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Biocontrol Efforts Against Avocado Pests in California: A Review and Recommendations for Future Proactive Programs for Identifiable Invasion Threats

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Introduction

Avocados (*Persea americana* Miller [Lauraceae]) have an evolutionary range that includes the eastern and central highlands of Mexico, Guatemala and the Pacific Coast of Central America as far south as Panama (Knight 2002). Because of its value as a food, humans domesticated avocados and moved them from this area of origin into northern South America by 4000 BC where it became naturalized (Knight 2002). In an effort to build new and diverse agricultural commodities in the US, "Agricultural Explorers" with the USDA's "Office of Foreign Seed and Plant Introduction" searched globally for new commodities with economic potential. Popenoe (1919) championed the avocado as a novel new crop for the US and California in particular.

The first commercial avocado grove was planted in Los Angeles County, California in 1908. Today, avocados are an iconic crop immediately identifiable with California. This image was reinforced in the California mindset when part of Interstate Highway 15 in San Diego County (~45% of the crop is grown here) between the junction of State Highway Route 78 in the City of Escondido and the City of Temecula was designated as the "Avocado Highway" in 1997 by a California State Assembly Resolution. More than 95% of total US avocado production is grown in California with the remainder being produced in Florida and Hawaii. The rough black-skinned 'Hass' cultivar accounts for ~95% of the ~330 million pounds of fruit produced annually (California Avocado Commission [CAC] 2008). This California Specialty Crop is grown on ~66,000 acres (mainly in San Diego, Riverside, Orange, Los Angeles, Ventura, Santa Barbara, and San Luis Obispo Counties), by ~6,000 growers and is worth ~$328 million each year (CAC 2008).

Pest Invasions and the Erosion of Naturally-Occurring Biological Control

Historically, pesticide use in California avocado orchards has been minimal. Important native and exotic pests like greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché) (Thysanoptera: Thripidae), avocado brown mite, *Oligonychus punicae* (Hirst) (Acari: Tetranychidae), six-spotted mite, *Eotetranychus sexmaculatus* (Riley) (Acari: Tetranychidae), and omnivorous looper, *Sabulodes aegrotata* (Guenée) (Lepidoptera: Tortricidae), *Amorbia cuneana* (Walsingham) (Lepidoptera: Tortricidae) have been kept below economically injurious levels by indigenous natural enemies (Ebeling, 1950; Fleschner, 1954; Fleschner *et al*., 1955; McMurtry 1992). Until relatively recently, California avocado production was world-renowned because it relied almost exclusively on biological and cultural control (e.g., selective fruit thinning for *H. haemorrhoidalis* control) for suppression of noxious pests and pesticide applications were rare.

Because of recent repeated invasions by exotic pests, the importance of natural enemies and naturally-occurring biological control of avocado pests has eroded in California and pesticide usage has steadily increased to mitigate the negative economic impacts invaders have caused

(Fig. 1). This declining dependence on biocontrol began in 1982, when red-banded whitefly, *Tetraleurodes perseae* Nakahara (Hemiptera: Aleyrodidae), established in San Diego County (Hoddle, 2006; Hoddle and Soliman, 2001; Nakahara, 1995; Rose and Wooley, 1984a,b). Following red-banded whitefly, persea mite, *Oligonychus perseae* Tuttle, Baker & Abbatiello (Acari: Tetranychidae), was discovered attacking avocados in San Diego County in 1990 (Bender, 1993). In 1996, avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), was discovered almost simultaneously in orchards 160 km apart in Ventura and Orange Counties (Hoddle and Morse, 1998). In 2004, two more leaf feeding exotic insects were added to the resident pest phalanx attacking California avocados; the avocado lace bug (ALB), *Pseudacysta perseae* (Heideman) (Hemiptera: Tingidae) and *Neohydatothrips burungae* (Hood) (Thysanoptera: Thripidae) were discovered feeding and reproducing on backyard avocados in San Diego County. Over a 22 year period (1982-2004), five new, host specific avocado leaf feeding arthropods established in California; an establishment rate of a new pest approximately every 4-5 years. According to this time table, the industry is possibly due for another new pest to establish in California.

Despite a long history of domestication and prodigious production of fruit for domestic and export markets, the pest fauna associated with avocados in the indigenous range of this plant is poorly documented and little studied. For example, three of the most significant avocado foliage feeding pests in California, *T. perseae*, *O. perseae*, and *S. perseae* were all species new to science at time of first discovery in the US. These three pests are specialists on avocados (as is *P. perseae*) and likely originated in the home range of *P. americana* (Hoddle 2004). Molecular evidence confirmed this for *S. perseae* (Rugman-Jones *et al*., 2007). Similar results from molecular studies are emerging for *P. perseae* which indicate that the California population likely originated from the Pacific Coast of México (Hoddle *et al*., unpublished data). The illegal movement of plant material, possibly out of México into Southern California, is probably responsible for the introduction of these phytophagous pests as they are unlikely to hitchhike and survive on mature fruit, a commodity of high interest to smugglers because of its value (Hoddle, 2004).

Biological Control Programs Targeting Recent Avocado Leaf Feeding Pests

<u>*Red banded whitefly, Tetraleurodes perseae:*</u> Interestingly, the red-banded whitefly, *T. perseae*, was controlled by *Cales noacki* Howard (Hymenoptera: Aphelinidae), a parasitoid that had been established in California for biological control of woolly whitefly, *Aleurothrixus floccus* (Maskell) (Hemiptera: Aleyrodidae) on citrus (Rose and Wooley, 1984a,b). This is an example of "fortuitous" biological control, where a natural enemy released for control of one particular pest serendipitously provides control of a different pest species even though this was not intended. *C. noacki* can parasitize up to 92% of *T. perseae* nymphs in coastal areas of California, but this level of control is inconsistent, and tends to be lower in hotter arid interior regions (Hoddle, 2006). Despite variable control of *T. perseae* by *C. noacki*, this whitefly is not

considered an important pest of avocados in California. Other species of natural enemies of *T. perseae*, *Encarsia* spp. and *Eretmocerus perseae* Rose and Zolnerowich (both Hymenoptera: Aphelindae [Rose and Wooley, 1984a,b; Rose and Zolnerowich, 2004]) have been discovered in the home range of this pest, Michoacán, México. The potential exists for future importation and establishment of these natural enemies against *T. perseae* should it be needed. *T. perseae* has also invaded other avocado producing regions, Israel (in 2002), and Lebanon (2005), possibly via the movement of plant material from California (Hoddle, 2006). Basic aspects of the field ecology, phenology, and biology of this pest in California have been studied (Hoddle, 2006).

<u>*Persea Mite, Oligonychus perseae:*</u> This mite is native to México and has invaded Costa Rica (established 1970 in San Antonio de Coronado, Province of San Jose, on *P. americana* and was misidentified as *O. peruvianus* [Salas 1978]), Israel (established 2001), and Spain (established 2002/03). Following its establishment in California in 1990, this phytophagous mite rapidly spread and caused substantial economic injury to the California industry. Economic damage is caused by primarily by high pest populations feeding in colonies on the undersides of leaves. Significant feeding damage appears as circular brown necrotic spots caused by families of mites feeding within the protection of silken nests (Aponte and McMurtry, 1997). Once feeding damage to leaves reaches and exceeds ~10% of the surface area premature leaf drop begins (Kerguelen and Hoddle, 1999a,b). Mite-induced defoliation can reduce yields by up to 20% (Palevsky *et al*., 2007). It is a perennial problem in coastal production areas, particularly Ventura, Santa Barbara, and San Luis Obispo Counties. This mite is not problematic in interior production areas like Riverside County where high summer temperatures are lethal to persea mite.

There have been no foreign exploration efforts for natural enemies of persea mite in its presumed evolutionary area of origin (México). Consequently, biological control of *O. perseae* in California has focused exclusively on evaluating the efficacy of augmentative releases of commercially available predatory mites (phytoseiids). This process followed a series of steps: (1) screening six commercially available phytoseiid predators for establishment and impact on trees following artificial hand releases (Hoddle *et al*., 1999); (2) demonstrating efficacy of the most the two promising predators (i.e., *Neoseiulus californicus* (McGregor) and *Galendromus helveolus* (Chant) [both Acari: Phytoseiidae]) identified from (1) (Kerguelen and Hoddle, 1999b); (3) optimizing release rates and timings of the cheapest and most efficacious predator identified from (2) (i.e., *N. californicus*) (Hoddle *et al*., 2000a), and (4) the development and evaluation of a modified back pack sprayer for mechanically applying predators to trees. This sprayer was designed to reduce labor costs and predator dispersion inefficiency associated with point-release hand liberations and was demonstrated to maximize tree coverage by spraying viable predators over the tree canopy (Takano-Lee and Hoddle, 2001).

