# CALIFORNIA AVOCADO COMMISSION
# PRODUCTION RESEARCH COMMITTEE
# MEETING MINUTES

## February 17, 2025

A meeting of the Production Research Committee (PRC) of the California Avocado Commission (CAC) was held on Monday, February 17, 2025, with the following people participating:

**MEMBERS PARTICIPATING:**
Danny Klittich, Chair
Victor Araiza
Allisen Carmichael
Jim Davis
Consuelo Fernandez
Leo McGuire
Daryn Miller
Ryan Rochefort

**CAC STAFF PARTICIPATING:**
Ken Melban
April Aymami

**OFFICIALLY PARTICIPATING:**
Dr. Tim Spann, Spann Ag Research & Consulting

**GUESTS PARTICIPATING:**
John Berns

**CALL TO ORDER**

Danny Klittich, Production Research Committee (PRC) Chairman, called the meeting to order at 2:01 p.m. with a quorum present.

**OPPORTUNITY FOR PUBLIC COMMENT**

There were no public comments.

**APPROVAL OF MINUTES OF JANUARY 29, 2025 PRODUCTION RESEARCH COMMITTEE MEETING**

***MOTION***
***To approve the minutes of the January 29, 2025 Production Research Committee meeting.***

***(Davis/Rochefort) MSC unanimous***

***Motion 25-2-17-1***

**ACTION ITEMS**

**A. Consider proposal "Surveys for avocado fruit feeding insect pests in Guatemala"**

Chairman Klittich reminded the Committee that the Board had previously approved the concept of reevaluating the fruit feeding pests in Guatemala and it was the Committee's role to evaluate the scientific merit of the proposal being considered. Discussion ensued and there was general agreement that the proposal was scientifically sound and would achieve the stated objectives. Questions were posed about what data Guatemala has and if any of that information could be shared. Dr. Spann reminded the Committee that the Pest Risk Assessment (PRA) for Guatemala that was compiled by the USDA listed 10 fruit feeding pests of concern, six of which were identified by Dr. Hoddle during his previous CAC-funded project in 2006-09, and it was unlikely that Guatemala had done any work themselves since that time.

It was mentioned that USDA's standard procedure when a country asks for access is to ask the country for whatever information they have and then supplement that data with what is present in the scientific literature. Thus, it falls on the California industry to help populate the literature with accurate information.

The idea of working with the international avocado community to establish an insect taxonomy lab in Mexico was discussed. Although this would not address the current issue with Guatemala, it would help to further develop the database of avocado pests present in the native range of avocado.

The consensus of the Committee was that it was just a matter of time before pests appeared in the U.S. through fruit shipments and being prepared and able to quickly identify the pests and act would be beneficial to all avocado growers.

***MOTION***
***To recommend funding the proposal "Surveys for avocado fruit feeding insect pests in Guatemala" as submitted for a total cost of $510,202.***

***(Davis/Miller) MSC unanimous***

***Motion 25-2-17-2***

**B. Consider proposal "Chemical Synthesis and Field Evaluation of an Enantiopure (+)-Grandisol, the Putative Avocado Seed Weevil (*Heilipus lauri*) Aggregation Pheromone"**

The Committee was aware of recent weevil finds in fruit in packinghouses in Mexico and immediately moved into discussion. The consensus was that the proposal was scientifically sound. Despite previous issues with trying to synthesize the pheromone, the chemist working on the project has two viable pathways to follow to overcome the challenges. There was agreement that having the pheromone would allow for a monitoring program in the U.S. for early detection of the seed weevils if they were to

arrive. But the pheromone was also a tool for use in countries of export for monitoring in export groves and packinghouses.

The Committee also considered the growing restrictions on pesticide use in California and the benefits of a tool like this pheromone will be essential if we ever need to manage this pest in California.

The question was asked whether the field testing of the pheromone could be conducted in Guatemala in conjunction with the pest survey work rather than having to make separate trips to Mexico for testing. Dr. Spann agreed to ask Dr. Hoddle to consider this option.

***MOTION***
***To recommend funding the proposal "Chemical Synthesis and Field Evaluation of an Enantiopure (+)-Grandisol, the Putative Avocado Seed Weevil (Heilipus lauri) Aggregation Pheromone" as submitted for the total amount of $349,212.***

***(McGuire/Davis) MSC unanimous***

***Motion 25-2-17-3***

**C. Consider proposal "Delimiting cryptic species within avocado seed moth, *Stenoma catenifer* for improved management and control of an economically important pest"**

There was general agreement among the Committee that we don't fully understand what is going on with this pest and the species complex that composes it. However, there was some dismay at why no other avocado producing countries seem to be willing to help support this work and why they don't see the importance of it.

