Lepidoptera and Associated Parasitoids Attacking Hass and Non-Hass Avocados in Guatemala

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ABSTRACT A 5-mo survey for fruit feeding Lepidoptera attacking Hass and non-Hass avocados (*Persea americana* Miller [Lauraceae]) was conducted in Guatemala from 1 November 2006 to 1 April 2007. In total, 6,740 fruit were collected from 22 different areas in Guatemala. Eight species of Lepidoptera, of which at least two are species new to science, were reared from avocado fruit. Reared Lepidoptera were *Amorbia santamaria* Phillips and Powell, *Cryptaspasma* sp. nr. *lugubris*, *Euxoa sorella* Schaus, *Histura* n. sp., *Holcocera* n. sp., *Micrathetis triplex* Walker, *Netechma pyrrhodelta* (Meyrick), and *Stenoma catenifer* Walsingham. Hymenopteran parasitoids were reared from larvae of *C.* sp. nr. *lugubris* and *S. catenifer*. One species of parasitoid, *Pseudophanerotoma* sp., was reared from field collected *C.* sp. nr. *lugubris* larvae. The dominant parasitoid reared from *S. catenifer* was a gregarious *Apanteles* sp. Other parasitoid species reared from *S. catenifer* larvae were *Brachycyrtus* sp., *Macrocentrus* sp., and *Pristomerus* sp. The oviposition preference of *C.* sp. nr. *lugubris* for avocado fruit hanging in trees, dropped fruit on the ground, or exposed avocado seeds was investigated by studying the oviposition preferences of adult female moths and determining egg hatch times in the laboratory, and by investigating the longevity of avocado fruit on the ground under prevailing field conditions. Together, data from these studies suggested that *C.* sp. nr. *lugubris* may be an unrecognized pest of avocados that causes hanging fruit to drop to the ground prematurely. The influence of season and altitude on the phenology and distribution of avocado feeding Lepidoptera in Guatemala is discussed.

KEY WORDS avocado pests, Braconidae, Ichneumonidae, *Persea americana*, quarantine

'Hass' is the most widely grown avocado (*Persea americana* Miller [Lauraceae]) in California, accounting for 95% of the annual harvest, which is valued at ≈$341 million (CAC 2006). Since the 1980s, California-grown avocados have been steadily acquiring host-specific exotic leaf-feeding arthropod pests that are native to the home range of *P. americana* in Mexico and Central America (Hoddle 2004). These folivores can cause substantial economic damage (Hoddle et al. 2003) and the increasing number of leaf-feeding pests has resulted in a significant increase in use of pesticides to manage populations of these arthropods below economic levels (Hoddle 2004). Presently, California has no specialist internally feeding frugivores (i.e., seed- and pulp-feeding curculionids and lepidopterans, or tephritid fruit flies) attacking fruit (Hoddle 2007a).

Successful invasion and establishment of specialist fruit feeding avocado pests in California would result in crop losses and associated control costs being substantially greater than those currently incurred for managing pest species which only attack leaves. The incursion threat posed by fruit feeding avocado pests to the California avocado industry has increased markedly with the recent legalization of fresh avocado fruit imports from Mexico which commenced in February 2007 (Hoddle 2007a). Importation of fruit from Mexico had been banned under U.S. Federal law since 1914 (CAC 2006), and this restriction on large-scale fruit movement from the native range of *P. americana* may have been significant in preventing the establishment of unwanted avocado fruit-feeding pests in California (Hoddle 2007a).