Even though predator mite releases were shown to be just as effective as pesticide applications (more so in some instances as resurgence was not observed [Kerguelen and Hoddle 1999b]), and they could be applied to trees quickly and effectively with a motorized back pack sprayer (Takano-Lee and Hoddle, 2001), predator mite releases have not been widely used by the avocado industry for persea mite control. Low adoption has been due to the relatively high cost (13-14 times more expensive than aerial applications of NR 415 petroleum oil, the industry standard when this work was done), and the need to monitor persea mites carefully to time releases when a low but steadily increasing pest population is detected in orchards thereby ensuring rapid establishment of predator populations on trees. If pest populations are too low when releases are made, predators go extinct because of lack of food. If pest populations are too high, predators can't exert control quickly enough and leaf damage results (Hoddle *et al*., 2000a). Because biological control is not cost competitive, and predator use requires careful monitoring of pest population dynamics, persea mite is controlled almost exclusively with pesticides in California because they are relatively easy to apply, often provide rapid knock down, and growers have invested significantly in application equipment which they feel needs to be used.

The standard industry control practice for persea mite is the application of abamectin with oil in summer. This product is also used against avocado thrips in the spring to control populations of this pest. Consequently, pest populations in avocado orchards can be exposed to two abamectin applications per year. Abamectin applications have a very long period of activity against targeted avocado pests, ~10-12 weeks (Morse *et al*., 2000) and resistance development is a concern (Humeres and Morse, 2005). To combat this potential problem, California avocado growers have invested resources in the evaluation of new miticides for registration in California to use in a rotation program with abamectin to manage resistance development. It is anticipated that within the next 1-2 years, three more pesticides will be available for use against persea mite in California: (1) Envidor (spirodiclofen), (2) Zeal (etoxozole), and (3) Fujimite (fenpyroximate).

Cultural control strategies have been evaluated for *O. perseae*. Use of (1) tanglefoot barriers on tree trunks, (2) removal of prematurely dropped leaves infested with mites from under trees, and a combination of (2) and (3) had no impact on mite densities on experimental trees. The main source of continued re-infestations was ballooning by mites abandoning leaves of deteriorating quality in an attempt to find better host leaves to colonize and not the climbing of tree trunks by mites on the ground that abandoned dropped leaves (Takano-Lee and Hoddle, 2002). Avocado varieties grown in California have been screened for resistance to *O. perseae*. The 'Hass' cultivar is highly susceptible to *O. perseae,* while 'Lamb Hass' is considered resistant (Kerguelen and Hoddle, 2000). Plant breeding may offer opportunities to better manage persea mite thereby reducing grower reliance on pesticides for managing this pest.

<u>*Avocado thrips, Scirtothrips perseae:*</u> This pest is the first invasive insect in California to directly damage avocado fruit. *S. perseae* builds to high densities on young avocado foliage and as this flush growth hardens off in late spring, thrips move to small developing fruit to feed (Yee *et al*., 2001a). The intensity and severity of fruit damage is correlated with population densities on flush growth and the amount of overlap between developing fruit susceptible to feeding damage

and the hardening off of leaf flush. Thrips (i.e., adults and larvae) feeding damage to the skin of the immature avocados (fruit < 2 cm in length are most susceptible, beyond this size skin thickness increases and damage declines markedly) results in extensive scarring as fruit mature reducing marketability. The insides of scarred fruit are not damaged. These brown skinned, or "alligator skin" avocados are graded as "papacados," a new sorting grade for avocados in California and they are used for fresh guacamole. This thrips has reduced industry revenues by 12% and increased production costs by 4.5%. Initial estimates suggest that economic losses to this pest may cost the California avocado industry ~$5-8 million per year (Hoddle *et al*., 2003), and reaching as high as $18.6 million in some years (Tollerup and Morse, 2005). Following its establishment in California in 1996, extensive foreign exploration efforts were rapidly undertaken for *S. perseae* with the goal of fulfilling two major objectives: (1) delineating its native range as this was unknown when this pest was described and named after establishing in California, and (2) to prospect for natural enemies for use in a classical biological control project (Hoddle *et al*., 2002). The home range was delineated and found to be restricted to avocados growing in the central plateaus of México and Guatemala. No specialist natural enemies were found in association with *S. perseae*, and generalist predators were considered unsuitable for use in a classical biological control program against *S. perseae* in California (Hoddle *et al*., 2002).

In addition to locating natural enemies and delineating the geographic distribution of *S. perseae,* foreign exploration allowed the compilation of a list of other phytophagous thrips species unknown in California, which could be serious pests should they invade and establish. For example, *S. perseae* was replaced by a congener, *S. astrictus*, in similar high altitude habits in Costa Rica and Panama. *Neohydatothrips burungae* was detected readily in México as part of these surveys and in some areas it was as abundant as *S. perseae*. Because of this relative abundance it was flagged as a species of potential invasive concern for California (Hoddle *et al.*, 2002). Interestingly, this thrips was detected on avocados in San Diego County for the first time in 2004. This find demonstrated the usefulness of these surveys for *S. perseae* and other thrips in México, Central and South America, and the Caribbean for short listing other potential invasive thrips pests. Of the 2,136 slide mounted thrips identified by Hoddle *et al*. (2002), 47 species in 19 genera were recorded, and at least four new species of *Frankliniella* were collected, a genus notorious for pest species. Some of these named and unnamed thrips species may represent future but currently unrecognized invasion threats to California.

Biological control efforts for *S. perseae* focused on a native California predatory thrips, *Franklinothrips orizabensis* Johansen (Thysanoptera: Aeolothripidae), and commercially available green lacewings, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *F. orizabensis* populations were observed to build naturally in avocado orchards in response to increasing *S. perseae* densities. However, predators appeared too late and in insufficient numbers to reduce avocado thrips densities to non-damaging levels. Despite this, augmentation of these naturally-occurring populations with mass reared *F. orizabensis* to boost predator populations early in the season was considered possible (Hoddle *et al*., 2000). To this end, the developmental and reproductive biology of *F. orizabensis* at different temperatures was studied to determine optimal rearing temperatures (Hoddle *et al*., 2000b); the suitability of 10 different diets were evaluated for mass rearing (Hoddle *et al*., 2001a); the pupation biology was studied, and harvesting and shipping techniques for pupae in plastic tubes were developed (Hoddle *et al*., 2001b) along with a mechanical sorter that used a venturi airstream to automatically separate tubes with predator pupae from empty tubes (Hoddle, 2002); effects of crowding on adult sex ratio under mass rearing conditions were quantified (Hoddle, 2003a); predation behaviors were analyzed (Hoddle 2003b), along with functional response attributes (Hoddle 2003c). Collectively these data and techniques were provided to commercial insectaries so the necessary information was available to mass rear and harvest *F. orizabensis* for sale to avocado growers. Unfortunately, large-scale replicated field releases of mass reared *F. orizabensis* purchased from insectaries failed to provide sufficient control of *S. perseae* in commercial avocado orchards (Hoddle *et al*., 2004). Similarly, replicated field trials evaluating releases of mass reared commercially-available *C. carnea* did not significantly reduce *S. perseae* populations in comparison to control treatments not receiving predator releases (Hoddle and Robinson, 2004). Natural enemies releases are not recommended for *S. perseae* control, and insecticides are the preferred tool for controlling this pest.

Consequently, significant effort has been invested in screening different pesticides in the laboratory and then evaluating the most promising in the field for control of *S. perseae*. Aerial applications of sabadilla and abamectin have been assessed in terms of spray volumes, concentrations, and coverage needed to get good control (Yee et al., 2001b,c). Resistance development to sabadilla in the field has been documented (Humeres and Morse, 2006) and resistance development to abamectin is also a concern, and this has prompted evaluations of additional classes of insecticides (i.e., spinosad, a macrocyclic lactone, and imidacloprid, a neonicotinoid) for avocado thrips control (Byrne *et al.*, 2005; Tollerup and Morse, 2005). Toxicity of neonicotinoid pesticides have been evaluated for *S. perseae* control on nursery stock (Byrne *et al*., 2007) and mature trees in orchards (Byrne *et al*., 2010) and ELISA assays have been developed to quantify levels of neonicotinoids in avocado leaves so toxicity can be accurately correlated with pesticide concentrations in leaves (Byrne *et al*., 2005). Because of long residual activity (~8-12 weeks) and its ability to kill additional avocado pests, e.g., persea mite, abamectin is a very popular pesticide with growers in Southern California.

