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# Results from Field Trials Refining the Use of the Sex Pheromone of *Stenoma catenife*r, the Avocado Seed Moth, in Commercial Hass Avocado Orchards in Guatemala


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**Project Rationale:** California avocado production is under an ever increasing threat from invasive arthropod species that originate in areas outside of California. Some of the most worrisome threats are specialist insects that have evolved to feed on avocado fruit in the home range of this plant in Mexico and Central America. Of most concern are moths, weevils, and fruit flies that feed internally on fruit and could be accidentally introduced with fresh fruit imports. These internal fruit feeding insects are poorly studied in their countries of origin and virtually no information is available on the biology, ecology, phenology, natural enemies, and management of these pests. With the legalization of fresh fruit imports into California from Mexico, which began for the first time in February 2007, the likelihood of new fruit feeding avocado pests hitchhiking unnoticed into California has markedly increased.

One group of avocado fruit feeding pests with high invasion potential into California, the fruit feeding moths, were selected for a proactive and intensive five month study **(Nov. 1 2006 – April 1 2007)** in Guatemala. Of particular interest in this study was the avocado seed moth, *Stenoma catenifer*. The rationale for studying *Stenoma* was that this moth would likely yield a sex pheromone that could be extracted and synthesized. The sex pheromone of *Stenoma* could be used for incursion monitoring in California, and in orchards of exporting countries to verify the validity of "pest free" certification.

The pestiferousness of *Stenoma* is well recognized in the majority of Central and South American countries with commercial avocado industries. *Stenoma* is considered the primary pest preventing a viable commercial avocado industry in Brazil, despite intensive management with broad spectrum pesticides.

The **November 2006 through April 2007** *Stenoma* study in Guatemala (reported upon to the CAC in a previous progress report: "An Investigation of Avocado Fruit Feeding Moths in Guatemala: A Report for the California Avocado Commission" submitted in July 2007) resulted in the isolation, synthesis, and preliminary lab testing of the *Stenoma* sex pheromone. The *Stenoma* sex pheromone was identified as an aldehyde, (9*Z*)-9,13-Tetradecadien-11-ynal, the first representative of a new class of natural products (Millar et al. 2008). The dienyne structure of the *S. catenifer* pheromone is remarkable, both in terms of the presence of the alkyne, which is a very unusual functional group in lepidopteran pheromones, and in terms of the overall high degree of unsaturation (Millar et al. 2008).

Following the elucidation of the *Stenoma* sex pheromone in the lab, the second phase of this project was to field test the attractiveness of the pheromone in commercial Hass avocado orchards in Guatemala for efficacy. Field tests of the *S. catenifer* sex pheromone were conducted over two different time periods, **November 13 to December 21 2007, and March 13 to May 1 2008.** The results of these studies have been provided to the California Avocado Commission.

These studies verified that the most efficacious pheromone blend tested was the aldehyde only, (9*Z*)-9,13-Tetradecadien-11-ynal, and higher concentrations on rubber septums worked better than lower doses. Addition of other components associated with pheromone extractions identified in the laboratory at any of the test concentrations did not have a synergistic or additive effect on the efficacy of the aldehyde. In some cases, the capture data suggested that the addition of other components may be slightly inhibitory to male *Stenoma*. Further, captures of male

*Stenoma* were greatest with pheromones that were two weeks or less of age. However, lures up to six weeks old were still attractive to male *Stenoma*, indicating that the pheromone has significant field longevity. All night field observations of pheromone traps clearly indicated that male *Stenoma* start arriving at traps around 2:30am in the morning, and flight arrivals peak around 4:30am before ceasing abruptly just before sunrise at 5:30am. Trap height and placement studies suggested an important effect on capture rates of male *Stenoma*. Traps at ground level between tree rows captured more moths than traps set at 2.0 and 4.0m between tree rows. Traps set in trees at 1.5-1.75m readily captured male moths. This discrepancy in height preferences, possibly because of placement (either in trees or between tree rows) needed resolving so trap placement recommendations can be optimized to increase the likelihood of captures and pest detection with the pheromone.

