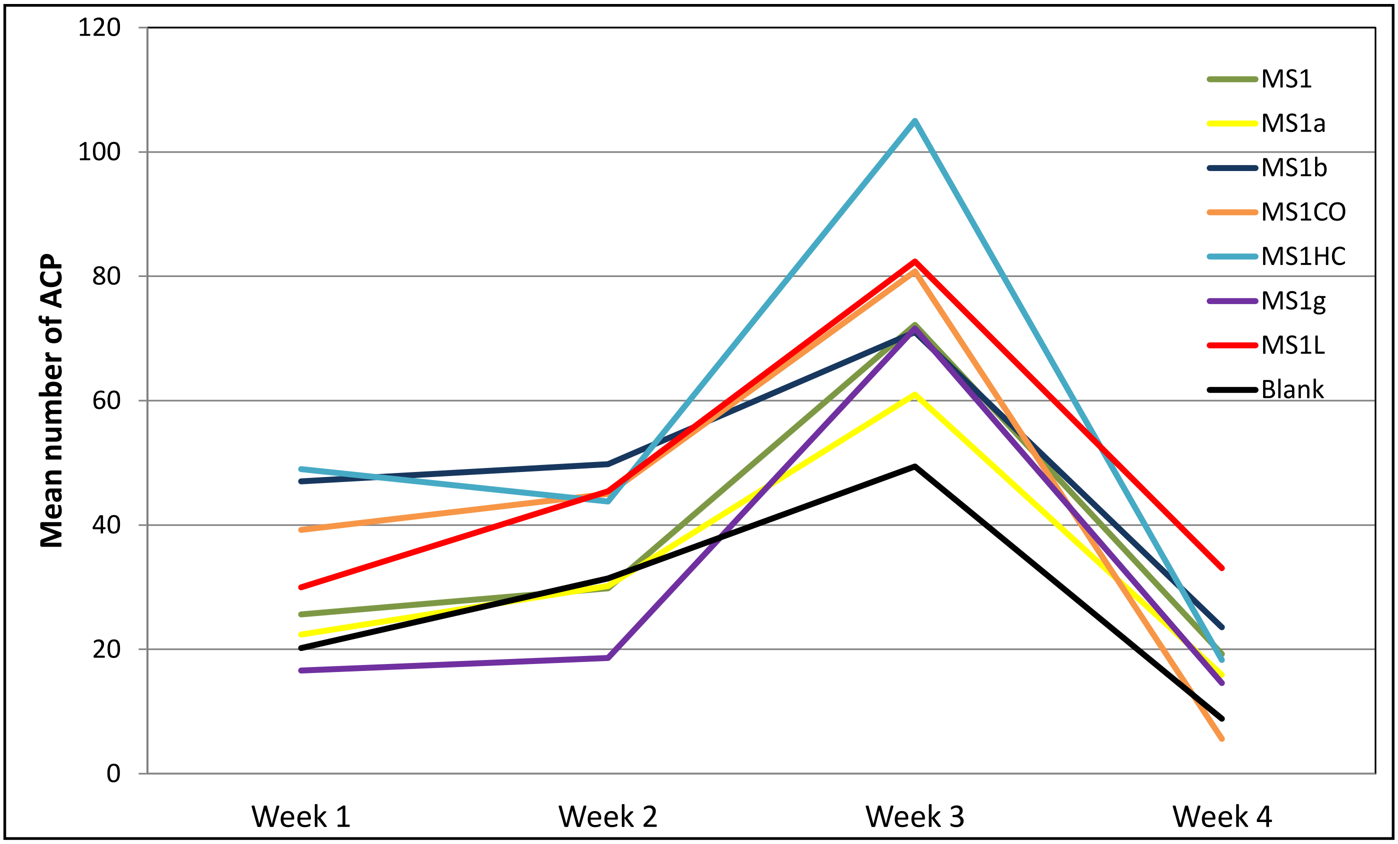

Figure 4. ACP captures, Lake Alfred, Florida, July 2014.