<u>*Avocado lace bug, Pseudacysta perseae:*</u> The avocado lace bug (ALB), was first described in 1908 from specimens collected from avocados in Florida. Emerging results from DNA work suggest that this insect is probably not native to Florida and was introduced from the Yucatán area of México (Hoddle unpublished). ALB nymphs and adults feed in dense aggregated colonies on the underside of predominantly mature leaves which results in the development of large necrotic areas (Hoddle *et al*., 2005). The exact impact of *P. perseae* on productivity is not known, but fruit yields are likely reduced because of lower photosynthetic rates and defoliation events that result from feeding damage. Until recently, ALB was considered a pest of sporadic

and minor economic importance (Mead & Peña, 1991). Population outbreaks of *P. perseae* on avocados have been observed since the mid 1990's in Florida and several countries in the Caribbean, and *P. perseae* has now emerged as a serious foliar pest of avocados in the Caribbean (Peña 2003). The known geographic range for *P. perseae* in the Caribbean includes Jamaica, Puerto Rico, the Dominican Republic, St. Lucia, St. Thomas, St. John, St. Croix, and Cuba (Hoddle, unpublished surveys). It has been recorded from the states of Chiapas, Michoacán, Nayarit, Veracruz, and Yucatán in México, and in Escuintla Guatemala (Hoddle, unpublished surveys). In South America, *P. perseae* is known from Venezuela and French Guyana (Mead & Peña, 1991; Medina-Gaud *et al*., 1991; Abreu, 1995; Diaz, 2003; Hernandez *et al*., 2004; Hoddle *et al*., 2005; Morales, 2005; Sandoval and Cermeli, 2005; Streito and Morival, 2005).

In the U.S., *P. perseae* has been recorded from the southeastern states of Florida, Georgia, Louisiana, and Texas (Hoddle *et al*. 2005). In September 2004, *P. perseae* was detected for the first time in California on two residential backyard avocado trees in Chula Vista and National City in San Diego County that were heavily infested and exhibiting premature leaf drop because of feeding damage (Bender and Witney 2005; Hoddle *et al*. 2005). Subsequent surveys conducted by the San Diego County Department of Agriculture, Weights & Measures and the California Department of Food and Agriculture indicated that this pest is restricted to residential areas in southern San Diego County and had not established in commercial avocado orchards in this area or spread beyond San Diego County.

Biological agents reported in Florida are two egg parasitoids including *Oligosita sp.* (a trichogrammatid wasp) and an unidentified mymarid wasp, but their impact is low. Green lacewings and other generalist predators may also attack ALB in Florida providing some control. A predatory thrips, *Franklinothrips vespiformis*, has been observed in high numbers feeding on avocado lace bugs on Hass avocados in the Dominican Republic (Hoddle pers. observ.). Surveys for natural enemies attacking avocado lace bug in California indicated a paucity of egg and nymphal parasitoids, and generalist natural enemies, in particular predators, have been rarely observed in association with high density ALB populations in San Diego County (Humeres *et al*., 2009a). Extensive foreign exploration efforts throughout the Caribbean and México for ALB natural enemies, in particular parasitoids attacking eggs, did not result in the successful rearing of one parasitoid species despite thousands of ALB eggs being shipped to the quarantine facility at UC Riverside (Hoddle unpubl.)

To rapidly meet the potential threat posed by ALB to commercial avocado producers in California, two lines of research were undertaken: (1) screening of pesticides in the laboratory and (2) evaluation of commercially-available natural enemies (Humeres *et al*., 2009b) for their respective abilities to kill ALB. Three natural enemies were evaluated: (1) a small predatory mite, *Neoseiulus californicus,* for its ability to attack ALB eggs and small nymphs (these are both small life stages that the mite could be expected to attack), (2) green lacewing larvae, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), and (3) the predatory thrips

Franklinothrips orizabensis, for their ability to attack small and large ALB nymphs, and adults (Humeres *et al*., 2009b). Green lacewing larvae were the most efficacious natural enemies in laboratory trials and when tested on small caged avocado trees they also significantly reduced ALB numbers in comparison to potted trees lacking predators (Humeres *et al*., 2009b). Large scale field trials with this predator to evaluate efficacy outside of the laboratory have not been undertaken. Insecticide tests revealed a very surprising result. Two insecticides commonly used for avocado thrips and persea mite control, abamectin and spinosad, were ineffective against ALB. While broadspectrum contact insecticides like carbaryl and pyrethrins were very effective (Humeres *et al*., 2009b) as was the systemic insecticide imidacloprid (Humeres *et al*., 2009b; Byrnes *et al*., 2010).

So far, ALB has not developed into a major new pest for California avocado producers. The reasons for this are not clear but could be related to an unsuitable climate or reduced vigor due to high levels of inbreeding depression that may have resulted from a small founder population that originally invaded California. In the future, should additional incursions occur and new genetic diversity be introduced, ALB may emerge as a more serious pest in need of management. Similarly, *N. burungae*, the phytophagous thrips found in San Diego around the same time as ALB, has not developed into a serious pest problem, possibly for reasons similar to those proposed for *P. perseae*.

<u>*Other leaf and stem feeding pests:*</u> Foreign exploration efforts for pest specimens for DNA work and natural enemies have provided immense opportunities to observe avocados and associated insects throughout the entire endemic, native, and naturalized range of this plant, in commercial orchards, backyards, and in natural areas (i.e., high altitude cloud forests). From these observations proactive work in the areas of origin for leaf galling *Trioza* spp. (Hemiptera: Triozidae) (see Hoddle 2011a) and the leaf and fruit feeding thrips *Pseudophilothrips perseae* (Watson) and *P. avocadis* (Hood) (both Thysanoptera: Phlaeothripidae, and formerly placed in *Liothrips*) is recommended. Stem and branch boring weevils in the genus *Copturus* are also extremely destructive. These three pest groups, *Trioza* spp., *Pseudophilothrips* spp., and *Copturus* spp., likely have high invasion potential because they are common on leaves or in twigs in many parts of the range of avocados in México, and Central America, and could easily be inadvertently introduced on plants moved illegally from these areas to California. If successful incursion and establishment occur the economic impacts would be immense.

Despite the obvious pestiferousness, abundance, and presumed detrimental economic impacts of *Trioza* spp., *Pseudophilothrips* spp., and *Copturus* spp. on avocados where these plants and insects are native, including countries with large export industries, e.g., México, there is an alarming dearth of information on the basic biology, ecology, phenology, natural enemies, and control practices for these pest species. Proactive work, especially on *Trioza* spp., and *Copturus* spp. in advance of the possible arrival of this pest complex into California could strongly position the CDFA and the avocado industry to manage small incipient populations should they be detected in a timely manner.

The Threat Posed to the California Avocado Industry by the Legalization of Fresh Fruit Imports into the USA

Legal imports of fresh avocado fruit entering the US, including California, are increasing steadily because of cumulative imports from several countries, e.g., México, Chile, Perú, New Zealand, and the Dominican Republic. Increasing avocado imports into the US are due to advertising and promotion under the Hass Avocado Promotion and Research Order, and by the California Avocado Commission, along with various import associations realizing new business opportunities (Hoddle *et al*., 2010). Another reason the US market is rapidly expanding is the growing US Hispanic population that regularly uses avocados as a food item, as well as increasing overall consumer awareness that avocados are highly nutritious, tasty, versatile, and easy to prepare. The overall growth in demand for Hass avocados in the US has long exceeded domestic production, and domestic US production simply cannot meet consumer demand. Consequently, imports of avocados from other countries are likely to increase for the foreseeable future to meet this growing demand for fresh fruit (Anon. 2006).

Importations of fresh produce into any area carry with them an easily identifiable risk; the threat of accidental introduction of unwanted arthropod pests, phytopathogenic diseases or a combination of both, i.e., the pest threat is both a vector which is accompanied by the pathogen it spreads. An excellent example of this latter possibility is the vector-pathogen complex represented by the red ambrosia beetle (*Xyleborus glabratus* Eichhoff [Coleoptera: Scolytidae]) and the laurel wilt fungus (*Raffaelea lauricola* Harrington and Fraedrich [Sordariomycetes: Ophiostomatales]) that is specific to native Lauraceae and avocados in the southeastern US. To minimize threats to importing regions, risk management programs are run by regulatory authorities representing the trading partners. The goal is to identify potential invasion threats which may be documented officially in risk assessment evaluations with subsequent reports and analyses quantifying threat levels. The investigatory and evaluation processes at work here are the basis of biosecurity practices enacted under law by countries that want to enforce quarantine restrictions. The application of these laws is to impose phytosanitary regulations governing imports and exports of commodities to prevent unwanted pest introductions while minimizing restriction of free trade between cooperating nations. This is an international trend and overseen, in North America by NAPPO (North American Plant Protection Organization), and specifically in the US by USDA-APHIS.

Preparation of risk assessment reports necessitates that potential invasive pests be assigned to one of two general categories; (1) well known pests in their home ranges that may or may not have a documented history of global movement; and (2) the wild cards, species either unknown to science because they are undescribed species or very poorly studied and not recognized as threats until they first establish outside of their home range and cause unprecedented problems. Consequently, depending on the commodity under evaluation, risk assessments that are developed under free trade agreements may not be as robust as would be expected because little effort has been invested in determining the risk posed by the unknown "wild cards." Realistically, there is little incentive for exporting nations to rigorously document their pest and disease fauna as this would likely make exporting produce more difficult. Additionally, importing nations and their regulatory agencies lack time and resources to send scientists to partner countries to construct full pest inventories for commodities of mutual interest. Therefore, pest lists and risk assessments are constructed from literature reviews of the scientific and gray literature, interception documents, and possibly museum collection records for the commodity under consideration. The threat posed by potential invaders associated with avocado fruit, especially lepidopteran pests that could be moved via international trade, will be examined here in greater detail.