Given the high economic value of avocados, it's long history of domestication, and increasing production levels and exports from Mexico and Central and South America, there is surprisingly little information on the identity, biology, ecology, phenology, biological control agents, and monitoring strategies of specialist fruit-feeding avocado pests that use *P. americana* in the home range of this plant. This dearth of information is especially important for Hass producers, which is the commercial cultivar of choice for all major avocado exporters in Mexico and Central America. The basic lack of information on internally feeding fruit pests is detrimental to the development of meaningful risk assessment reports for exporting countries and their importing partners. As a first step in developing a better understanding of fruit feeding pests associated with Hass avocados, surveys for Lepidoptera attacking fruit were conducted in three commercial Hass avo-

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cado orchards in Guatemala. In addition to surveying Hass fruit, surveys for fruit feeding Lepidoptera were simultaneously conducted for non-Hass avocados. For this study, non-Hass avocados were noncommercial cultivars of indeterminate parentage that were noncloned trees grown from seeds that were largely unmanaged and either growing in residential backyards, along roadsides, or as shade trees in coffee plantations. These collections of Hass and non-Hass avocados were used to fulfill two objectives. First, to rear and identify Lepidoptera from field-collected fruit picked from trees and dropped fruit gathered from the ground. Dropped fruit were collected from the ground because previously published work indicated that avocados infested with Lepidoptera larvae are likely to be prematurely aborted (Ventura et al. 1999). Second, to document the larval parasitoid fauna associated with Lepidoptera reared from Hass and non-Hass avocados. The results of surveys for Lepidoptera and associated parasitoids conducted in commercial Hass avocado orchards and for unmanaged non-Hass fruit grown in Guatemala are presented here.

Materials and Methods

Field Sites and Hass Fruit Collections. Surveys for Lepidoptera associated with avocado fruit in Guatemala were conducted over a 5-mo period, 1 November 2006 to 1 April 2007. During this time, fruit on trees were reaching maturity and selective harvesting was underway. Three commercial Hass orchards were surveyed for fruit-feeding Lepidoptera. The first orchard was ≈5 ha consisting of ≈800 trees in San Miguel Dueñas, Sacatépequez Guatemala (Table 1). Fruit surveys were conducted from 19 January 2007 to 31 March 2007. Trees were ≈5 yr old, ≈4–5 m in height, and canopies were separated by ≈5–7 m of clear ground, allowing full sun exposure. The orchard was treated with Malathion three times (rate of application and interval frequency data were not available) in December 2006, and no subsequent treatments were made during this study. Every 2–4 d, at least 50–60 randomly selected trees in the orchard were examined for fruit showing feeding damage. From each sampling event, ≈200 damaged fruit exhibiting frass accumulations, whitish perseitol exudates, or obvious holes were harvested from trees, returned to the laboratory, and placed in labeled cages for rearing of Lepidoptera associated with fruit.

The second commercial Hass avocado orchard was in Najauitov, Verapaz, Guatemala (Table 1). This orchard was of similar size and tree age to the previously described orchard, and it was sampled twice. On 18 December 2006, 101 fruit were removed from this orchard and included fruit harvested from trees and dropped fruit picked up off the ground. Fruit from trees and the ground were commingled and combined in a single cage for rearing of Lepidoptera larvae and associated larval parasitoids. The second harvest was made on 20 March 2007 and 77 fruit were picked from trees only.

The third commercial Hass orchard was in Antigua, Sacatépequez (Table 1). This orchard was 1 ha and comprised ≈300 15–20-yr-old trees that were in various stages of heavy pruning. As with the previous two orchards, all trees were separated by bare ground and tree canopies were not interlaced. This orchard was sampled once on 6 February 2007 and 48 damaged fruit were picked from trees and returned to the laboratory for rearing.

Collection of Non-Hass Avocados. Non-Hass avocados were either picked from backyard plants, or trees grown as an overstory for commercial coffee plantations. In addition, any dropped fruit in yards or coffee plantations were collected from the ground, and 15 different sites were sampled in this manner (Table 1). In four instances, locally grown non-Hass fruit that had been picked from local neighborhood trees were purchased from vendors. These fruit were either sold at the roadside, in supermarkets, or at farmer's markets (Table 1).

Rearing of Lepidopteran Larvae and Associated Parasitoids. Hass and non-Hass avocados were returned to the laboratory and kept for 10–14 d in collapsible ventilated insect rearing cages (Bug-Dorm-2120, [60 by 60 by 60 cm], MegaView, Taichung, Taiwan) that were labeled by collecting date and location. Rearing of larvae was conducted in a well lit room under natural daylight and lengths (≈12 h) at 22.14 ± 0.13°C (temperature measured with a Hobo data logger [Onset Corp., Bourne, MA] at 30-min intervals).