The remainder of this report contains results of studies conducted over **<u>November 14 2008 to January 14 2009</u>** in Guatemala on the sex pheromone of *Stenoma catenifer*. Phase III of *Stenoma* research for the California Avocado Commission had the following five objectives:

1) Determine which pheromone dispenser type is the most efficacious for attracting male *Stenoma*.
2) Determine optimal trap height in avocado trees for deploying pheromone traps to maximize capture likelihood of male *Stenoma*.
3) Determine how far male *Stenoma* can fly in avocado orchard.
4) Determine the number of pheromone traps needed in an orchard to detect the presence of *Stenoma* when damage is visually undetectable.
5) Determine if the pheromone is attractive to *Stenoma* in other avocado growing areas outside of the immediate vicinity of Antigua Guatemala where moths were sourced for pheromone analyses.

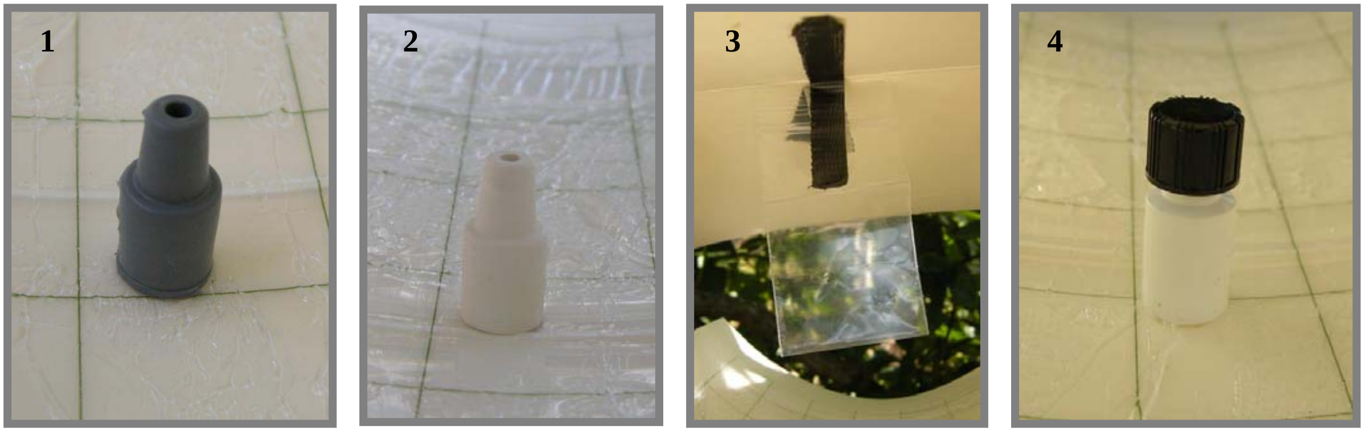
**<u>Experimental Orchards: All experiments were conducted in the following five orchards:</u>**

1) A ~ nine acre commercial Hass avocado orchard consisting of ~800-900 trees in San Miguel Dueñas, Sactepéquez, Guatemala. Trees in the orchard were ~7-8 yr old, around 6-8 m in height, and canopies were separated by ~5-7 m of clear ground allowing full sun exposure. This orchard had no fruit.
2) A ~ seven acre orchard in Santa Ana, Sactepéquez, Guatemala with ~ 700 five year old trees. This commercial Hass orchard had mature fruit.
3) A ~1.25 acre orchard with ~100 trees with no fruit, and about 30 trees were stumped. This orchard was in Antigua, Sactepéquez.
4) A ~2.0 acre orchard with ~150 trees ~six year old trees with mature fruit in Antigua, Sactepéquez.
5) A ~187 acre Booth avocado orchard with nine year old trees in Escuintla Guatemala. This orchard had no fruit.