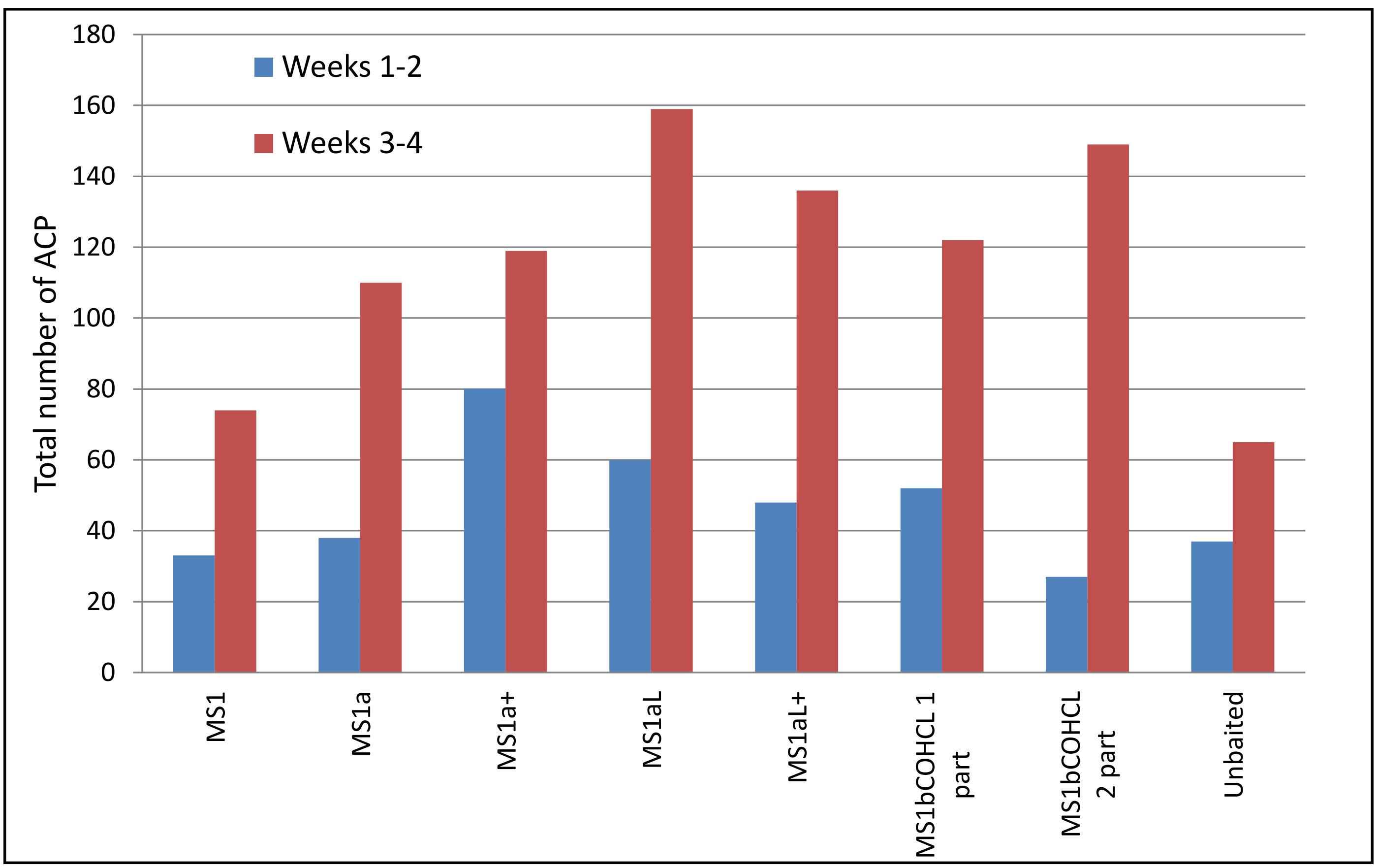

Figure 5. ACP captures, Weslaco, Texas, September - October 2014.