Fruit were inspected daily and emerged mature Lepidoptera larvae searching for pupation sites were isolated and kept in clear labeled plastic cups with ventilated lids that were lined with paper towel to provide a pupation substrate. After the 10–14-d holding period, all fruit from a particular harvest date and location were opened. The pulp and seeds were examined for Lepidoptera larvae. Larvae, in particular, the avocado seed moth, *S. catenifer*, found feeding in avocado pulp were removed and used to artificially inoculate clean uninfested avocado seeds. A number 2 cork borer (4.0 mm in diameter) was used to punch a ≈1.5-cm hole into a clean uninfested avocado seed. The head of the walking larva was aligned with the artificial hole and ≈99% of larvae readily entered seeds in this manner and commenced feeding. Artificially inoculated avocado seeds and naturally infested seeds extracted from fruit were kept individually in labeled ventilated plastic cups until moth or parasitoid pupae were observed in the cup. Infested seeds from which no life stages emerged were opened and feeding galleries examined for moth and parasitoid pupae. All pupal stages were kept in the labeled plastic cups until either adults emerged or the immature life stages died. Lepidoptera larvae found feeding externally on avocado fruit were isolated in ventilated plastic containers with fruit and left to complete development on the exterior of fruit. The fruit holding and insect rearing technique described here was used in preference to fruit cutting to locate Lepidoptera larvae in avocados. Fruit cutting failed to detect very small larvae bur-

Table 1. Avocado fruit collection data from field sites in Guatemala and rearing records for Lepidoptera and associated parasitoids