**<u>Results:</u>**

*1)* *<u>Evaluation of Pheromone Dispenser Type for Efficacy.</u>*

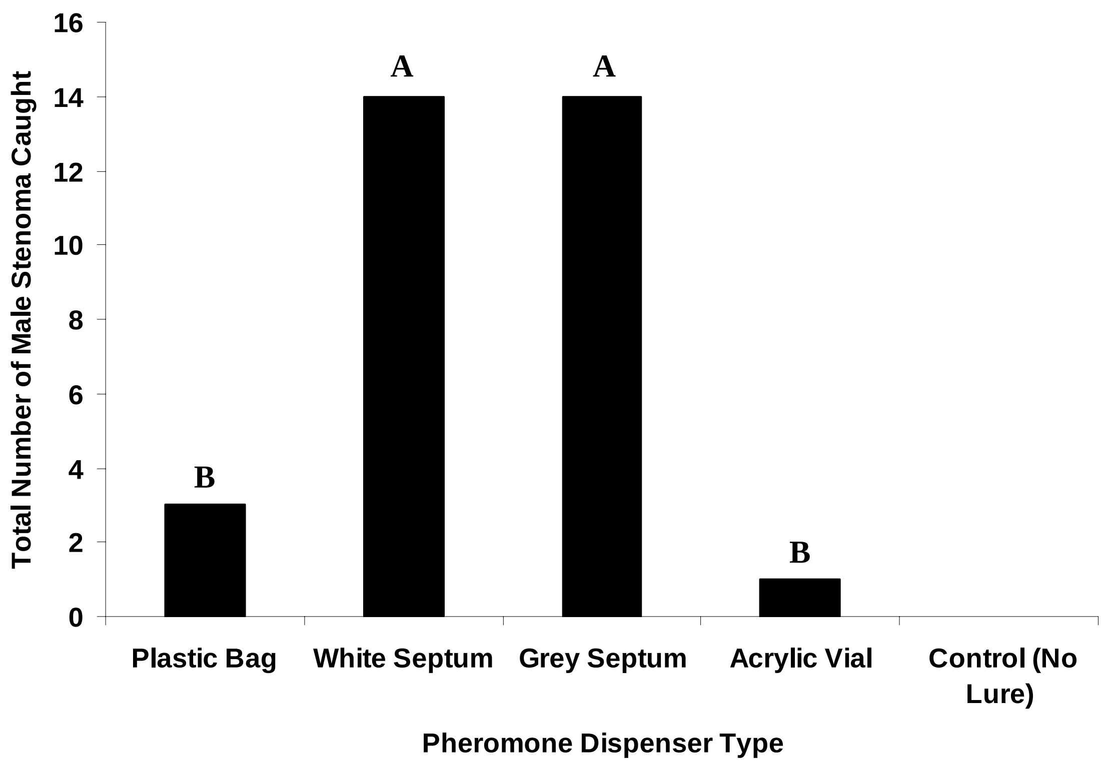
Four different pheromone dispensers were tested for their efficacy in emitting the sex pheromone of *Stenoma*: (1) grey rubber septums (these had been used in all previous studies), (2) white silicon septums, (3) small plastic bags, and (4) porous acrylic vials (see immediately below for photos of the different *Stenoma* pheromone dispensers field tested for efficacy in commercial avocado orchards in Guatemala).




Pheromone traps without lures acted as experimental controls. All lures were charged with 1 ng of the sex pheromone. In each experimental orchard, blocks of five lures were set up. Each lure type in a block was separated by at least two trees, and experimental blocks were separated by at least 3-5 trees. Every four days lures were examined, the number of moths caught in each trap by lure type was recorded. Traps in blocks were then re-randomized and re-deployed for an additional four days. The number of blocks of lures deployed per orchard varied and depended on orchard size.

There were significant differences between lure types impregnated with pheromone in their ability to attract male *Stenoma*. The worst performing lures were the porous acrylic vials and plastic bags. Both of these lure types were statistically equivalent in their ability to attract male *Stenoma* – capturing around 1-2 male moths over a two week period (Fig 1 below). The grey rubber septum and the white silicon septum were statistically equivalent in their attractiveness to male *Stenoma*. Over a two week period both lure types each caught exactly the same number of male *Stenoma*, 14 moths per lure type (Fig. 1 below). Because all previous studies with the *Stenoma* pheromone had been conducted using the grey septum, this lure type was used for all additional studies because the white silicon septum had no demonstrable advantages to warrant changing to a new lure and complicating use and interpretation of previous results obtained with the grey septum.