Global avocado production statistics for 2009 indicated that 4 of the top 10 producing nations are located in the native and naturalized range of this plant: Mexico (ranked number 1); Central America (Guatemala [10]) and South America (Colombia [6] and Perú [8]) (FAOSTAT 2010). California in the US, is the fourth largest producer of avocados in the world (FAOSTAT 2010). Importation of avocado fruit from Mexico and Central America into the US had been banned since 1914 to protect US producers from the unwanted introductions of fruit feeding pests, especially specialist internally feeding moths, weevils, and tephritid flies, which have evolved with avocados (Hoddle and Hoddle 2008a). However, recent legislative changes have allowed imports of fresh avocado fruit into the US from areas where they had originally been banned. In 1997, the US began importing avocados from México into restricted areas, and by 2007 year round importations into all 50 US states were permitted (Morse et al., 2009).

Recently, eight species of armored scales were found feeding on the skin of imported avocado fruit entering California from México (Morse *et al.*, 2009). Of these eight species, at least one was previously unknown and subsequently described (Evans *et al*., 2009), and two more are probably new species needing description (Morse *et al*., 2009). Only one of the eight scale species, *Hemiberlesia lataniae* Signoret (Hemiptera: Diaspididae), intercepted on fresh avocados exported from México is established in California (Morse *et al*., 2009). This clearly suggests that imports of fresh avocados into the US originating from the area of origin of this plant are contaminated with living insects, some of which are unknown species, and may pose a substantial new pest risk to California and other countries that receive fruit from these export areas.

The potential pest threat fresh avocado fruit imports pose to local avocado producers is not unique to California. Israel and Spain, for example, are countries with domestic avocado industries which also import fruit from countries where this crop is naturalized, e.g., Perú, and they have suffered invasions from specialized avocado pests that are native to the home range of this plant, e.g., *O. perseae*. This trend of increasing fruit importation from overseas, especially countries where avocados are indigenous and likely to have a rich co-evolved but little studied insect fauna into nations where avocados are not native but domestic production exists is unlikely to stop or reverse itself as long as market demand and good financial returns exist. Consequently, in addition to established import sources for the US, other countries including Colombia, Guatemala, South Africa, Spain, and Uganda are currently seeking entry into the US market for fresh avocado fruit (USDA-APHIS, April 30 2010). To meet the threat that invasive pest species associated with fresh avocados pose, importing countries with domestic markets need to develop forward-leaning policies to simultaneously manage increasing import loads and the pest threats that will likely accompany an expanding pool of exporting countries that are both within, e.g., México, and Central America, and outside, e.g., Uganda and Africa in general, the evolutionary area of origin of the avocado. Each exporting country presents a unique set of pest risks because invaders could be co-evolved specialists from the home range of the avocado that may have a very limited host range and represent a risk only to avocados and close relatives, or generalist pests from within and outside the area of origin of the avocado that can feed and breed on avocados as well as pose a threat to other existing crops and native flora. The next section of this document will focus on potential invasion threats posed by well recognized and poorly known lepidopteran pests associated with avocado fruit in the home range of this plant.

Lepidopteran Pests Associated with Avocado Fruit

Globally, 111 species of Lepidoptera in 73 genera representing 22 families have a documented association with avocados (HOSTS 2010). The pest status and invasion potential of the majority of these species is not well understood. The most notorious of these fruit feeding lepidopteran pests is *Stenoma catenifer*, but other additional species have recently been discovered attacking small and large avocado fruit in part of the native range of this plant (Hoddle and Brown 2010).

<u>*Stenoma catenifer Walsingham (Lepidoptera: Elachistidae)*</u>: Over 350 species of moths are described in the genus *Stenoma* (Becker, 1984) but this number could exceed 730 species (zipcodeZoo.com, 2010). Three species of *Stenoma* have been recorded in association with avocados, *S. catenifer*, *S. invulgata*(?) Meyrick (in Trinidad and Tobago), and *Stenoma vacans* Meyrick (in the Neotropics) (HOSTS 2010).

Stenoma catenifer is native to Neotropical areas, and it is considered to be one of the most important pests of avocados in México, and Central and South America (Wysoki *et al*., 2002). In total, there are at least 16 countries with commercial avocado industries that have native *S. catenifer* populations, and collectively they produce ~25% of the world's avocados (FAOSTAT, 2010). Several of these countries with native *S. catenifer* populations, e.g., México and Perú, export avocados from certified areas to countries that have domestic avocado production, but are outside the native range of *S. catenifer*. This pest has been recorded attacking avocados in México (Arellano 1975, Mendez Villa 1961, Muniz Velez 1958, Wolfenbarger and Colburn

1966, 1979), Guatemala (Popenoe, 1919), Belize (CABI, 2001), Honduras (Sasscer 1921), Nicaragua (CABI, 2001), El Salvador (Wolfenbarger and Colburn, 1966), Costa Rica and Panama (CABI 2001), Venezuela (Boscán de Martínez and Godoy 1984), Argentina, Colombia and Ecuador (CABI 2001), Guyana (Cervantes Peredo et al. 1999), and Perú (Wille, 1952). In Brazil, *S. catenifer* is the major pest limiting commercial avocado production (Hohmann *et al*. 2003).

All life stages of *S. catenifer* have been described and line illustrated (Cervantes Peredo *et al*., 1999), and colored photographs are available (Hoddle 2011b).

<u>Biology and Ecology:</u> *Stenoma catenifer* is a specialist on plants in the family Lauraceae (Cervantes Peredo *et al*., 1999). In avocados, primary economic damage results from larvae tunneling in fruit as they bore towards the seed where the majority of the feeding typically occurs. Infested fruit are characterized by accumulations of frass on surface, and perseitol, a seven carbon sugar alcohol, appears as a chalky white residue that often oozes from feeding tunnel entrances. Feeding tunnel entrances (53%) are most often situated in the bottom third of the fruit distal to the pedicel (Hoddle and Hoddle, 2008b). On average, ~1-2 larvae infest a single avocado fruit, but up to seven or eight larvae can be found in one fruit (Hoddle and Hoddle, 2008b; Nava *et al*., 2006). Fruit of *Chlorocardium rodiei* (Schomb.) Rohwer, Richter, and van der Werff are infested with ~3 larvae per fruit on average, with a maximum of 21 larvae being recorded (Cervantes Peredo *et al*., 1999). This suggests that large numbers of larvae can develop in single fruit and a founding population of this size could be sufficient for establishment in a new area. In Brazil, within tree distribution of fruit damaged by *S. catenifer* differs between study sites with infestations either being most concentrated in the upper half of trees (Hohmann *et al*., 2003) or in the middle to lower parts (Nava *et al*., 2006).

Larvae of this pest can also mine developing buds, green and woody avocado twigs and stems, and fruit pedicels (Wille, 1952; Wolfenbarger and Colburn 1966; Wolfenbarger and Colburn, 1979). Larval attacks can kill small trees and can cause considerable crop loss by inducing small fruit that are attacked to be dropped prematurely (Ventura *et al*., 1999; Wille 1952). Because fruit are not present on trees year round, *S. catenifer* probably maintains constant populations in orchards by feeding inside twigs and stems which are always present. In the field, evaluation of 20 different avocado varieties showed varying levels of susceptibility to *S. catenifer* infestation, with 5-54% of fruit infested depending on the cultivar (Hohmann *et al*., 2000). Commercial 'Hass' avocado orchards in Guatemala, under regular pesticide treatments can have ~45% of fruit in damaged by *S. catenifer*, suggesting that this cultivar is vulnerable to *S. catenifer* (Hoddle and Hoddle, 2008b). Similar high levels of damage have been observed in avocado orchards in Brazil (up to 60% fruit infestation) (Hohmann *et al*., 2003; Nava *et al*., 2005c; 2006), Venezuela (~80%; Boscán de Martínez and Godoy, 1982), Perú (~80-100% on non-managed farms; Hoddle pers. obs. 2010) and México (Wysoki *et al*., 2002).

In addition to avocados and *C. rodiei*, other hosts include, *Persea schiedeana* Nees and *Beilschmedia* sp. (Cervantes Peredo et al., 1999), *Nectandra megapotamica* Mez and *Cinnamomum camphora* (L.) (Link and Link 2008). In a non-avocado host, *C. rodiei*, fruit infestation rates did not exceed 10% (Cervantes Peredo *et al*., 1999). Similarly for *N. megapotamica* and *C. camphora*, fruit infestation rates did not exceed 5% (Link and Link, 2008). Because of high infestation rates, avocado fruit may be a highly preferred host for *S. catenifer*, especially commercial varieties.