The question came up about whether fully understanding the species complex would allow for better pest management should these pests arrive in the U.S. Similarly, would this information be of benefit from a regulatory standpoint or would it be better to have a general classification of this pest and place the burden on the importing country to prove what species may be present there?

The consensus was that, although this is important work, it likely would not affect how we would manage the pest should it arrive in California and could be conducted at a later date. There was no support to recommend funding for this project.

## ADJOURN MEETING

Danny Klittich, Production Research Committee (PRC) Chairman, adjourned the meeting at 2:54 p.m.

Respectfully submitted,

Timothy Spann
Timothy Spann

**EXHIBITS ATTACHED TO THE PERMANENT COPY OF THESE MINUTES**

EXHIBIT A February 17, 2025, Production Research Committee AB 2720 Roll Call Vote Tally Summary

EXHIBIT B Proposal, “Surveys for avocado fruit feeding insect pests in Guatemala”

EXHIBIT C Proposal, “Chemical Synthesis and Field Evaluation of an Enantiopure (+)-Grandisol, the Putative Avocado Seed Weevil (Heilipus lauri) Aggregation Pheromone”

EXHIBIT D Proposal, “Delimiting cryptic species within avocado seed moth, Stenoma catenifer for improved management and control of an economically important pest”

EXHIBIT A

# CALIFORNIA AVOCADO COMMISSION
## Production Research Committee
## AB 2720 Roll Call Vote Tally Summary

*To be attached to the Meeting Minutes*

| **Meeting Name:** | **Meeting Location:** | **Meeting Date:** |
|---|---|---|
| *California Avocado Commission Production Research Committee Meeting* | *Hybrid*<br>*In-person – Ventura County Cooperative Extension Office, Ventura*<br>*Online – Zoom* | *February 17, 2025* |

| **Attendees Who Voted** | ***MOTION 25-1-29-1*** | ***MOTION 25-1-29-2*** | ***MOTION 25-1-29-3*** |
|---|---|---|---|
| Danny Klittich, Chair | *Did not vote* | *Did not vote* | *Did not vote* |
| Victor Araiza | *Yea* | *Yea* | *Yea* |
| Allisen Carmichael | *Yea* | *Yea* | *Yea* |
| Jim Davis | *Yea* | *Yea* | *Yea* |
| Consuelo Fernandez | *Yea* | *Yea* | *Yea* |
| Leo McGuire | *Yea* | *Yea* | *Yea* |
| Daryn Miller | *Yea* | *Yea* | *Yea* |
| Ryan Rochefort | *Yea* | *Yea* | *Yea* |
| ***Outcome*** | ***Unanimous*** | ***Unanimous*** | ***Unanimous*** |

**PROJECT SUGGESTION:** Surveys for avocado fruit feeding insect pests in Guatemala

**PI:** Mark Hoddle, Department of Entomology, University of California Riverside

**1. Pest Research Highlight Priority Topic:** This project addresses *High Priority Research Topic 24*: Imported fruit poses a risk of introducing unknown pests and diseases that could be detrimental to the California avocado industry. Avocado fruit from Guatemala was recently approved for importation into the US by USDA-APHIS and insects associated with fruit in Guatemala may pose an unknown risk to California avocado growers.

**2. Concept Synopsis:** At the request of the California Avocado Commission, surveys for fruit feeding pests associated with avocado fruit in Guatemala will be conducted. This project will build on previous research conducted on avocado fruit feeding moths in Guatemala from 2006-2009. However, unlike previously completed studies, fruit surveys will focus primarily on areas in Guatemala that were not intensively surveyed in earlier studies that were conducted around Antigua, Chimaltenango, Santiago Atitlán, and Escuintla over 2006-2009. One large previously unsurveyed area to the north of previously surveyed areas that is of particular interest is encompassed by moderate to high elevation avocado growing areas such as Quetzaltenango, Huehuetenango, San Marcos, Chichicastenango, San Cristóbal Verapaz, and one lower elevation production area to the west, Mazatenango. Hass avocado production within parts of this circumscribed area was “up and coming” when earlier surveys for fruit feeding pests were conducted.

**3. Project Objectives:** This project has one simple objective:

i. To collect as much avocado fruit as possible from six distinct geographic locations: (1) Quetzaltenango, (2) Huehuetenango, (3) San Marcos, (4) Chichicastenango, (5) San Cristóbal Verapaz, and (6) Mazatenango that have not been previously surveyed for avocado fruit pests (Fig. 1).