Collection date(s)/period	Location	GPS coordinates and elevation (m)	No. fruit harvested from trees (and ground)	Lepidoptera species reared	Parasitoid species reared
Hass					
12/18/2006	Najauitov, Santa Cruz, Verapaz	N15°19.012; W90°25.756 (1,609)	6 (95)	[1A]*Cryptaspasma* sp. nr. *lugubris*	[1F]*Pseudophanertoma* sp.
3/20/2007	Najauitov, Santa Cruz, Verapaz	N15°19.012; W90°25.756 (1,609)	77		
1/24/2007–2/23/2007	San Miguel Dueñas, Sacatépequez	N14°31.461; W90°46.579 (1,494)	2,080 (50)	[B]*Amorbia* sp. *Euxoa sorella* [C]*Holcocera* sp. [D]*Histura* n. sp. *Micrathetis triplex* *Netechma pyrrhodelta* [1E]*Stenoma catenifer*	[1]*Apanteles* sp. [1G]*Brachycyrtus* sp. [1H]*Macrocentrus* sp.[1?I]*Orthocentrus* sp. [1J]*Pristomerus* sp.
2/6/2007	Antigua, Sacatépequez	N14°32.717; W90°44.603 (1,496)	48	[1]*S. catenifer*	[1]*Apanteles* sp.
		Total Hass fruit	2,211 (145)		
Non-Hass					
11/5/2006–12/29/06	Palín, Guatemala	N14°26.127; W90°40.881 (1,402)	26 (157)	[1]*S. catenifer* [2]*C.* sp. nr. *lugubris*	[1K]*Apanteles* sp.[2]*Pseudophanertoma* sp.
11/6/2006–1/6/2007	Sumpango, Sacatépequez	N14°40.292; W90°43.195 (1,995)	156 (66)		
11/10/2006	Calle 5, Antigua, Sacatépequez	N14°33.115; W90°44.064 (1,532)	25		
11/11/2006	Parramos, Chimaltenango	N14°36.071; W90°48.147 (1,789)	56 (2)		
11/11/2006–1/22/2007	San Pedro Las Huertas, Sacatépequez	N14°31.930; W90°44.073 (1,497)	243 (352)	[1]*C.* sp. nr. *lugubris*	[1]*Pseudophanertoma* sp. [1?L]*Hymenochaonia* sp.
11/13/2006	El Torre Supermercado (purchased)		2	*S. catenifer*	
11/14/2006–1/25/2007	Alotenango, Sacatépequez	N14°28.245; W90°48.702 (1,321)	1,025 (557)	[1]*S. catenifer* [2]*C.* sp. nr. *lugubris*	[1]*Apanteles* sp. [1M]*Pristomerus* sp. [2N]*Pseudophanertoma* sp.
11/14/2006	Iztapa, Escuintla (purchased)	N13°55.234; W90°47.116 (3.4)	126	[1]*S. catenifer*	[1]*Apanteles* sp.
11/18/2006	Cemizal, Sacatépequez	N14°24.568; W90°55.714 (946)	6 (3)		
11/28/2006	Panajachel, Sololá	N14°41.593; W91°04.351 (1,943)	70 (74)	[1]*C.* sp. nr. *lugubris*	[1]*Pseudophanertoma* sp.
12/11/2006	San Lucas Tolimán, Sololá	N14°38.034; W 91°07.943 (1,849)	62 (49)	-	
12/11/2006	Santiago Atitlán, Sololá	N14°37.528; W91°08.725 (1,586)	55 (115)	[1]*S. catenifer* [2]*C.* sp. nr. *lugubris*	[1O]*Apanteles* sp. [2P]*Pseudophanertoma* sp.
12/11/2006	Santiago Atitlan, Sololá (purchased roadside)	N14°37.581; W91°13.557 (1,592)	354	[1]*S. catenifer* [2]*C.* sp. nr. *lugubris* *Histura* n. sp.	[1]*Apanteles* sp. [2Q]*Pseudophanertoma* sp.
12/18/2006	Tactic, Verapaz	N15°19.537; W90°23.168 (1,473)	19 (306)	[1]*C.* sp. nr. *lugubris*	[1R]*Pseudophanertoma* sp.
12/18/2006	Tactic, Verapaz		80 (6)		
12/30/2006	Mercado de Antigua, Sacatépequez (purchased)		104		
1/5/2007	Pastores, Sacatépequez	N14°35.177; W90° 45.013 (1,647)	153		
2/5/2007	Escuintla, Escuintla	N14° 23.954; W91°06.354 (423)	5	*S. catenifer*	
3/7/2007	Aldea Santa Ana, Antigua, Sacatépequez	N14°32.992; W90°43.216 (1,498)	68 (62)	[1]*S. catenifer*	[1]*Apanteles* sp.
		Total non-Hass fruit	2,635 (1,749)		

[1,2] Indicate host–parasitoid association; [?] uncertain with which host species parasitoid is associated.

University of California Riverside ascension numbers for representative voucher specimens: [A]UCRC ENT 142783-791; [B]UCRC ENT 154992; [C]UCRC ENT 160946; [D]UCRC ENT 160947-960; [E]UCRC ENT 142771-782; [F]UCRC ENT 160926; [G]UCRC ENT 161015; [H]UCRC ENT 161011-14; [I]UCRC ENT 160000; [J]UCRC ENT 161016-017; [K]UCRC ENT 160928-934; [L]UCRC ENT 160999; [M]UCRC ENT 161018; [N]UCRC ENT 161006-007; [O]UCRC ENT160935-941; [P]UCRC ENT 161005; [Q]UCRC ENT 162843-844; [R]UCRC ENT 162848-865, 161001.

rowing through pulp to seeds, larvae were occasionally sliced accidentally and killed, and depending on the angle of the cut, feeding damage to seeds was easily missed too. Consequently, fruit cutting to determine infestation rates of avocados failed to reliably find larvae and under estimated numbers of infested fruit from collection sites.