In the laboratory, female *S. catenifer* display strong oviposition preferences for different avocado substrates with the majority of eggs being on woody branches to which fruit are attached (~68% of eggs are laid here), and ~9%, ~10%, and ~12% of eggs being laid on the fruit pedicel, between the pedicel and the fruit, and directly on the fruit, respectively (Hoddle and Hoddle 2008b). Additionally, female moths appear to discriminate between different types of avocado fruit when ovipositing. In choice tests, 'Hass' fruit are strongly preferred (2.69 times more favored) for egg laying when smooth-skinned non-Hass fruit are available simultaneously in experimental cages (Hoddle and Hoddle 2008b). Avocado fruit are needed to stimulate ovipositional activity, and when fruit is present, female moths will oviposit onto artificial substrates with textured surfaces, of which quilted paper towels are most preferred. In the laboratory with a 14:10 L:D phase (scotophase initiated at 2000 hr), oviposition commences at 1800 hr and ends at 0600 hr the following morning. Peak ovipositional activity occurrs from 2000 to 2400 hr when 80% of eggs were laid (Nava *et al*., 2005a). Egg laying habits appear to be mainly crepuscular or nocturnal (Nava *et al*., 2005a).

Adult *S. catenifer* are nocturnal, and flight activity begins immediately at dusk. During the day adult moths hide on the ground, the beige coloration and black spots on the forewings help camouflage moths, especially when they are resting amongst dried grasses in avocado orchards (Hoddle, 2011a; Hoddle and Hoddle 2008c). Resting moths will fly short distances when disturbed during the day (Cervantes Peredo *et al*., 1999).

Developmental and Reproductive Biology: Life history characteristics of *S. catenifer* have been well studied in the laboratory (Cervantes Peredo *et al*., 1999; Boscán de Martínez, 1984; Nava *et al*., 2005a,b). Eggs can hatch in as few as 4 days at temperatures around 28°C or higher (Nava *et al*., 2005b), the five larval instars (Cervantes Peredo *et al*., 1999) can take 19-40 days to reach the pupal stage depending on the temperature (Nava *et al*. 2005b). In the laboratory, mature larvae, easily identified by the turquoise blue color of the ventral surface, abandon seeds and fruit and typically walk for ~24-36 hrs before settling in a protected place to pupate within the protection of loosely spun bivouac of silk (Hoddle and Hoddle 2008b; Hoddle 2011a). Larvae apparently pupate in the upper ~2 cm of soil in orchards (Boscán de Martínez and Godoy, 1984). Pupae need 8-20 days to complete development when temperatures range 18-30°C, and the entire life cycle (egg to adult) requires 31-70 days over the same temperature range (Nava *et al.*, 2005b). Adult males can live for 11-18 days, and female longevity is similar at temperatures that range 20-30°C (Nava *et al*., 2005b). Female moths can lay 133-319 eggs when reared on avocados at 20-30°C and when fruit of *C. rodiei* is used as the developmental substrate, average female fecundity is 206 eggs (Cervantes Peredo *et al*., 1999). The sex ratio is slightly female biased, e.g., 1.46 female (Cervantes Peredo *et al*., 1999), and longevity and fecundity of adult male and female moths is not enhanced through the provisionment of carbohydrate resources (10% honey water) in comparison to adults given access to water only (Milano *et al*., 2010).

Laboratory-derived developmental data have been used to develop a degree-day model for *S. catenifer* and up to five generations a year may be possible in some parts of Brazil (Nava *et al*., 2005b).

Control Measures:

Sex Pheromone: The sex pheromone of *S. catenifer* is an unsaturated aldehyde, (9Z)-9,13-tetradecadien-11-ynal. The presence of the alkyne is a very unusual functional group in lepidopteran pheromones as is the high degree of unsaturation (Millar *et al*., 2008). The pheromone is a new class of natural compound as the terminal conjugated dienyne has no precedent amongst known natural products (Millar *et al*., 2008). Synthesis instructions for the pheromone are available (Hoddle *et al*., 2009; Zou and Millar, 2010). The components of the pheromone, i.e., different ratios, and blends of the corresponding alcohols and acetates, have been field tested and results indicate that only (9Z)-9,13-tetradecadien-11-ynal is needed to attract male moths. Concentrations of pheromone ranging from 10 μg to 1 mg are efficacious and lures retain field activity for several weeks (Hoddle *et al*., 2009). Males show temporal responses to the pheromone with first arrival to traps in the field occurring at 0230 and ceasing abruptly at 0430 hours (Hoddle et al., 2009).

Operational parameters for the sex pheromone have been optimized under field conditions. Gray rubber septa are superior to polyethylene vials and small plastic bags for releasing pheromone. Trap height placement in avocado trees in commercial orchards is not important as traps capture statistically equivalent numbers of moths when hung at ~0.15 m, ~1.75 m, and ~4.5 m above the ground. Hanging traps at 1.75 m is recommended as this height is convenient for placement and inspection (Hoddle *et al*., 2011). Probabilistic modeling of *S. catenifer* capture data from different sized commercial avocado orchards (~2 ha - ~76 ha in size) with varying levels of infestation suggests that 10-13 randomly placed traps will capture at least one male *S. catenifer* with 90% confidence over a seven day sampling period if the pest is present (Hoddle *et al*., 2011). It is likely that small orchards, e.g., ≤ 2 ha, could be adequately monitored with fewer traps, e.g., 2-5 randomly placed traps could be sufficient, where as for large orchards, 10-13 traps could be sufficient.

The pheromone has attracted males in Guatemala, México, Brazil, and Perú, indicating that geographically separated pheromone races of *S. catenifer* probably don't exist (Hoddle *et al*., 2011). Interestingly, in avocado orchards in Guatemala and México, the pheromone was also highly attractive to another closely related moth, *Antaeotricha nictitans* (Zeller) (Lepidoptera: Elachistidae: Stenomatinae) (see below for more on this moth). This result suggests that the *S. catenifer* sex pheromone is conserved amongst new world stenomatines and differing temporal responses to the pheromone separate sympatric species (Hoddle *et al*., 2011).

Management applications with the pheromone: With the sex pheromone, there may be opportunities to development new control strategies for *S. catenifer*. Such approaches include mating disruption via the distribution of pheromone dispensers in orchards, which collectively make it difficult for males to find females to mate with. Alternatively, the sex pheromone allows implementation of "attract and kill" management for this pest. This concept is simple. A wax-based matrix impregnated with pheromone and a small amount of pesticide is applied as a few small droplets to trunks of avocado trees. The pheromone attracts male moths which pick up a debilitating dose of insecticide from contact with the droplets. Pest populations collapse because males are rapidly removed thereby reducing mating rates with females.

It is recommended that the *S. catenifer* pheromone be used to monitor for this pest in orchards exporting fresh avocado fruit from countries with native populations, e.g., México and Perú, and year round pheromone monitoring should be required for exporters as part of a certification program. Additionally, regions with domestic avocado production that also import fresh fruit from high risk areas, e.g., California (USA) and Spain, could use the pheromone to detect *S. catenifer* incursions. Early detection could facilitate a rapid response to contain or eradicate the pest while populations are small and highly localized. In addition to monitoring *S. catenifer* in avocado orchards, the sex pheromone could be used to monitor population phenologies in natural areas to better understand the basic ecology of this insect. Geographical and altitudinal distributions could be identified in countries with native populations of *S. catenifer* which may delineate areas naturally free of this pest, e.g., perhaps at altitudes > 2000 m where the climate is not favorable but avocados are able to grow.

Natural Enemies: The eggs, larvae, pupae, and adults of *S. catenifer* are attacked by a variety of natural enemies in the home range of this insect. In Brazil, eggs are parasitized by species of *Trichogramma* and *Trichogrammatoidea* (Hymenoptera: Trichogrammatidae). Although naturally-occurring parasitism may exceed 60% in avocado orchards, it is not sufficient to prevent severe yield losses (Hohmann *et al*., 2003). In an attempt to remedy this shortcoming, Nava *et al*., (2007) evaluated the life history traits in the laboratory of four different egg parasitoids (all Trichogrammatidae) and up to eight strains of individual species for mass rearing to determine which would be most efficacious against *S. catenifer*. The best performing parasitoids determined by egg parasitism rates were *Trichogramma atopovirilia* Oatman and Platner and *Trichogrammatoidea annulata* De Santis. However, release rates of 28-30 parasitoids per host egg were estimated to be necessary to obtain satisfactory levels of parasitism (~70%).