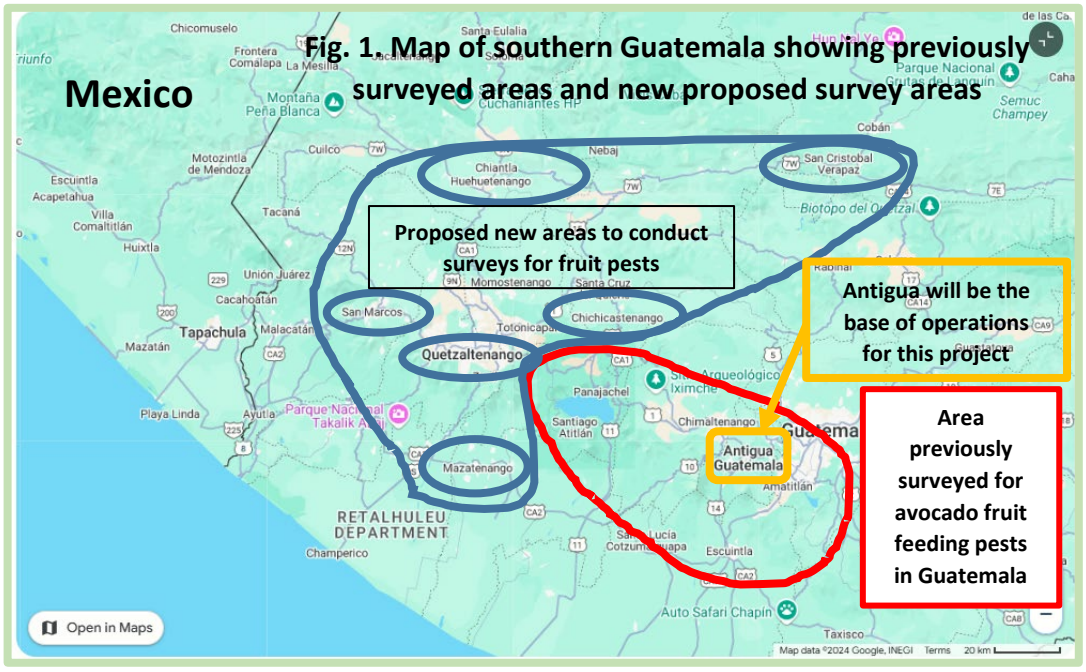



**Fig. 1. Map of southern Guatemala showing previously surveyed areas and new proposed survey areas**

ii. Fruit from these six areas of interested will either be collected with permission from small scale low input avocado growers, or as was done previously and with great success, purchased in large quantities from street vendors (Fig. 2A). Fruit will be placed in bugdorms (Fig. 2B) labeled by locality and date and all insects infested fruit will be reared out and preserved in 95% ethanol for future identification and DNA analyses. Fruit collections will be made in Guatemala over the "dry season," December – June.

**Fig. 2A.** Hoddle holding a sack of avocado fruit purchased from roadside vendors. These fruit were held in bugdorms and insects were subsequently reared from them.

**Fig. 2B.** A spare bedroom in a rented house in Antigua was used as a laboratory to rear out insects from avocado fruit held in labeled bugdorms. The plastic tables, rearing bins under tables, and free standing heater/fan were all purchased in Guatemala to set up this lab.

**4. Anticipated Project Duration:** 2 years. Starting December 2025.

**5. Total Estimated Project Cost:** $510,202

**Personnel:** Funds to hire a Specialist II at 100% time for years 1 and 2 of this project are requested. Annual salary is $90,000 in year 1 and $92,700 in year 2.The Specialist is fluent in Spanish, is very familiar with Guatemala having worked with the PI on fruit pest surveys in this country previously. Specialist will assist with all aspects of field and lab work in Guatemala and will take the lead in logistics organization pre and post travel and for curating and processing all collected insects after returning from Guatemala. Total salary request = $182,700.

**Benefits**: Benefits are set at 43.7% and are estimated to be $39,330 in year 1 and $40,510 in year 2. Total benefits = $79,840.

**Total Salary and Benefits:** Year 1 = $129,330, year 2 = $133,210. Grand total requested = $262,540.

**Supplies:** Funds to purchase bugdorms (light weight collapsible insect rearing cages) for rearing insects from collected fruit are requested and estimated by to be $6,000 in year 1 and $4,000 in year 2. Total request for bugdorms = $10,000. Funds are requested to purchase fruit from roadside vendors in areas

being surveyed for avocado fruit pests. $1,500 is estimated in year 1, and $1,575 for year 2. Total request for fruit purchases = $3,075. Field supplies (i.e., vials, note books, ethanol, flagging tape, indelible markers, Stenoma pheromone, heaters and fans to moderate temperature in the insect rearing "labs" (i.e., the spare bedrooms) is estimated at $2,200 in year 1 and $2,310 in year 2 for a grand total of $4,510 for both years of this project.

**Total Supply Costs:** Year 1 estimated at $9,700, year 2 at $7,885 for a grand total of $17,585 for both years of this project.

**Publications:** One publication in a peer reviewed entomological journal is anticipated in year 1 at $3,800 and year 2 at $3,990. Total publication costs for two peer reviewed articles on the results of fruit surveys is estimated at $7,790.