**Fig. 1.** Total number of male *Stenoma* caught per lure type in commercial Hass avocado orchards over a two week evaluation period in Antigua, Guatemala. Bars with same letters indicate statistical equivalency, while different letters indicate significant statistical differences at the 0.05 level (ANOVA).



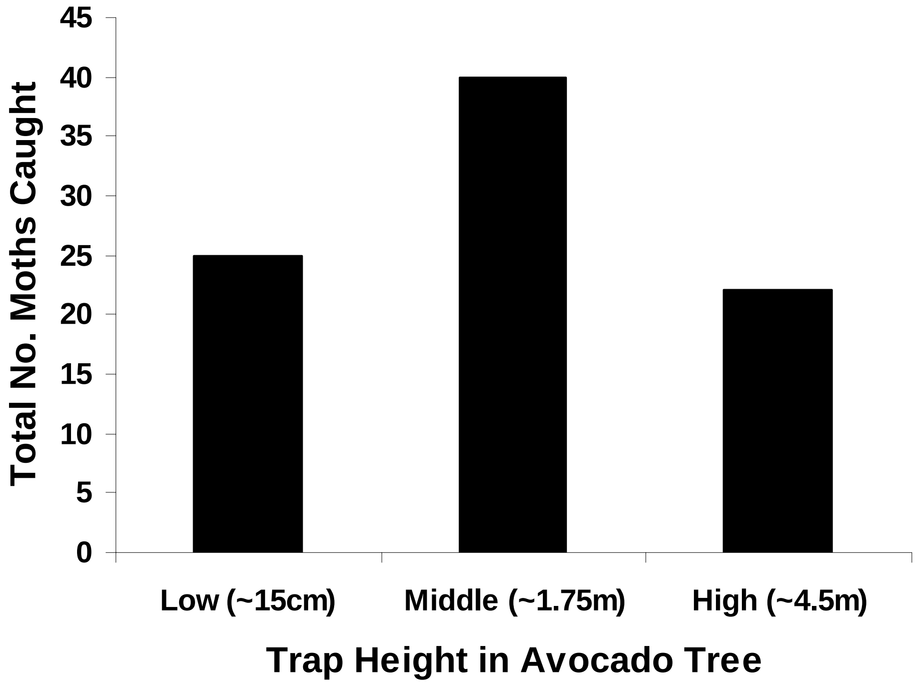

2) *Evaluation of Pheromone Trap Height in Avocado Trees for Attracting Male Stenoma.*

Pheromone trap height has been demonstrated for some species of moths to be very important for attracting male moths for monitoring pest species (e.g., codling moth). Preliminary work on trap height placement for *Stenoma* indicated that traps in trees at ~1.75 meters of height caught moths, while traps hung on poles between trees were most attractive at ground level (see *Stenoma* Report number 2 prepared for the California Avocado Commission). Determination of optimal trap height for *Stenoma* pheromone trap deployment is critical if the pheromone is to be used most effectively for monitoring this pest. Trap height studies were conducted in all five experimental orchards in Guatemala over a six week period. In each experimental orchard blocks of traps were deployed. Depending on orchard size, one to three blocks per orchard were deployed, and each block consisted of two to eight trees per block. Each experimental tree in a block had three traps placed in it. One trap was placed at the foot of the tree on a metal stake approximately 15 cm above the ground, a second trap was placed in the tree at ~1.75 meter above the ground, and the third trap was placed at ~4.5 meters above the ground near the top of the tree canopy. Because all three traps were deployed in a single tree to reduce data analysis complications because of tree effects, grey septa were treated with 0.33 ng of pheromone to give a total pheromone charge of 1.0 ng per tree, a concentration shown to be attractive to male *Stenoma*. Trap deployment pattern for this study is shown in Fig. 2.

**Fig. 2.** Deployment of three pheromone traps per Hass avocado tree at three different heights to determine which trap height is optimal for capturing male *Stenoma* attracted to pheromone lures.