Larvae are attacked by a diverse guild of hymenopteran, and to a much lesser extent, dipteran parasitoids. In Guatemala, a gregarious *Apanteles* sp. (Hymenoptera: Braconidae) dominates the parasitoid fauna accounting for >95% of parasitism (Hoddle and Hoddle 2008b). Solitary *Macrocentrus* sp. (Braconidae), *Pristomerus* sp. and *Brachycyrtus* sp. (all Hymenoptera: Ichnuemonidae) collectively account for < 5% parasitism (Hoddle and Hoddle, 2008b,c; Hoddle *et al*., 2011). In South America, *S. catenifer* larvae are attacked by an *Apanteles* sp. in Venezuela which inflicts ~30% parasitism (Boscán de Martínez and Godoy, 1982). In Perú parasitism by *Apanteles* sp. is considered inconsequential (Wille, 1952). In Guyana, *Eudeleboea* sp. (Hymenoptera: Ichneumonidae) and *Chelonus* sp. (Braconidae) attack *S. catenifer* larvae infesting *C. rodiei* at very low levels (Cervantes Peredo *et al*., 1999). Five species of braconids, *Dolichogenidea* sp., *Hypomicrogaster* sp., *Apanteles* sp., *Chelonus* sp., and *Hymenochaonia* sp., and two species of ichneumonids, *Eudeleboea* sp. and *Pristomerus* sp., have been recorded attacking *S. catenifer* larvae in Brazil. *Dolichogenidea* sp. and *Apanteles* sp. dominate this guild and inflict peak parasitism of 30-40% (Nava *et al*., 2005c).

In Perú, the dominant larval parasitoid is an *Apanteles* sp., and a tachinid fly, *Chrysodoria* sp. has been reared from *S. catenifer* pupae (Hoddle unpublished). Spiders, in particular *Hogna* sp. (Lycosidae), are voracious predators of *S. catenifer* larvae, pupae, and adults in the laboratory (Hoddle and Hoddle 2008c). These spiders are extremely common on orchard floors in Guatemala and may be important natural enemies attacking wandering larvae that have abandoned avocado seeds or fruit in search of pupation sites, of pupae in the soil, or adults resting on the orchard floor (Hoddle and Hoddle 2008c). These life stages do not appear to be chemically protected via the sequestration of toxic avocado furans (Hoddle and Hoddle 2008c).

Cultural Control, Sterile Insect Technique, and Insecticides: Cultural control recommendations for *S. catenifer* include removal and destruction (burning) of infested fruit, branches, and shoots (Wille, 1952), or placing dropped fruit infested with larvae in clear plastic bags that are airtight. After about 4 days at 20-23^{o}C, larval mortality is almost 100%. Elevated temperatures or the buildup of noxious gases inside sealed bags may contribute to increased rates of larval mortality (Nava *et al*., 2006). Control of weeds on the orchard floor may reduce hiding places for moths during the day thereby lowering adult densities and subsequent mating and oviposition events (Hoddle pers. obs.). Planting of highly susceptible avocado varieties in orchards could act as trap crops protecting economically valuable cultivars (Ventura *et al*., 1999), or resistant varieties should be grown instead of susceptible cultivars (Hohmann *et al*., 2000).

Gamma radiation from cobolt-60 can reduce egg viability by 81% at doses of 50 Gy and 100% when eggs are irradiated with $\geq$ 75 Gy (Gy = gray, the absorption of one joule of energy, in the form of ionizing radiation, by one kilogram of matter). When eggs are treated with 25 Gy, a dose sufficient to kill 12% of individuals, the levels of sterility in males and females that develop from these eggs are 93% and 100%, respectively (Silva *et al*., 2006). Doses of 75 Gy have been recommended for treating avocado fruit that could be contaminated with *S. catenifer* eggs (Silva *et al*., 2006). **A** higher dose, 150 Gy, is recommended for controlling larvae and pupa within fruit, a dose that is also sufficient to sterilize ~70% of treated pupae (Silva *et al*., 2007). At 200 Gy, irradiated males or females that breed with radiated or unradiated partners produce eggs with 0-3% viability, a result statistically equivalent to adults treated with 300 or 400 Gy. Therefore, for sterile insect production, a treatment dose of 200 Gy is recommended for *S. catenifer* (Silva et al., 2007).

Insecticides are the primary control strategy for *S. catenifer*, although there are seemingly little published data documenting efficacy and application rates and timings for different products. Foliar applications will only be effective against eggs, small larvae penetrating fruit and stems, and adults landing on treated foliage. Once inside fruit and twigs, larvae are likely protected from sprays, while pupae will be protected in the soil. It is possible that soil applications of systemic neonicotinoid insecticides, like imidacloprid, may not be able to provide adequate protection of avocado twigs and branches, or small and large avocado fruit from fruit-feeding pests such as *S. catenifer*. Field tests in California indicate that uptake of imidacloprid can be slow, probably because of binding to organic matter in soils, and mobilized concentrations are often inadequate to control small phytophagous pests like thrips (Byrne et al., 2010). Furthermore, neonicotinoids are generally not effective against lepidopterous pests due to a low affinity of the nicotinic receptor for imidacloprid binding.

Additionally, residue tests have shown that imidacloprid levels in large avocado fruit at harvest are extremely low (less than 0.1 ppm) and this may occur because quickly growing leaves are more efficient sinks for this insecticide than slower growing fruit. Although residue levels of imidacloprid in fruit pose no human consumption risk, this also implies that concentrations may be too low to kill larvae feeding inside large fruit (F. Byrne pers. comm. 2011). The ability of systemic insecticides to enter and protect small fruit from insect attack, even those known to be susceptible to imidacloprid in bioassays, has not been well studied (F. Byrne pers. comm. 2011).

Applications of pyrethroids can significantly reduce fruit infestation rates with applications being made at either 15 or 60 day intervals having statistically equivalent efficacy (Hohmann *et al*., 2000). Monthly applications of endosulfan and malathion on a rotating calendar resulted in 45% of fruit in a commercial 'Hass' avocado orchard in Guatemala being infested with *S. catenifer* (Hoddle and Hoddle 2008b). Similar calendar spray regimens have been reported from México as being necessary to minimize economic losses (Wysoki *et al*., 2002). Spray applications are affected by increasing tree height and architectural complexity which can significantly limit control because coverage is reduced (Hohmann et al., 2000). Additionally, it is assumed that broad spectrum insecticides have severe negative effects on *S. catenifer* natural enemies, especially egg parasitoids, and applications may induce outbreaks of other avocado pests, e.g., mites (Hohmann *et al*. 2000). To maximize impacts, it is recommended that pesticides be applied at times of the year when *S. catenifer* are naturally low in avocado orchards, e.g., December, January, and February in Brazil, thus prematurely retarding population growth in advance of favorable conditions that promote pest outbreaks (Nava *et al*., 2006).

<u>Invasion Potential:</u> *S. catenifer* has demonstrated an ability to invade and establish in new areas in which it has not been previously recorded either through the movement of infested fruit or potentially via the movement of new planting material with infested branches, twigs, or shoots.

In 2000, *S. catenifer* was recorded for the first time from Isla Santa Cruz, in the Galápagos Islands, Ecuador (Landry and Roque-Albelo, 2003). By 2003, the moth had established on additional islands in the Galápagos where avocados are grown, e.g., San Cristóbal (Landry and Roque-Albelo, 2003). The mode of entry into the Galápagos is uncertain, but could have been from the importation of infested avocados from mainland Ecuador, an item intercepted previously by quarantine officials (Landry and Roque-Albelo, 2003). As recently as 2009, infested avocado fruit imported from mainland Ecuador were found for sale in a local supermarket on Santa Cruz indicating that quarantines are not robust enough to prevent continued introductions (Hoddle 2011a). Sasscer (1921) reports interceptions of *S. catenifer* entering the US in fruit imported from Honduras. Importation of fruit from areas with native or invasive *S. catenifer* populations increases the risk that this pest will be inadvertently introduced into new areas.

The establishment of *S. catenifer* in the Galápagos has reduced the viability of a commodity that could be locally produced and sold. It has also necessitated increased imports of fresh avocados from mainland South America which have elevated costs of a popular food for local people, and further increased the risk of additional pests entering the Galápagos Islands. Fortunately, there are no endemic Lauraceae in the Galápagos and avocados are the only representative of this plant family in these islands.

Limited fruit surveys suggest that infestation levels are extremely high, possibly reaching 90% around Bella Vista on Santa Cruz. In October 2009, 40 dropped fruit and exposed seeds were collected from under avocado trees around Bella Vista. Of these 40 fruit, 21 *S. catenifer* larvae were reared. Twenty larvae developed into pupae. One larva died from parasitism caused by a gregarious *Apanteles* sp. (?). However, all nine parasitoid larvae died as pupae, suggesting that parasitism may have been the opportunistic exploitation of an unsuitable host. Seven of the 20 pupae died and 13 adults emerged, i.e., 62% larval to adult survivorship rate (Hoddle, 2011a). *S. catenifer* may be flourishing in the Galápagos because of natural enemy free space.

<u>Other Lepidoptera Associated with Avocados in Parts of the Native Range of this Plant:</u> Comprehensive rearing studies for Lepidoptera associated with avocado fruit have been conducted in Guatemala (Hoddle and Hoddle, 2008a; Hoddle and Brown, 2010). Over the period November 2006 to January 2009, 1,078 small ‘Hass’ avocado fruit (5 – 25 mm in length) and 7,742 ‘Hass’ and non-Hass avocados ≥ 100 mm in length (for a total of 8,820 fruit) were collected and held for the emergence of Lepidoptera. A total of 1,098 specimens representing 10

moth species in 10 genera and four families were reared (Table 1) (Hoddle and Brown, 2010). *S. catenifer* accounted for 91% of the reared material. However, this result is possibly biased because this pest was the primary focus of collecting and rearing efforts and other species associated with avocado fruit could be under-represented. Tortricidae had the most representatives with six species. Two new moth species were discovered and subsequently described, *Histura perseavora* Brown (Tortricidae) (Brown and Hoddle, 2010), and *Holcocera plagatola* Adamski (Coleophoridae) (Adamski and Hoddle, 2009).