**Travel:**

**Airfares and excess luggage costs:** Round trip airline tickets, LAX to Guatemala City are estimated at $800 per person. Cost of tickets for two people in year 1 is estimated at $1,600 and in year 2 at $1,648. Excess baggage to move all necessary materials from California to Guatemala is estimated at $120/bag and up to four extra suitcases per person are likely to be needed. Cost is estimated at $960 in year 1 and $989 in year 2 for a total of $1,949 for both years of this project.

**Accommodation:** This project will be based in Antigua, a relatively safe city in Guatemala and one that is popular with foreigners. Consequently, accommodation in this city is expensive. An Air BnB search for a 4 bedroom house (spare bedrooms are needed as labs for setting up rearing cages [see Fig. 2B above]) in Antigua in a secure guarded compound (24 hr security) returned a monthly rental estimate of $5,500. For six months in year 1, rental accommodations are estimated at $33,000 and in year 2 at $33,900. Total rental estimates for year 1 and year 2 are estimated at $66,990.

Because the proposed new survey areas are long distances from Antigua (See map, Fig. 1) it will be necessary to rent hotel rooms/Air BnB outside of Antigua for multiple multi-day excursions into areas where fruit will be collected. Decent and secure accommodation in these towns can be expensive, and may require stays within private and secure residences. Estimated cost per night of overnight stays is estimated at $120/night, with 10 nights per month for each of 6 months being required to achieve survey goals. Total cost of hotel stays outside of Antigua is estimated at $7,200 in year 1 and $7,416 in year 2. Total hotel costs for years 1 and 2 are estimated at $14,616.

**Incidentals:** The Federal Govt. daily incidentals and expenses cost for areas in Guatemala outside of Guatemala City are set at $80/day/person. Total incidentals/meals costs for two people for 6 months is estimated at $26,880 in year 1 and $27,686 in year 2 for a grand total of $54,566 for both years of this project.

**Transportation:** Overland travel in Guatemala is difficult. Roads are often in poor condition (the exceptions are toll roads which can be expensive to travel on) and drivers are outrageously dangerous. Therefore to accommodate the demands of bad roads, especially in rural areas where avocados are grown a 4WD SUV will be needed to get to sites and to have "secure" storage for purchased fruit. Vehicle rental and insurance costs are very high in Guatemala because of risk of theft and accidents. The best estimate for a mid-size SUV hired at Guatemala City International Airport with full insurance is

$200/day. Total rental cost for 6 months for year 1 of this project will be $33,600 and in year 2 the cost is estimated at $34,608. Total vehicle rental for both years of this project is estimated at $68,208.

Gas in Guatemala is expensive and a lot of driving is anticipated to get to field sites each month of this project. Total gas cost is estimated at $450/month and will cost $2,700 in year 1 and $2,835 in year 2 for a total cost of $5,535 for both years of this project.

Whenever possible toll roads will be taken in and out of major cities. Toll roads tend to be in reasonable condition and are regularly patrolled by police which reduces risks to drivers. Additionally, parking of vehicles on streets is very risky and secure 24 hr parking areas are needed to park vehicles overnight, especially when staying in hotels or even when visiting small towns to buy fruit during the day. Cost of tolls and secure parking in year 1 is estimated at $500 and in year 2 at $525. Total cost of tolls and secure parking for year 1 and 2 of this project is estimated at $1,025.

**Professional Meeting:** Funds are requested to attend one professional entomology conference in years 1 and 2 of this project to present results of field surveys. Cost of meeting attendance (registration, flights, hotel, meals) is estimated at $3,000 in year 1 and $3,150 in year two for a grand total of $6,150.

**Total Travel Costs:** Six months in Year 1 = $109,440, six months in year 2 = $112,847. Grand total for 12 months of travel in Guatemala spread over 2 years = $222,287.

**Grand Totals:** Total project cost in year 1 is estimated at $252,270, year 2 = $257,932, total project costs for years 1 and 2 combined = $510,202.

California Avocado Commission

**PROJECT SUGGESTION:** Chemical Synthesis and Field Evaluation of an Enantiopure (+)-Grandisol, the Putative Avocado Seed Weevil (*Heilipus lauri*) Aggregation Pheromone

**PI:** Kevin Kou, Department of Chemistry, UC Riverside

**Co-PI:** Mark Hoddle, Department of Entomology, UC Riverside

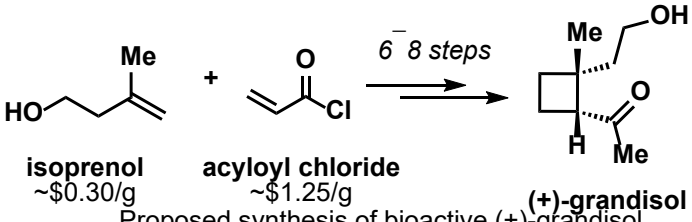
**1. Pest Research High Priority Topic:** *Risk of importing Heilipus weevil-infested avocadoes that could be detrimental to the California avocado industry.*