Pheromone trap height had an effect on capture rates of male *Stenoma*. Experimental traps hung at ~1.75m, caught approximately two times more moths than traps set at 0.15m and ~4.5m (Fig. 3). The intermediate trap height is easy to deploy and is extremely easy to inspect because it is around head height and does not require kneeling on the ground (~0.15cm height above the ground) or climbing into the tree (~4.5m height above the ground).

**Fig. 3.** Total capture numbers for male *Stenoma* in pheromone traps across five different experimental orchards. This study indicates that pheromone traps hung at 1.75m above the ground are at an optimal height for capturing male *Stenoma*.




3) *<u>Determination of Flight Distances for Male Stenoma in Avocado Orchards.</u>*

To determine how far male *Stenoma* can fly in one night, newly emerged (~48 hrs of age) male moths were dusted with day-glo dust and released in the center of a study plot that had pheromone traps deployed at various distances from a central release point. Male moths were reared from infested Hass avocados collected from three orchards around Antigua (San Miguel Urias – remnant fruit that were missed during harvest; an orchard in Santa Ana, and one orchard in Antigua; these source orchards are 1, 2, and 4 in the list of study orchards above). Approximately 500 fruit with *Stenoma* damage were collected, held in bug dorms in the laboratory, and the fate of reared larvae was recorded as emerging as an adult moth, parasitized by either *Apanteles* or *Macrocentrus*, or dying of unknown causes. Adult moths were used for two studies: (1) female moths were dusted with day-glo dust to determine if day-glo had some level toxicity. Average survivorship in days of dusted female moths was compared to non-dusted female *Stenoma*. Four different day-glo dusts were used in this study; (i) hot pink, (ii) electric blue, (iii) lime green, and (iv) hot orange. No toxicity was observed in the lab for female moths and it was assumed that the day-glo dust would likely be safe for male moths as well. (2) Male

*Stenoma* were dusted and released in the study plot set up to determine the flight distance of male moths. The study site for the male moth flight distance study was set up in a 3 acre field planted with 3 year old criollo avocados (~2-3 meters in height and non-fruit bearing) and intercropped with asparagus. Pheromone traps were deployed around a central release point in the center of the field at the following distances: 2m (1 trap), 8m (1 trap), 16m (1 trap), 32-40m (5 traps), 64m (6 traps), 85m (8 traps), and 100m (1 trap). Dusted male moths were released at dusk each night, and the following morning traps were examined and captured moths were examined in the closed trunk of the field car with a black light for the presence of day-glo dust on male moths. On each different release date male moths were dusted a different color so as not to be confused with earlier released moths should they be caught at a later date.

A total of 15 laboratory-reared male moths were released, and 4 males were recaptured at the following distances from the central release point: (a) 40m, (b) 65m, (c) 70m, and (d) 85m. All males were caught the night of release. If a released male was not caught the same night it was released, it was not caught at any time after its release. The average distance a male *Stenoma* flies in one night is 65m ± 9m. These flight data may be extremely important for optimizing trap placement distances in orchards exporting fruit to the USA that are originating from countries with endemic *Stenoma* populations. Further, these data could prove useful for developing eradication/monitoring plans should *Stenoma* be detected in California in the future.


**Fig. 4.** The layout of the flight distance study is shown in the Google Earth image to the left. Male *Stenoma* dusted with day-glo dust were released in the center of the study plot. The moth release site is shown with an orange arrow and is pointing at a lime green marker. The yellow circles indicate the traps in which male *Stenoma* were caught the night of their release from the central release point.

It is interesting to note that no male *Stenoma* were caught in traps close to the central release point. The likely reason for this is that moths begin to fly immediately at dusk, but males are not receptive/responsive to pheromone plumes until 2:30am in the morning. Consequently, males can disperse some distance from a flight initiation point before being attracted/responding to the sex pheromone.