Ovipositional Observations for *Cryptaspasma* sp. nr. *lugubris* in the Laboratory and Longevity of Avocado Fruit on the Ground. During the course of this study, *C.* sp. nr. *lugubris* (Lepidoptera: Tortricidae: Olethreutinae) was unexpectedly reared from Hass and non-Hass avocados. It has been hypothesized that species in this genus of moths are specialists on hard seeds inside fallen fruit (Brown and Brown 2004) and subsequently would not be classified as pests of hanging avocado fruit. This hypothesis that *Cryptaspasma* species only attack recently dropped fruit on the ground was investigated. A colony of *C.* sp. nr. *lugubris* was established using adult male and female moths reared from field-collected fruit and maintained in a Bug-Dorm. Adult moths were provisioned with 10% honey water solution in plastic vials with cotton wicks, and fresh honey water was provided every 3–4 d. The rearing cage was provisioned with avocado fruit, and clean uninfested avocado seeds were placed either in clusters on the floor of the cage or in clear smooth plastic cups. Observations were made as to where and how many eggs were laid by female moths, and the average length of time taken for eggs to hatch in the cage at room temperature (22.14 ± 0.13°C). The colony was maintained in the same room under the same conditions as the Bug-Dorms used for rearing lepidopteran larvae and associated parasitoids.

As part of this study on *C.* sp. nr. *lugubris*, eight mature green-skinned non-Hass avocado fruit were picked from one tree and placed in pairs in four different places on the ground and monitored every three days for 12 d to determine the longevity of fruit skin and pulp surrounding the seed. Observations were made from 29 December 2006 to 10 January 2007. Temperature was recorded in the orchard at 1-h intervals with a Hobo data logger over this time. This avocado skin-pulp longevity study was conducted in a commercial coffee plantation with a non-Hass avocado overstory in Alotenango Sacatépequez from which *C.* sp. nr. *lugubris* had been reared from non-Hass fruit during this survey (Table 1).

Results and Discussion

In total, 6,740 fruit were collected from 22 different areas in Guatemala (Table 1). Of this total, 2,211 Hass (from three orchards) and 2,635 non-Hass fruit (from 19 sites) were picked from trees. Fruit collected from the ground numbered 145 for Hass (two orchards), and 1,749 for non-Hass (from 13 sites) (Table 1). Eight species of Lepidoptera from four families were reared from Hass avocados: 1) *Amorbia santamaria* Phillips and Powell (Tortricidae: Tortricinae: Sparganothini) ($n \approx 5$); 2) *Cryptaspasma* sp. nr. *lugubris* ($n \approx 50$); 3) *Histura* n. sp. (Tortricidae: Chlidanotinae: Polyorthini) ($n \approx 30$); 4) *Holcocera* n. sp. (Coleophoridae: Blastobasinae: Holcocerini) ($n = 2$); 5) *Netechma pyrrhodelta* (Meyrick) (Tortricidae: Tortricinae: Euliini) ($n = 1$); 6) *Stenoma catenifer* Walsingham (Elachistidae: Stenomatinae) ($n \approx 800$); and 7 and 8) were one specimen each of two different species of Noctuidae, *Euxoa sorella* Schaus (Noctuinae: Agrotini) and *Micrathetis triplex* Walker (Condicinae: Leuconyctini). From non-Hass avocados, three species of Lepidoptera were reared: 1) *C.* sp. nr. *lugubris* ($n \approx 80$), 2) *Histura* n. sp. ($n \approx 5$), and 3) *S. catenifer* ($n \approx 30$) (Table 1).

The two dominant species of Lepidoptera reared from avocados were *C.* sp. nr. *lugubris* and *S. catenifer.* The latter is a well-known pest of commercially grown avocados grown in Mexico and Central and South America (Hoddle 2007b, Hoddle and Hoddle 2007). *C. lugubris* has been recorded previously from Hass avocados in Michoacán Mexico where larvae were initially confused with *S. catenifer.* This moth also has been reared from non-Hass avocados in Puerto Rico (Brown and Brown 2004). Other species of *Cryptaspasma* have been recorded from fruit of *Quercus* sp., *Macadamia integrifolia* Maiden & Betche, *Beilschmidia pendula* (Sw.) Hemsl., *Persea borbonia* (L.), and *P. palustris* (Raf.) Sarg. (the latter three host species are Lauraceae) (Brown and Brown 2004). The third most common lepidopteran reared from avocados in Guatemala was an undescribed species of *Histura.* This is a poorly known genus, specimens of *Histura* are extraordinarily rare, and the life history and host plants for members of this genus are unknown (J. Brown, personal communication). The record presented here is the first species of *Histura* that has been documented from Guatemala and this is the first record of avocados being attacked by any member of this genus (J. Brown, personal communication).