**2. Concept Synopsis:** The aggregation pheromone of the avocado seed weevil, *Heilipus lauri* has been identified from a previously supported CAC project. This complex molecule is grandisol and simple syntheses result in mixtures of several different forms (i.e., enantiomers) of this molecule. Unfortunately, field evaluations of these impure mixtures were not attractive to weevils in heavily infested Hass orchards In México. This finding has led us to conclude that weevils may only respond to the pure biologically-active form of grandisol and the impurities when present do not attract weevils. Despite over thirty synthetic strategies reported for the preparation of grandisol, most yield a mixture of biologically-active and inactive enantiomers, while methods targeting a single enantiomer produce impractical milligram quantities of the pheromone.[1,2] We propose developing a concise synthesis approach to access significant quantities of bioactive (+)-grandisol. The pure bioactive form of grandisol will be field tested in weevil infested Hass avocado orchards in México. If successful, this aggregation pheromone will be a very powerful tool for monitoring for weevil presence in export areas of México where this weevil is native, and Colombia where this pest is invasive. Additionally, the pheromone will be invaluable for monitoring for weevil incursions into California.

**3. Project Objectives:**

I. Synthesize >1 gram of (+)-grandisol starting from racemic (±)-grandisol. We will develop a kinetic resolution method using the Corey-Bakshi-Shibata reagent to separate the inactive (–)-enantiomer. This is a low-risk approach to quickly generate enough of the active pheromone for initial field studies.

II. Advance a short synthesis route to (+)-grandisol starting from readily accessible reagents isoprenol and acryloyl chloride (Figure 1). Two approaches are envisioned: one incorporates the method established in **Project Objective I**, while the other explores a novel chemical reaction to construct the pheromone's four-membered ring as a single enantiomer. The latter approach offers a 100% increase in yield, as it avoids the 50% yield loss from the kinetic resolution method, which requires removal of the inactive (–)-pheromone that accounts for half the material. Both methods are designed to be scalable for manufacturing.




Proposed synthesis of bioactive (+)-grandisol

**Figure 1.**

III. Conduct field studies in México to evaluate the efficacy of enantiopure (+)-grandisol in trapping *Heilipus* weevils, as previous field testing showed racemic (±)-grandisol to be ineffective.

**4. Anticipated Project Duration:** 2 years

**5. Estimated Total Project Cost:** Total $349,212

I. **Salaries and Benefits:** One post-doctoral fellow ($71,362) + employee benefits ($15,974 @ 22.4%) at 100% time in year 1 and 50% time in year 2. Post-doctoral fellow will conduct

experiments, record observations and results, as well as analyze data. Total salary ($108,113) and benefits ($24,217) = $132,331. Specialist II, Entomology, to assist with field work in México at 33% time in yr 1 and 2, salary = $29,568 (yr 1) and $30,455 (yr 2) and benefits at 26% = $7,688 (yr 1) and $7,918 (yr 2). Total salary and benefits for yrs 1 & 2 = $75,629. **TOTAL SALARIES FOR PROJECT = $219,615**

II. **Supplies**: Consumable supplies and reagents ($12,000 in yr 1 and $4,000 in yr 2. Total reagents = $16,000) will support chemical synthesis experiments. Sticky cards for field trials (white, blue, green, yellow, and purple) to test attraction for weevils with pheromone = $6,000 yr 1 and $3,000 in yr 2. Total cost of sticky cards = $9,000. Field supplies for pheromone testing in México (flagging tape, vials, ethanol, note books, markers), $1,500 in yr 1 and $500 in yr 2. **TOTAL SUPPLIES FOR PROJECT = $27,000**

III. **Equipment:** UV Photoreactor ($7,000). The photoreactor provides ultraviolet (UV) irradiation for photochemical experiments and synthesis of grandisol and this equipment safely encloses and isolates reactive UV radicals. **TOTAL EQUIPMENT COST = $7,000**

IV. **Analytical Services:** Analytical Chemistry Instrumental Facilities needed for confirming and characterizing chemical intermediates and products synthesized. Estiamated cost is $5,000 in yr 1 and $2,500 in yr 2. **TOTAL ANALYTICAL SERVICES COST = $7,500**

V. **Publication Costs:** Estimated publication costs in yr 2 of project for grandisol synthesis = $3,000 and for entomological field trials with grandisol in México = $3,000. **TOTAL PUBLICATION COSTS = $6,000.**