Cryptaspasma sp. nr. *lugubris* was reared in large numbers across multiple collection sites from fruit that was collected intact from the ground with fruit picked directly from the same trees and commingled but separated by locality It was also reared from fruit purchased from commercial venders (Hoddle and Hoddle 2008a). It is uncertain whether this moth is an unrecognized avocado pest that attacks fruit hanging in trees causing them to drop prematurely, or a specialist of hard seeds that attacks fruit once it has dropped to the ground (Brown and Brown, 2004). Laboratory studies indicated that this moth would oviposit on intact fruit and exposed avocado seeds. Field trials with intact fruit placed on the ground in avocado orchards indicated that fruit had a high probability of being eaten by small animals within several days. The length of time from placement in the field prior to consumption was inadequate for egg hatch to occur and for *C.* sp. nr. *lugubris* larvae to bore into the seeds where they would be safe from accidental predation (Hoddle and Hoddle 2008a). Oviposition on hanging fruit seems a likely infestation route since this would allow larvae sufficient time to reach the protection of the avocado seed prior to fruit prematurely dropping to the ground (Hoddle and Hoddle 2008a). The pest status of *C.* sp. nr. *lugubris* urgently needs resolving as this species has been reared from avocados in Michoacán México, an area with major 'Hass' production and international exports. Photographs of *C.* sp. nr. *lugubris* eggs, larvae, pupae, adults, feeding damage, and its parasitoids are available (Hoddle 2011b).

Field testing of the *S. catenifer* sex pheromone in México and Guatemala resulted in a noteworthy result with respect to the identification of a potential new avocado pest. Pheromone traps baited with (9Z)-9,13-tetradecadien-11-ynal attracted substantial numbers of a related moth, *Antaeotricha nictitans* (Zeller) (Lepidoptera: Elachistidae: Stenomatinae). This moth was very abundant in traps set in avocado orchards at 400–700 m elevation in Escuintla Guatemala and Tapachula México. Captures of *A. nictitans* were 7–9 times higher than captures of *S. catenifer* at these sites. *A. nictitans* is easily separable from *S. catenifer* based on size (*A. nictitans* is larger) and numbers of spots on the forewings (*A. nictitans* has just one central spot; *S. catenifer* has many, especially at the distal margin of the forewing). Photographs comparing *A. nictitans* and *S. catenifer* are available (Hoddle, 2011a). The significance of this moth in avocado orchards is unknown because there is very little information about its host-plant preferences (it has not been reared from avocados) or its associated natural enemy fauna. The avocado orchard in Guatemala that yielded *A. nictitans* was surrounded by pineapple and rubber tree plantations. In México, Hass avocados were planted as a cover crop for coffee, and surrounding this coffee farm were native forest remnants and additional coffee plantations. Trapping results suggest that *A. nictitans* has a close association with avocados because this is the only plant common to sampling sites in Guatemala and México. If this assumption is correct, *A. nictitans* may be a previously unknown pest of avocados grown at low elevations. This possibility needs investigation.

Recommendations for Proactively Managing Potentially Invasive Lepidopteran Pests that Threaten California Avocado Producers: The primary lepidopteran pest attacking avocados in the native range of this plant is *S. catenifer*. This pest has the potential to be accidentally moved into new areas via the importation of infested fruit or plants that originate in the home range of this pest. This risk is highest from countries with native populations of *S. catenifer* and commercial avocado industries that export fruit to countries with domestic avocado production outside of the natural range of this pest. In the absence of natural enemies, abundant hosts, and a permissive climate, *S. catenifer* is likely to flourish should it invade new areas. This has been observed in the Galápagos Islands. The sex pheromone for *S. catenifer* has been identified and field efficacy has been demonstrated in multiple countries. Mandatory monitoring with the pheromone should be implemented for all producers in countries with native populations of *S. catenifer* that export fresh avocados and planting stock. This moth is not only a risk to commercial avocado producers and home owners with backyard fruit trees, but it could also pose a significant incursion threat to native species of Lauraceae in countries that have no evolutionary association with insects that cause similar feeding damage.

Rearing studies for Lepidoptera associated with avocados in the native range of this plant have been very instructive, especially for Guatemala. Survey results have clearly illustrated how poorly known the moth fauna (and associated natural enemies) is in countries where avocados are native and the biodiversity associated with this plant is expected to be greatest. At a minimum, the potential pest status of *C.* sp. nr. *lugubris*, *H. perseavora*, and *A. nictitans* needs resolving. It is recommended that surveys for these insects in other countries where avocados are native and have export industries, e.g., México, be undertaken to determine their presence or absence, geographic distribution and abundance, associated natural enemies, and to develop monitoring and management tools, e.g., pheromones, whether they are pestiferous, and pose an invasion risk to other countries.

Other Fruit Pests with Invasion Potential

Coleopteran Fruit Pests Associated with Avocado Fruit: The most important potential beetle threats to the California avocado industry are the large seed weevils (*Heilipus* spp.), and the lesser seed weevils (*Conotrachelus* spp.). The risk these weevils posed to the incipient US avocado industry was recognized at a very early stage (Popenoe, 1919). The genus *Heilipus* consists of about 86 species that are restricted to the Americas (~39 species are known from north and central America, and ~53 are from South America) and of these ~ 8 species are associated with avocados. Weevil larvae bore into branches or fruit, causing premature fruit drop (Ebeling, 1950), and potentially killing young plants (Castañeda-Vildózola *et al*., 2007). Recently, the morphology of these weevils in México and Costa Rica was revised to improve the identification and taxonomy of three of these pestiferous *Heilipus* species associated with avocados (Castañeda-Vildózola *et al*., 2007). Very little is known about these important pests. Females drill holes into fruit with their long rostrums and ovipoist eggs into to these wounds. Upon hatching, larvae bore through the pulp to the seed where they feed, and later, tend to pupate inside damaged fruit. Adults feed on leaves, buds, and young avocado shoots (Ebeling, 1950). There is an alarming paucity of information on *Conotrachelus* species associated with avocados, a fact recognized long ago by Whitehead (1979). The situation does not appear to have improved during the intervening 32 years. Given the importance of these pests and how little is known about their basic biology,, proactive research programs on *Heilipus* and *Conotrachelus* species of weevils would be a prudent investment to mitigate the invasion threat posed by these pests to the California avocado industry.

<u>Dipteran Fruit Pests Associated with Avocado Fruit:</u> Tephritid fruit flies are notorious fruit pests have received considerable research attention on their potential to use avocados for reproduction. Tephritid flies have been the subject of intensive quarantine disputes between the California Avocado Commission vs. the USDA-APHIS and México (CAC 2004). Field and laboratory experiments in México have concluded that four species of *Anastrepha*, *A. ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann), and *A. striata* (Schiner) are not likely to infest Hass avocados produced in commercial production areas of Michoacán even though these flies are found in commercial orchards (Aluja *et al*., 2004). This finding cemented APHIS' decision to allow importation of fresh Hass avocados from Michoacán into California (CAC, 2004). Similarly, avocados that originate from within the US, in particular Hawaii, where there are populations of invasive fruit fly species that threaten California, have also been subject to considerable research on their suitability as hosts for tephritid reproduction (Follet *et al*., 2010). In comparison to fruit feeding moths and weevils, the importation risk associated with tephritids and avocados appears to be much better understood and managed.

