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# Oviposition biology, behavior, and avocado cultivar preferences of *Heilipus lauri* Boheman (Coleoptera: Curculionidae)

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*Heilipus lauri* is a specialist avocado seed feeding weevil native to parts of México and is an invasive pest in Colombia. This weevil is considered an incursion risk because possible unintended introductions into areas outside of its native range can result in establishment, which threatens avocado production in invaded regions. Despite being a well-recognized pest of avocados, relatively little is known about oviposition biology, behavior, and cultivar preferences of this weevil. Field studies in commercial Hass avocado orchards in México and laboratory studies in a quarantine facility in California (USA) indicated that *H. lauri* prefers to oviposit into middle and bottom thirds of fruit. In the laboratory, an average of 1.7 eggs (range 1–6 eggs) are laid over a 24-h period in oviposition chambers by individual females. Conspecifics add eggs to these clutches, with up to 17 eggs being recorded in 1 oviposition chamber. This finding suggests that *H. lauri* does not use an oviposition deterring pheromone. Female weevils readily oviposit in artificial holes representing mechanical wounds on fruit. In comparison to the commercially dominant Hass variety, fruit from Lamb Hass and G22, a native Guatemalan variety, and to a lesser extent Gem, may be less preferred for oviposition when females have a choice of fruit in which to oviposit. Videography studies indicated that *H. lauri* exhibits predominantly diurnal activity with respect to oviposition behaviors, feeding, and walking.

**Key words:** avocado, oviposition biology, cultivar preference, videography

## Graphical Abstract



## Introduction

The introduction of invasive pests into new areas is a global and perennial problem that threatens agricultural productivity and food security (IPBES 2023). Increasing tourism and trade (Seebans et al. 2018), including international movement of fresh produce, are conduits that introduce invasive pests into new areas where they did not previously exist (Morse et al. 2009, Lu et al. 2021, Nnzeru et al. 2021). With respect to international movement of fresh fruit, global trade in avocados (*Persea americana* Miller [Lauraceae]) was valued at \$7.35 billion (US) in 2022, and México, the world's largest producer, accounted for ~48% of exported fruit internationally (Workman 2023). Avocados are native to Mesoamerica (e.g., México and Guatemala) and evolved within geographical tropical latitudes 23.5°N to 23.5°S (Schaffer et al. 2013). Within this highly climatically varied geographical range, 2 subtropical races, "Mexican" and "Guatemalan," evolved in the highland montane cloud forests of México and Guatemala, respectively (Schaffer et al. 2013). The commercially and globally dominant 'Hass' cultivar is likely a hybrid between Mexican and Guatemalan races (Schaffer et al. 2013).

Within this native range, insect biodiversity associated with avocados is high but relatively understudied (Peña et al. 2013, Jones et al. 2019). This is worrisome given the economic importance of this crop, especially when considering the large volumes of fruit exported from countries where *P. americana* is native (e.g., México and Guatemala) (Hoddle 2006). California is the largest US producer of avocados, and a recognized threat associated with importation of avocado fruit into the United States, and California in particular, is the risk of inadvertent introductions of specialist internal feeding fruit pests, such as moths (e.g., the avocado seed moth, *Stenoma catenifer* Walsingham [Lepidoptera: Depressariidae] and weevils (e.g., the large avocado seed weevil, *Helipus lauri* Boheman [Coleoptera: Curculionidae]), that consume avocado seeds which renders fruit unmarketable (Hoddle 2006).

Among the potential invasive insect pests associated with imports of fresh fruit that enter California, *H. lauri* is a recognized quarantine

threat associated with fruit that originates from México (USDA-APHIS 2004), where this insect is native (Castañeda-Vildózola et al. 2013a, 2017, Ortega-Licona et al. 2019), and Colombia (USDA-APHIS 2016a, b), where this weevil is an invasive pest of economic importance (Caicedo et al. 2010). Guatemala is also considered a risk source (USDA-APHIS 2024) even though *H. lauri* has not been recorded from this country (Arévalo and Cano 2019). Ecological niche modeling indicates that *H. lauri* has potential to extend its range within México that could result in infiltration of commercial avocado production areas that currently lack this pest (Luna et al. 2017). Similar predictive modeling that assesses incursion risk for other avocado production areas, such as California, is lacking. This is a serious impediment for the development of detection and incursion management programs for *H. lauri*. The establishment of a specialized fruit-feeding pest in California, such as *H. lauri*, which is currently absent, would significantly disrupt and threaten the long-term economic viability of the state's avocado industry (Hoddle 2006, Hoddle et al. 2022).