VI. **Travel to Meetings and México for Field Work:** Cost to attend one professional O-Chem conference, PacifiChem, to present results of grandisol synthesis work = $3,000. Travel to one professional entomology meeting to present results of field work in México = $3,000. Return air flights from LAX to México City for 2 people at $600 round trip = $1,200 in yr 1 and $1,236 in yr 2, total air flights =$2,436. Air BnB, three bedroom house in Malinalco at $250/night for 56 nights (i.e., 8 weeks) = $14,000 in yr 1 and $14,420 in yr 2, total accommodation = $28,420. Meals and incidentals for two people in Malinalco based off of the US Federal rate for México City = $122/day. Malinalco is close to MX City and estimated at $80/day per person for 56 days = $8,960 for yr 1 and $9,229 in yr 2, total for yr 1 and 2 = $18,189. Rental car to get to field sites in and around Malinalco with full insurance estimated at $150/day. For 56 days rental car cost = $8,400 in yr 1 and $8,652 in yr 2, total rental car cost = $17,052. **TOTAL TRAVEL COSTS FOR YR 1 AND 2 = $72,097.**

VII. **Contract with Cooperators in México:** We have previously conducted field work on *Heilipus* weevils in México with Dr. Armando Martínez-Equihua, a professor in entomology at the post-graduate college in Texcoco México. This has been an excellent arrangement. Dr. Martínez-Equihua is a preeminent avocado pest researcher in México whose previous assistance has been invaluable in procuring access to field sites for medium-term field studies on *Heilipus*. Assistance with Mexican cooperators for this project will be essential if it is to be successful. To facilitate this cooperation, Dr. Martínez-Equihua will require $5,000 in yr 1 and yr 2 for a **total of $10,000** to help with negotiations with Comite Estatal de Sanidad Vegetal del Estado de México, in Malinalco and Coatepec-Harinas, México to locate and access field sites and to get permission from orchard owners, to assist with on the ground logistics, and to participate in planning and execution of field trials.

**References Cited**: 1) dos Santos, M. K. *et al. Mini-Rev. Org. Chem.* **2021**, *18*, 690.

California Avocado Commission

2) Bartlett, W. R. *et al. Synthesis* **2022**, *54*, 3209.

**Delimiting cryptic species within avocado seed moth, *Stenoma catenifer* for improved management and control of an economically important pest**

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*Alica Timm, Department of Agricultural Biology, Colorado State University, Fort Collins, CO 80523 alicia.timm@colostate.edu 1-970-490-4495*

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**California Avocado Commission priority research area**

Pest Research High Priority number 24: Identification of unknown pests that could be introduced into the U.S. that would be detrimental to the California avocado industry

**Concept synopsis**

The avocado seed moth, *Stenoma catenifer*, is an avocado pest of major economic importance in the Neotropics, which encompasses its native range. This pest is a significant threat to the California avocado industry, where it is not yet established. Identifying this species is challenging, as adults possess few distinguishing external morphological characteristics besides a "C" pattern of black dots on the forewings. The lack of identifying characteristics may have led to an overly broad and phylogenetically uninformative species description of *S. catenifer*, which may be better defined as a species complex.

Nucleotide sequences of the mitochondrial DNA barcoding region (cytochrome oxidase 1) that are available in publicly available databases provide preliminary but compelling evidence that cryptic species can be resolved within the current delimitation of *S. catenifer*. Based on these 32 mtDNA sequences, *S. catenifer* is comprised of six Barcode Index Numbers (BINs). Although recognition of BINs does not always indicate discrete species, they do indicate that further investigation into their species status is necessary. A phylogenetic tree based on available sequences for specimens considered to be *S. catenifer* is shown in Figure 1. The tree shows evidence for three major clades – one from French Guiana, a second from South America, and a third from Mexico and Central America. The three sequences from Mexico can be divided into two well supported lineages (likely species), and the nine from Peru can be divided into three well supported lineages (likely species). Photographs of the sequenced specimens all show the pattern of dots on the forewing that is characteristic for *S. catenifer*. Since these sequencing results are based on a small number of specimens from a limited part of the geographic range for *S. catenifer*, extensive sampling and sequencing of a much greater number of individuals throughout the range of the species, including at different elevations, is crucial. With this increased sampling, it can reasonably be expected that *S. catenifer* could be divided into more species than are shown on the tree. The primary objective of this study is therefore to sample *S. catenifer* from Mexico, Guatemala, Costa Rica, Belize, Honduras, Panama, Colombia, Venezuela, Ecuador, Peru, Brazil, Bolivia, Guyana, Argentina, Paraguay, and Uruguay, and to evaluate and delimit cryptic species from these regions based on DNA barcode sequences (including ITS, CO1, and EF1-

alpha). Following this objective, the morphology of each cryptic species will be described in detail, including images of the morphological characters most useful for separating species. Species complexes that include morphologically similar species can vary remarkably in aspects of their biology, including their host range, specificity, and behavior. Recognizing cryptic species is therefore essential not only for effective targeted pest management, but also has important implications for agriculture since each lineage may cause differing degrees of damage. The confirmation of cryptic species and the delimitation of each species within *S. catenifer* would justify a revision of current quarantine regulations.