4) *How Many Pheromone traps are Needed in an Orchard to Detect the Presence of Stenoma when Damage is Visually Undetectable?*

The purpose of this study was to ascertain if it is possible to detect the presence of *Stenoma* in avocado orchards that have no obvious *Stenoma* damage. To determine the number of traps needed to detect *Stenoma* in the study orchards listed above, different numbers of traps were deployed for one week and then inspected for *Stenoma*. If *Stenoma* was detected, the number of traps deployed was decreased and deployed for a further seven days. This process of trap elimination was repeated until *Stenoma* was not caught, and at this point it was assumed that the minimum trap number had been attained for an orchard of a particular size. If *Stenoma* was not detected, the number of traps was increased until *Stenoma* was detected. Once *Stenoma* was detected, it was assumed that the minimum number of traps needed for detecting *Stenoma* was attained for that particular sized orchard.

i) Results for the 187 acre Booth Orchard in Escuintla. The initial deployment of 6 randomly placed traps at this site caught *Stenoma* in the first week of deployment. If this number of traps had been unsuccessful, 12 traps were to be deployed the following week. This doubling of trap numbers was to be repeated until *Stenoma* was caught, and traps were to be deployed in a GPS grid system based on a design overlaid on a Google Earth map. Because 6 traps caught *Stenoma*, the number of traps was reduced by 50%, and just three traps were deployed the following week. Three traps failed to catch moths over this 1 week period. Trap number was increased to four and at this trap density, four traps captured moths on three different occasions, a 100% success rate. The three trap deployment was repeated for a total of three replicates, and all three times a three trap deployment failed to detect *Stenoma*. Other traps deployed in different parts of the orchard were catching moths, so we know that over this time that the three trap density was being assessed *Stenoma* was still present in the orchard. It was concluded that 4-6 traps need to be deployed in a 187 acre orchard to reliably detect *Stenoma*.

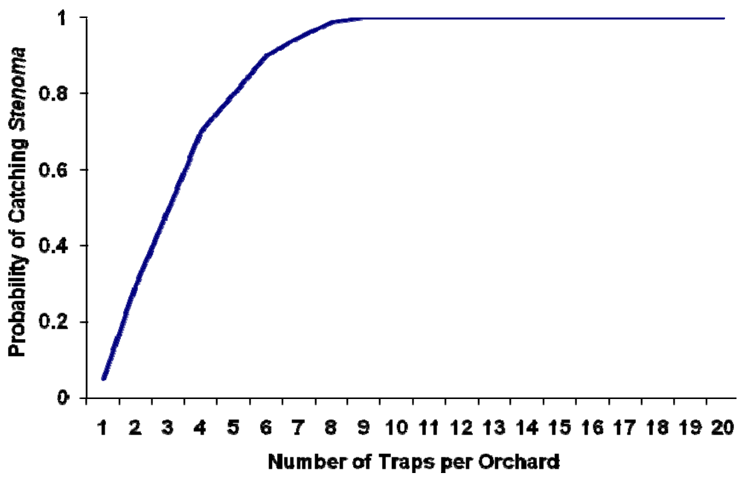
ii) In a nine acre orchard, the same experimental procedure revealed that 1-6 traps can detect *Stenoma* over a one week period. However, 50% of the time a single trap will not catch *Stenoma* over a 7-day period.

iii) A seven acre orchard required 3 traps to reliably capture *Stenoma* over a 7-day period.

iv) In orchards 1.5-2.5 acres in size, three traps captured *Stenoma* 100% of the time, but one trap only caught *Stenoma* 66% of the time over a 7-day period.

The trap data we have accumulated from all of these trials will be subjected to a probability analysis with assistance from statisticians in the Department of Statistics at UC Riverside. The goal of these analyses will be to develop a probabilistic relationship between the likelihood of trap capture and the number of traps per orchard. Ideally, this relationship will be independent of orchard size and will require the random deployment of traps along roads and maintenance trails in orchards, and not require a sophisticated grid-GPS deployment strategy (see Fig. 5 below).

**Fig. 5.** It may be possible to develop a probabilistic relationship similar to the graph shown below, that any given trap density has a certain probability of catching *Stenoma* in a one week period independent of orchard size. The possibility of developing this probabilistic model will be investigated with statisticians at UCR.