Conclusions

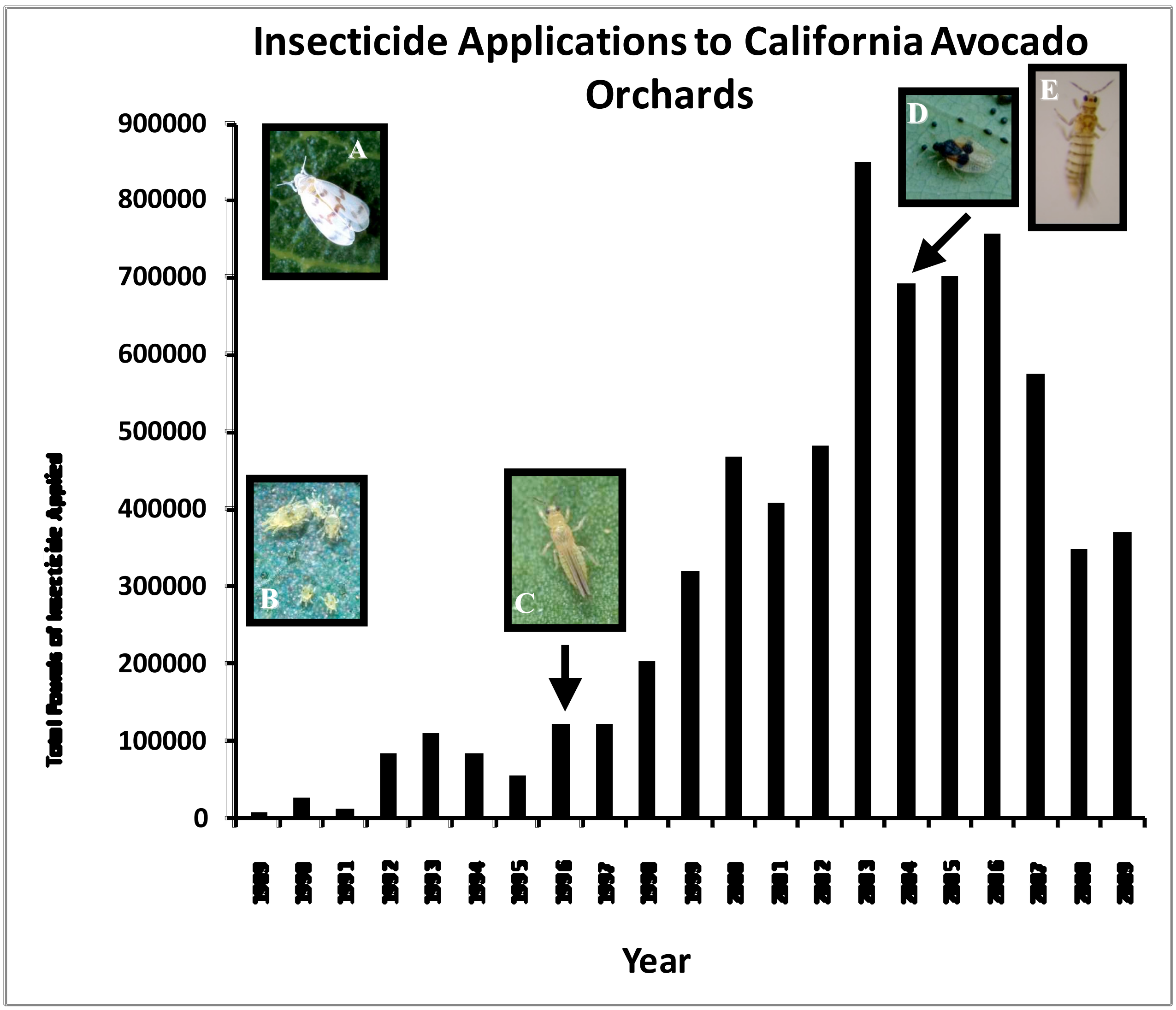
California avocados were once world renowned for production that used minimal insecticides and relied almost exclusively on biological control agents to suppress a variety of pests, e.g., leaf feeding mites, moths, and some thrips. Beginning in 1982, a steady trickle of exotic leaf feeding pests, all native to the area of origin of avocado, have successfully invaded California and reduced grower reliance on natural enemies for pest control. The consequence of these invasions have been: (1) greatly increased pesticide use and research on new products to combat the most destructive of these pests, the persea mite and avocado thrips. This trend would be considerably worse should specialist fruit feeding pests (e.g., moths or weevils) establish in California. (2) Reduced reliance by growers on biological control for pest suppression, and (3) industry sponsorship of proactive studies to identify future potential invasive insect pests, in particular, fruit feeding moths, that may be accidentally introduced with fresh fruit imported from countries with native populations of these pests, e.g., México and Perú. Work in this area has demonstrated conclusively that little is known about fruit feeding moths and weevils associated with avocados in its native range (fruit flies are much better studied). This is particularly worrisome as countries that comprise the native range of avocados have been granted privileges to export fresh avocados to the US, and the specialist co-evolved insects associated with this crop in these countries is very poorly studied and represent a largely unknown risk. This paucity of information about specialist fruit feeding pests that have evolved with avocados in its natural range is surprising given that this plant has a long history of domestication and fruit are widely exported from these countries to other regions that are not in the native range of the avocado but have domestic avocado production, e.g., California. It is reasonable to assume that it is only a matter of time before these poorly known fruit pests catch up with their host plant that is growing outside of its native and naturalized range. To counter this obvious threat, the California Avocado Commission sponsored research programs to develop monitoring tools for invasive Lepidoptera, in particular the avocado seed moth, *Stenoma catenifer*. This work isolated the sex pheromone for monitoring, allowed detailed studies on the ecology of *S. catenifer* in its native range (Guatemala and Perú), and permitted the construction of a detailed inventory of natural enemy species and their impacts. The value of this natural enemy inventory for *S. catenifer* cannot be overstated: should this pest establish in California a rapid biological control program could be launched as University of California Riverside scientists have considerable familiarity with the natural enemies of this pest, collecting strategies, locations, and times of year to search. The savings in time (and money) are hard to estimate, but could likely be in the order of 2-5 years. These types of studies need to be conducted for other moths including *Cryptaspasma* sp. nr. *lugubris*, *Histura perseavora*, and *Antaeotricha nictitans.* Similar challenges exist for incursion threats posed by seed feeding weevils.

The proactive approach to surveying for newly arrived avocado pests, e.g., avocado thrips, and avocado lace bug, in their native range has enabled the development of an extensive list of additional pest species that feed on foliage or branches. For example, thrips (*Neohydatothrips burungae* was recognized as in invasion threat in 2002 because of proactive thrips surveys, and in 2004 it was discovered in San Diego), galling psyllids, and stem feeding weevils should also be considered for proactive studies in their areas of origin so that the ecology, biology, behavior, and natural enemy fauna is well studied, documented, and understood before possible future arrival in California. Forward leaning and aggressive efforts to manage potentially invasive avocados pests native to the home range of this crop are strongly recommended, especially if the rapid development of control programs is desired and biological control agents are envisioned as the cornerstones for sustainable Integrated Pest Management programs.

Table 1. Species of Lepidoptera reared from 1,078 small (5 – 25 mm in length) 'Hass' avocado fruit and 7,742 large (≥ 100 mm in length) 'Hass' and non-Hass avocados collected in Guatemala. Rearing data for this table are from Hoddle and Hoddle (2008a) and Hoddle and Brown (2010). Also included is *Antaeotricha nictitans*, a species caught in *S. catenifer* pheromone traps placed in avocado orchards in Guatemala and México.

Species	Family	Pest Status
Argyrotaenia urbana (Busck)	Tortricidae	Small fruit specialist? First record from Guatemala
Polyortha n. sp.	Tortricidae	Small fruit specialist? First record from Guatemala
Netechma pyrrhodelta (Myerick)	Tortricidae	Opportunist? First record from Guatemala and avocados
Euxoa sorella Schaus	Noctuidae	Opportunist?
Micrathetis triplex Walker	Noctuidae	Opportunist?
Holcocera plagatola Adamski	Coleophoridae	Opportunist? New species and first record of this genus from Guatemala
Amorbia santamaria Phillips and Powell	Tortricidae	Known avocado pest, originally described from Guatemala
Histura perseavora Brown	Tortricidae	Previously unrecognized avocado pest? Only known from Guatemala. First host plant record for this genus
Cryptaspasma sp. nr. *lugubris* (Myerick)	Tortricidae	Previously unrecognized avocado pest?
Antaeotricha nictitans (Zeller)	Elachistidae	Previously unrecognized avocado pest? Captured in *S. catenifer* pheromone traps in Guatemala and México
Stenoma catenifer Walsingham	Elachistidae	Well recognized avocado pest with high invasion potential

Figure 1. Pesticide applications to California avocados have steadily increased since the California Department of Pesticide Regulations commenced data collecting in 1989. The primary reason for this increase has been the invasion of exotic foliar feeding pests from the home range of avocados (i.e., México) and the investment of substantial funds (>$100,000 per year) by the California Avocado Commission to register new pesticides for growers to use against these pests, and to increase the diversity of products available to manage the threat of insecticide resistance by perseae mite (photograph B below) and avocado thrips (C). The decline in pesticide use that started in 2007 may be due to the economic recession, and low crop production in 2008-2009 (this harvest was 54% of that the 2007-2008 harvest, and value per bearing acre declined by ~40%). (Photo A) Red banded whitefly, invaded California in 1982 and was fortuitously controlled by a resident parasitoid, *Cales noacki*. (B) Persea mite arrived in 1990. (C) Avocado thrips invaded in 1996. (D) Avocado lace bug and (E) *Neohydatothrips burungae* were found in California in 2004 (these last two pests have not been significant). Persea mite and avocado thrips have been serious perennial problems for California avocado growers requiring insecticidal management. ***A, B, and C were species new to science at time of first discovery in the USA. None of these five pests are specialist fruit feeders. Establishment of specialist fruit feeding moths, weevils, or flies would drive pesticide use in California avocados to unprecedented levels in an attempt to protect the marketable commodity of this industry.***



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