Nucleotide sequence data potentially allows the identification of the geographic origin of a specimen. Haplotype network analysis and the identification of single nucleotide variants (SNVs) that are associated with geography are helpful for identifying the source of an invasive species. For *S. catenifer*, this data would be particularly useful for individuals from Mexico and Guatemala, since both countries are now exporting fruit into California from different areas. The DNA barcode sequences that will be generated as part of the first objective of the study can be used to examine genetic variation within each cryptic species, and this variation could pinpoint the origin of each haplotype. If *S. catenifer* is introduced into California, knowledge of geographic variation within each species would be crucial for identifying the origin of the invasion, and could inform future quarantine and pest eradication measures, as well as the development of biological control programs with natural enemies from the pest's accurately identified native range.

The objectives of this study are therefore to confirm cryptic species within *S. catenifer* using genetic data to delimit species, to provide comprehensive morphological descriptions for each species, and to associate *S. catenifer* genotypes with their geographic origin to allow the determination of the area of origin of a specimen. The resulting data would identify new pest species in the Neotropics that threaten the California avocado industry via the potential risk of invasions through commercial fruit export and improved understanding of this putative species complex would be an essential foundation for the development of biological control programs should such an effort be needed in the future.

**Main project objectives**

1. Evaluate cryptic species within *S. catenifer* based on DNA barcode sequences from across the range of the species. A limited number of sequences are already available. This project will generate additional sequence data and use a variety of species delimitation methods to confirm that *S. catenifer* is a species complex, and to delimit cryptic species.
2. Describe the external morphology of *S. catenifer*, including all cryptic species, to enable unequivocal identification. High-resolution images will be taken of all relevant morphological characteristics to enhance identification.
3. Examine within-species variation based on nucleotide sequence data that will include DNA barcodes and potentially other genes. This information would facilitate the identification of the geographic origin from which an interception originated.

**Anticipated project duration**

The project is anticipated to be conducted over a 2-year period. During the first year, specimens will be collected, their DNA will be sequenced, and the results will be analyzed. These genetic data will be used to inform morphological descriptions during the second year of the project.

**Estimated project cost**

| ITEM | DOLLAR AMOUNT | DETAILS AND JUSTIFICATION |
|---|---|---|
| **PERSONNEL**: | | |
| Postdoctoral fellow | $130, 000 | Base salary for two years for a post-doctoral fellow to collect specimens, conduct laboratory work, analyze data, and write manuscripts |
| **Subtotal** | **$130, 000** | |
| **FRINGE BENEFITS:** | | |
| 13% of post-doctoral fellow salary during the first year | $8,450 | Fringe benefits associated with post-doctoral fellow salary, in accordance with CSU policies |
| 28% of post-doctoral fellow salary during the second year | $18,200 | |
| **Subtotal** | **$26,650** | |
| **TRAVEL:** | | |
| *International travel* | | |
| Airfare | $20,000 | Foreign travel for two researchers to collect *S. catenifer* throughout its range in Mexico, Central, and South America. Potential collecting locations include Mexico, Guatemala, Costa Rica, Belize, Honduras, Panama, Colombia, Venezuela, Ecuador, Peru, Brazil, Bolivia, Guyana, Argentina, Paraguay, and Uruguay. |
| Accommodation | $16,000 | |
| Ground transportation | $15,000 | |
| Gas, tolls and parking | $2,000 | |
| Per diem / meals | $15,000 | |
| Incidentals | $5,000 | |
| *Domestic travel* | $3,000 | Travel to entomological meetings to present findings |
| **Subtotal** | **$76,000*** | |
| **SUPPLIES** | | |
| *Molecular laboratory supplies* | | Supplies necessary for extracting DNA from specimens and generating sequence data. |
| Capillary array cartridges | $6,000 | |
| DNA extraction kits | $3,000 | |
| Flowcells and kits for high throughput sequencing | $4,000 | |
| Tips, tubes, gloves, etc | $2,000 | |
| *Imaging supplies* | | Supplies necessary for imaging species to provide high resolution images of relevant morphological characteristics |
| Mounting media, imaging software, slides, etc. | $1,000 | |
| *Insect collecting supplies* | | Supplies required for collecting *S. catenifer* throughout its native range |
| Pheromones for attracting moths | $1,200 | |
| Traps for collecting moths | $500 | |
| Material to remove moths from traps | $30 | |
| Vials and ethanol to preserve moths | $250 | |
| Shipping specimens to partner institutions | $1,000 | |
| **Subtotal** | **$18,980** | |
| **OTHER** | | |
| Page charges for publications | $8,000 | Page charges for 2-3 publications resulting from this study |

| | | |
|---|---|---|
| Subtotal | $8,000 | |
| **TOTAL COSTS** | **$129,630** | |

***SUBCONTRACT TO UC RIVERSIDE:** We estimate that approximately 50% (~ $38,000) of the travel funds for this project will be used by Mark Hoddle, Department of Entomology UC Riverside, to collect *Stenoma* specimens for this project.