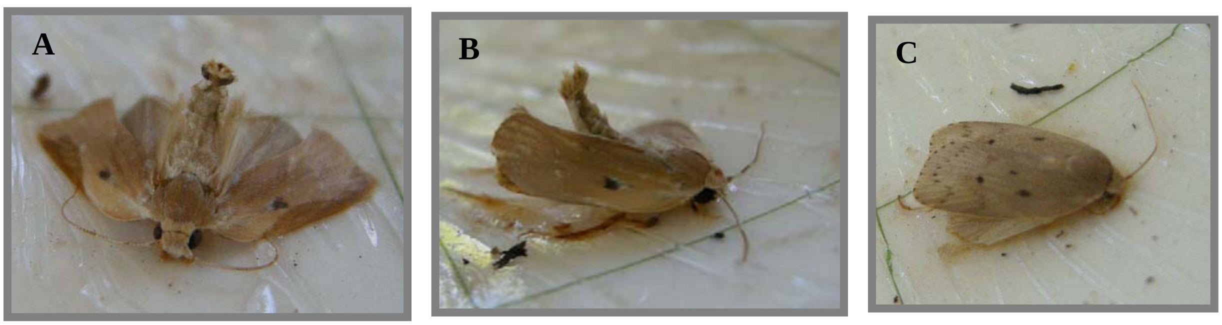

5) *Evaluation of the Attractiveness of the Stenoma Sex Pheromone to Stenoma Populations Outside of Antigua, Guatemala.*

One potential limitation affecting the utility of the *Stenoma* sex pheromone could be the possibility that *Stenoma* populations distant from Antigua Guatemala would be unreceptive to the pheromone because of the allopatric evolution of species races that respond to different blends, concentrations, etc. of the pheromone. To test this possibility, pheromone traps with the *Stenoma* sex pheromone were deployed in Hass avocado orchards in Quetzaltenango Guatemala (~90 kilometers [~56 miles] from Antigua) and Tapachula in the state of Chiapas, Mexico (~192 kilometers [~118 miles] from Antigua). In both locations, the pheromone was attractive to male *Stenoma*. This finding supports earlier work that has demonstrated that the pheromone can attract male *Stenoma* in Brazil (Millar unpublished field results). It would appear that race specific blends/concentrations of pheromone are probably not needed to attract *Stenoma* in orchards, and the active compound identified from *Stenoma* mass reared from colonies established from Antigua Guatemala should be attractive anywhere *Stenoma* is present.

**Additional Observations:**

In addition to *Stenoma*, the pheromone was extremely attractive to another species of moth, *Antaeotricha nicititana* (Lepidoptera: Elachistidae: Stenomatinae) (identification provided by Dr. John Brown, USDA-ARS Systematics Entomology Laboratory, Smithsonian Institute, Washington DC), a close relative of *Stenoma* (same tribe). This moth was very abundant in traps and present in avocado orchards at around ~1500-1700ft in Escuintla Guatemala and Tapachula Mexico. Captures of *A. nicititana* were often 7-9 times higher than captures of *Stenoma*. The significance of the presence of this moth in avocado orchards is unknown. There is very little available information about this moth, its biology, host plant preferences, or associated natural enemy fauna. A photo of this moth is shown in Fig 6A. The orchard in Escuintla that yielded *A. nicititana* was surrounded by pineapple and rubber tree plantations. In Tapachula, the Hass avocado orchard was planted as a cover crop for coffee. Based on trapping results it may be reasonable to assume that *A. nicititana* has a close association with avocados as this is the only plant common to both sampling sites, and *A. nicititana* could be an unknown pest of avocados.

**Fig. 6.** *Antaeotricha nicititana* (A and B) was caught in traps baited with the *Stenoma* pheromone in Escuintla Guatemala and Tapachula Mexico. This moth is easily identified by the single black spot on the forewing. (C) *Stenoma* has many more black spots on its forewings in comparison to *A. nicititana*, and male *Stenoma* tend to be smaller as well.