There are ≈60 described species of *Netechma* in the new world tropics that have been recorded from Costa Rica through to Argentina and Venezuela (Brown and Adamski 2002, Brown 2005). *Netechma pyrrhodelta* (Meyrick) is known from Costa Rica where it has been reared from an *Inga* sp. (Fabales: Fabaceae). The record presented here for *N. pyrrhodelta* is the first time that this species has been reared from avocado and the first record of this species from Guatemala (J. Brown, personal communication). The new species of *Holcocera* from the new world tropics reared from Hass avocados is a first record for this genus from Guatemala (D. Adamski, personal communication). *Holcocera* spp. have been reared from a diverse variety of plant species in the Americas, including *Acer* sp. (Aceraceae), *Caesalpina* sp. (Fabaceae), *Eriobotrya* sp. (Rosaceae), and *Yucca* sp. (Liliaceae) (Adamski 1998). *Holcocera concolor* Adamski & Maier is a minor pest of conifers (Pinaceae), in particular *Pinus* spp., in the northeast United States and parts of Canada (Adamski and Maier 2003). In Japan, *H. sakura* Ohshima has been reared from Japanese flowering cherry, *Prunus yedoensis* Matsumura (Rosaceae) (Ohshima 2003). Larvae of *Amorbia* species (e.g., *A. cuneana* [Walsingham] and *A. emigratella* Busck) are well-

known avocado pests that damage leaves and the skin of avocado fruit. These tortricids can be problematic after pesticide applications release well controlled populations from suppression by hymenopterous and dipterous natural enemies (Oatman et al. 1983). Malathion applications at the study site where this moth was reared may have contributed to this pest being more prevalent than it would otherwise have been.

Hymenopteran parasitoids were reared from larvae of *C.* sp. nr. *lugubris* and *S. catenifer.* One species of parasitoid, *Pseudophanerotoma* sp. (Braconidae: Cheloninae), was reared from ≈30% of field-collected *C.* sp. nr. *lugubris* larvae (Table 1). This is the first host record for a *Pseudophanerotoma* sp. Members of the Cheloninae are generally egg–larval parasitoids of tortricoids and pyraloids (J. Luhman, personal communication). The dominant parasitoid reared from *S. catenifer* was a gregarious *Apanteles* sp. (Braconidae: Microgastrinae) that accounted for ≈99% of the reared parasitoids, and this natural enemy parasitized ≈53% of reared *S. catenifer* larvae. Other parasitoid species reared from *S. catenifer* larvae were *Macrocentrus* sp. (Braconidae: Macrocentrinae; $n = 4$); *Pristomerus* sp. (Ichneumonidae: Cremastinae; $n = 3$); and *Brachycyrtus* sp. (Ichneumonidae: Labeninae; $n = 1$). Labenines are a Gondwanian group with most known species being endemic to Australia. The record presented here for *Brachycyrtus* sp. is a very rare New World find (J. Luhman, personal communication). A single specimen of *Orthocentrus* sp. (Ichneumonidae: Orthocentrinae) emerged from fruit from which *S. catenifer* was predominantly reared. *Hymenochaonia* sp. (Braconidae: Macrocentrinae; $n = 1$) emerged into a cage from which *C.* sp. nr. *lugubris* was reared. The exact host association of these latter two parasitoids is not certain, but given the size of these parasitoids and that *S. catenifer* or *C.* sp. nr. *lugubris* was the most prevalent lepidopteran in samples at sites from which fruit were collected, it is possible that *Orthocentrus* sp. and *Hymenochaonia* sp. possibly parasitize the larvae of *S. catenifer* and *C.* sp. nr. *lugubris,* respectively. However, *Hymenochaonia* sp. has been reared previously from *S. catenifer* (Nava et al. 2005a), and this parasitoid may not therefore be associated with *C.* sp. nr. *lugubris.*