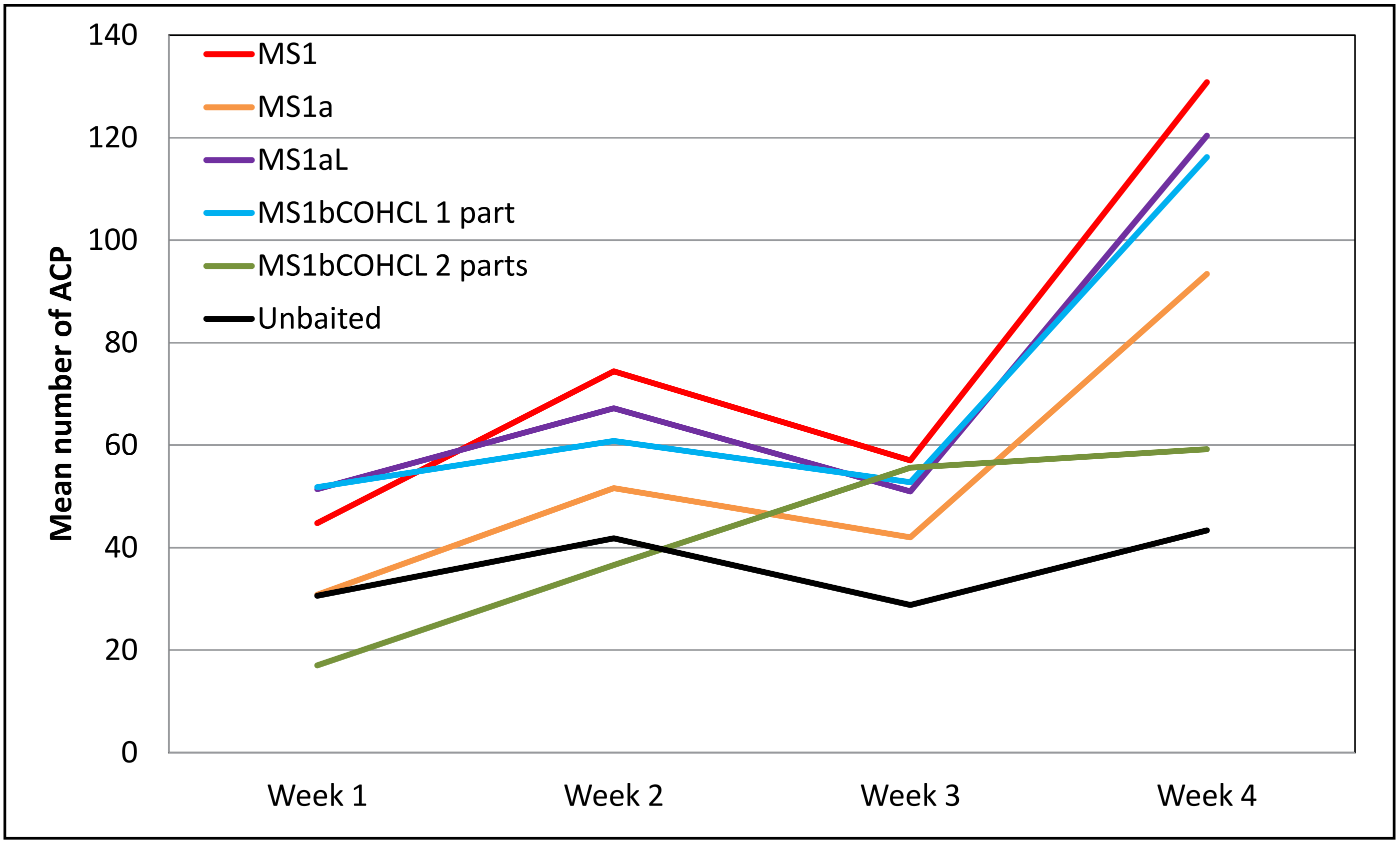

Figure 6. ACP captures, Lake Alfred, Florida, August - September 2014.

were active in the field for more than four weeks. Alpha Scents' ACP trap attracts three to four times more ACP than standard yellow trap; and in addition; the lure will attract three to four times more psyllids. Taking this into consideration, we can state that monitoring ACP with Alpha Scents' ACP trap and lure is six to eight times more effective than the currently-used un-baited yellow traps. The commercial ACP lures will be available from Alpha Scents in the 2015 season.

Acknowledgements

This research was partially co-funded by the Citrus Research Board, Texas Citrus Mutual and Alpha Scents, Inc. We acknowledge the assistance of Anna Soper, USDA-APHIS, with the California studies.

Darek Czokajlo, Ph.D., is the president and an entomologist and insect chemical ecologist at Alpha Scents, Inc., West Linn, Oregon. Kris Godfrey, Ph.D., is an associate project scientist at the University of California at Davis. Mamoudou Setamou, Ph.D., is a professor of entomology at Texas A&M University, Kingsville. Lukasz L. Stelinski, Ph.D., is a professor of entomology at the University of Florida, Lake Alfred.