**Conclusions:**

1) The sex pheromone for *Stenoma catenifer* has been successfully isolated, synthesized, and field tested for efficacy in Guatemala, Mexico, and Brazil.
2) The grey rubber septum that has been used in all previous trials and evaluated for efficacy in this report is one of the best lure dispensers available for releasing the *Stenoma* sex pheromone.
3) The optimal height for pheromone trap placement is ~1.75 meters in trees.
4) Male *Stenoma* can fly 40-85 meters in one night, and average ~65 meters per night.
5) It would appear that very few randomly deployed traps are necessary for detecting extremely low density *Stenoma* populations in commercial avocado orchards. Depending on orchard size, as few as 1-6 traps may be all that are required for determining the presence of this pest. Further analysis of trap capture is planned with statisticians at UC Riverside to determine if a probabilistic model can be developed relating trap number to likelihood of detecting *Stenoma*.
6) In addition to the pheromone work, we have investigated and published on the natural enemy fauna associated with *Stenoma* in Guatemala, and two new species of avocado feeding moth, *Histura persevora* and *Holcocera plagatola*, have been described and published in the Proceedings of the Entomological Society of Washington.
7) In addition to *Stenoma catenifer*, the active compound in the pheromone of this pest is also very attractive to *Antaeotricha nicititana,* a moth found in avocado orchards Escuintla Guatemala and Tapachula Mexico. This moth is closely related to *Stenoma*

*catenifer,* and may be a pest of avocados. Nothing is known about the biology of this insect, and it is possible that this moth is restricted to hot, humid, low altitude areas that are marginal for Hass production.

**Recommendations:**

1) Once the work reported on here is published in a peer-reviewed journal (anticipated time line for this is will around 6-8 months, which includes writing, submission, peer-review, revision, and publication) then the California Avocado Commission will be in a strong position to request that avocado exporting countries with endemic populations of *Stenoma* monitor their orchards for the presence of *Stenoma* with the sex pheromone. Consideration of the potential pestiferousness of *Antaeotricha nicititana* should be taken seriously and included in monitoring requests of exporting orchards.
2) A monitoring system in California should be set up and the *Stenoma* sex pheromone deployed around packing houses, and especially packing houses/receiving facilities that are embedded in avocado orchards (e.g., CMT in Carpinteria). About 30-50 traps could be deployed from San Diego County (possibly including cooperating homeowners in San Diego County [e.g., Master Gardeners who have participated in the avocado lace bug project]) to San Luis Obispo. UC Riverside personnel could be hired to change out traps every two months and to identify captured moths. Trap capture information for California would be confidentially provided to the California Avocado Commission.

**Publications Resulting from this Work on *Stenoma catenifer*:**


Adamski, D. and M. S. Hoddle. 2009. A new *Holcocera* Clemens from Guatemala and redescription of *H. iceryaeella* (Riley) from the US (Lepidoptera: Coleophoridae: Blastobasinae: Holcocerini); two congeners with incidental preference to avocado. Proceedings of the Entomological Society of Washington (in press).

Brown, J. W. and M. S. Hoddle. 2009. A new species of *Histura* Razowski (Lepidoptera: Tortricidae: Polyorthini) from Guatemala attacking avocados (*Persea americana*) (Lauraceae). Proceedings of the Entomological Society of Washington (in press).

Hoddle, M. S. and C. D. Hoddle. 2008. Bioecology of *Stenoma catenifer* (Lepidoptera: Elachistidae) and associated larval parasitoids reared from Hass avocados in Guatemala. Journal of Economic Entomology 101: 692-698.

Hoddle, M. S. and C. D. Hoddle. 2008. Lepidoptera and associated parasitoids attacking Hass and non-Hass avocados in Guatemala. Journal of Economic Entomology 101: 1310-1316.

Hoddle, M. S. and C. D. Hoddle. 2008. Aspects of the field ecology of *Stenoma catenifer* (Lepidoptera: Elachistidae) infesting Hass avocados in Guatemala. Florida Entomologist 91: 693-694.

Hoddle, M. S., J. G. Millar, C. D. Hoddle, Yunfan Zou, and J. S. McElfresh. 2009. Field evaluation of the sex pheromone of *Stenoma catenifer*. Journal of Economic Entomology (in press).

Millar, J. G., M. S. Hoddle, J. Stephen McElfresh, YunFan Zou, and C. D. Hoddle. 2008. (9*Z*)-9,13-Tetradecadien-11-ynal, the sex pheromone of the avocado seed moth, *Stenoma catenifer*. Tetrahedron Letters 49: 4820-4823.