Nava et al. (2005a) and Cervantes Peredo et al. (1999) list *Apanteles* sp., *Dolichogenidea* sp., *Hypomicrogaster* sp., *Chelonus* sp. *Hymenochaonia* sp., (all Braconidae), *Eudeleboea* sp., and *Pristomerus* sp. (latter two are Ichneumonidae) parasitizing *S. catenifer* larvae in South America. In Brazil and Venezuela, solitary *Apanteles* spp. have been documented as being among the most common parasitoids parasitizing *S. catenifer* larvae in avocados at rates ranging from 31 to 33% in Venezuela (Boscán de Martínez and Godoy 1982) and contributing to 30–40% parasitism levels observed in Brazilian avocado orchards that had been treated heavily with insecticides (Nava et al. 2005a). The relatively high level of parasitism observed by *Apanteles* sp. in Hass avocados in Guatemala that had been treated with a broad-spectrum insecticide strongly suggests that this natural enemy may play an important role in regulating populations of *S. catenifer* in commercial orchards despite insecticide use. Similar to the survey results presented here from Guatemala, there are no reports of dipteran parasitoids (e.g., tachinids) having been reared from *S. catenifer* in South America.

Ovipositional Observations for *Cryptaspasma* sp. nr. *lugubris* in the Laboratory and Longevity of Avocado Fruit on the Ground. Examination of the placement of 633 eggs in 30 randomly selected egg masses laid by *C.* sp. nr. *lugubris* in the laboratory revealed that the average egg mass size was 21.10 ± 3.52 eggs (range 2–73 eggs) and that ≈80% of eggs were laid on smooth plastic, which included clear plastic cups and lids holding avocado seeds or fruit, and the floor and walls of the Bug-Dorm used for ovipositional studies. The remaining ≈20% of eggs were distributed evenly across seeds and fruit in the cage. At 22.14 ± 0.13°C, it took 10.78 ± 2.53 d for *C.* sp. nr. *lugrubis* eggs to hatch, and of 301 larvae reared on avocado seeds, 72% reached adulthood of which 54% were female. In the field, the longevity of avocado fruit on the ground was brief. Within 6–9 d at least 75% of skin and pulp had been removed and by day 12, 75% (i.e., six of eight fruit) of fruit were exposed seeds with fragments of dried pulp adhering to seeds. The agents responsible for attacking fruit on the ground were not observed but may have been rodents or birds. The average temperature at the field site over the course of the fruit observation study was 18.11 ± 0.13°C (range 12.55–23.24°C). Photographs of all life stages of *Cryptaspasma* sp. nr. *lugubris* and the appearance of avocado fruit exposed to foraging animals in Guatemala are available on the web (Hoddle 2007c).

The purpose of these two studies, *C.* sp. nr. *lugubris* egg hatch times and fruit longevity, was to investigate the hypothesis that *Cryptaspasma* species associated with avocados are specialists of recently dropped fruit on the ground as proposed by Brown and Brown (2004), and these moths are not pests that attack avocado fruit hanging in trees. Because fruit picked from trees and dropped fruit removed from the ground were commingled for sites from which *C.* sp nr. *lugubris* was reared, it was impossible to discern from which fruit this moth emerged. Consequently, experiments were conducted to investigate the Brown and Brown (2004) hypothesis. Oviposition studies in the laboratory demonstrated that *C.* sp. nr. *lugubris* would oviposit on avocado seeds and fruit. However, the strength of any egg-laying preferences as they may occur in the field as predicted from laboratory observations was overwhelmed by the predilection of *C.* sp. nr. *lugubris* for laying eggs on smooth plastic in the oviposition cage. Extreme preferences for nonhost material during ovipositional studies in the laboratory have been observed with numerous other lepidopterans (Nava et al. 2005b).