**Figure 1.** Maximum likelihood tree, of *S. catenifer* mtDNA sequences (CO1) available in public databases, along with three outgroup species. BIN numbers, each indicating a putative species, are indicated next to brackets.

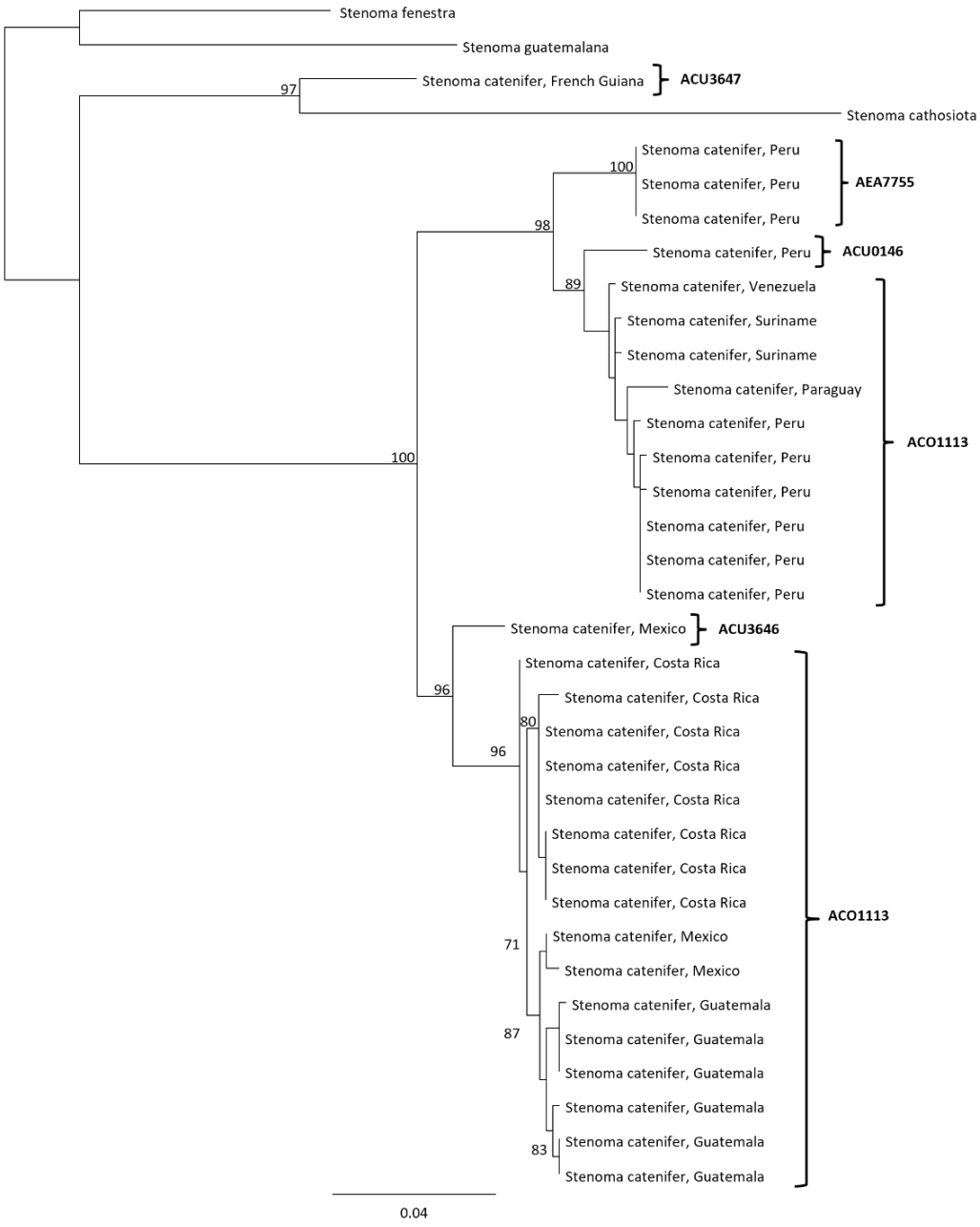

**Figure 2.** Map showing the known distribution of *S. catenifer*. Countries indicated in blue show localities from which samples have been sequenced, along with the number of sequenced samples from each. Countries indicated in orange show unsampled localities.