Assuming that *C.* sp. nr. *lugubris* does lay eggs on the skin or pulp of dropped fruit in the field as indicated by the laboratory results presented here, it is reasonable to suggest that under prevailing field conditions the majority of these eggs would be consumed before they had procured sufficient degree-days to hatch and

insufficient time would pass for larvae to bore through fruit pulp before tunneling into protective seeds. Results presented here have demonstrated that under field conditions avocado skin and fruit pulp is almost entirely removed by foraging animals within ≈12 d at ≈18°C, whereas it takes ≈10 d for *C.* sp. nr. *lugubris* eggs to hatch at ≈22°C. Therefore, laying eggs on the skin or exposed pulp of dropped avocados lying on the ground would seem to be a maladaptive reproductive strategy for *C.* sp. nr. *lugubris* because of risk from accidental predation. Consequently, oviposition by *C.* sp. nr. *lugubris* on dropped avocado fruit may be unlikely to occur, and detection of this moth in avocado fruit collected from the ground may result from feeding larvae causing premature abortion and dropping of hanging fruit. Eggs oviposited on exposed seeds on the ground would be at less risk of accidental predation by animals consuming fruit and could be a viable reproductive strategy because destructive seed consumption by vertebrates was not observed in the field.

In this study, support for *C.* sp. nr. *lugubris* ovipositing on avocados hanging in trees came from the purchasing of 354 fruit from a local roadside vendor who had harvested avocados from backyard trees in Santiago Atitlán on the day they were purchased (these fruit were being carried in a basket strapped to the vendor's head and the vendor was carrying the fruit picker at time of purchase; Hoddle 2007c) (Table 1). Examination of purchased fruit in the laboratory failed to detect any rotten, cracked, or otherwise damaged avocados (damage of this nature would have been indicative of fruit being damaged upon hitting the ground after dropping), which strongly suggested that these fruit had not been collected from the ground, and were recently picked from trees as claimed.

The obvious way to address the *C.* sp. nr. *lugubris* egg laying habitat to determine if this moth is a pest of hanging avocados is to harvest fruit directly from trees for rearing and not to commingle picked fruit with those collected from the ground. Direct fruit harvesting to address this question was attempted as part of this project in March 2007, but it was unsuccessful. There were two possible reasons why *C.* sp. nr *lugubris* was not reared from picked fruit harvested at sites that had yielded this moth from earlier surveys. First, by March when picked fruit surveys for *C.* sp. nr. *lugubris* were initiated the dry season in Guatemala was well underway and this climatic change was accompanied by a noticeable decrease in the collection of this moth (i.e., complete lack of detection). This seasonal transition was accompanied by an increase in the detection frequency of *S. catenifer* in fruit. Second, post priori analysis of collection data suggests that in addition to time of year, altitude may be important in determining the distribution of *C.* sp. nr. *lugubris* in Guatemala. This tortricid was only reared from avocados that were collected at altitudes exceeding 1,300 m, and it coexisted with *S. catenifer* up to 1,592 m; above 1,609 m, only *C.* sp nr. *lugubris* was reared from avocados (Table 1). Conversely, *S. catenifer* was reared from avocados grown in hot and humid conditions at sea level up to 1,592 m, and the prevalence of this moth at higher altitude sites increased as the dry season progressed (Table 1).

Future work on the potentially pestiferous *C.* sp. nr. *lugubris* and *Histura* n. sp., and the well-recognized avocado pest *S. catenifer*, in Guatemalan-grown avocados may need to consider time of year and altitude when studies are conducted. Detailed phenological studies on these moths conducted over a 2–3-yr period and across different altitudes where avocados are grown would be extremely useful to fully understand the effect of climatic seasonality and elevation on population dynamics, natural enemy fauna, and impacts, pestiferousness, and preferences for Hass and non-Hass avocados. The deployment of species-specific pheromone traps would greatly assist with monitoring studies for pest moths because it would eliminate the labor-intensive need for rearing moths from harvested fruit to determine seasonal phenology. If altitude is scientifically demonstrated to be important in delineating species distributions (especially high-altitude sites for *S. catenifer*), certified "pest free zones" that meet requirements of importing countries could be established for commercial avocado production areas in Guatemala.

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