Female *H. lauri* oviposit into perforations that females chew into fruit with mandibles located on the distal end of the rostrum. Economic damage to fruit results when larvae, upon emerging from eggs, bore through avocado pulp to reach seeds within which they tunnel, feed, and pupate (Diaz Grisales et al. 2017). Fruit damage in poorly managed Hass orchards from *H. lauri* can reach 30% (Ortega-Licona et al. 2019), whereas in Hass orchards under intensive management, damaged fruit ranges 4%–8% (Caicedo et al. 2010). Control of damaging feeding stages is difficult because *H. lauri* larvae concealed within fruit are protected from insecticides (Caicedo et al. 2010). Even though *H. lauri* is a well-recognized avocado pest and importation risk, and has demonstrated an ability to invade and establish in areas outside of its native range (i.e., Colombia), basic aspects of the biology of this weevil are poorly understood. For example, there is a lack of information on *H. lauri* oviposition and mating behaviors and whether this pest exhibits feeding and oviposition preferences for fruit from different avocado cultivars.

These knowledge deficits significantly affect the development and implementation of detection, monitoring, and management programs for *H. lauri*. To address these shortcomings, frequency and placement of oviposition holes in Hass fruit in commercial orchards in México were quantified. In a quarantine facility in California, 3 different investigations were conducted. Oviposition studies investigated the number of eggs laid into oviposition holes, reuse of oviposition holes by conspecific females, and the propensity of female weevils to oviposit into artificial holes representing mechanical damage to fruit. Female oviposition preferences for fruit from 5 different cultivars, Bacon, Gem, G22, Hass, and Lamb Hass, were assessed. Digital videography was used to quantify oviposition, feeding, and mating behaviors. The results of these studies are reported here and the implications of these findings are discussed.

## Materials and Methods

### Field Surveys for Hass Avocado Fruit Damaged by *H. lauri* in México

Two commercial Hass avocado orchards in the Municipality of Coatepec-Harinas in the State of México, México were surveyed over 11–12 February 2020 for fruit exhibiting damage from oviposition by female *H. lauri* and subsequent larval feeding. Survey orchards were located in Meyuca de Morales (huerta La Palapa; N18.87494; W99.89690; elevation 2,100 m) and Almoloya de Alquisiras (huerta La Barranca; N18.84188; W99.77203; elevation 1,825 m). Visual surveys of 250 (i.e., 125 fruit per orchard) hanging Hass fruit indicated that 38% (i.e., 95 fruit) of fruit exhibited damage representative of *H. lauri* damage. Damaged fruit hanging in trees were picked, and the number and position of oviposition holes made by female weevils were recorded. Position of oviposition holes was assigned to 1 of 3 strata, top (i.e., holes were found in the top proximal third of the fruit which was identified from the attached fruit pedicel), middle (holes were found in the central equatorial region of the fruit), and bottom (holes were located in the distal bottom third of the fruit). From La Palapa and La Barranca, 299 and 50 weevil damaged fruit, respectively, for a total of 349 collected fruit were assessed for perforations.

### Collection of Weevil Infested Avocado Seeds for Colony Initiation and Colony Maintenance in Quarantine

A total of 67 seeds infested with immature *H. lauri* (i.e., larvae or pupae) were excised from fruit collected from La Palapa and an additional 54 infested seeds were collected from La Barranca, for a total of 121 seeds. Infested seeds were moved under USDA-APHIS permit number P526P-19-04341 to the Insectary and Quarantine Facility (IQF) at the University of California, Riverside for rearing. In quarantine, seeds were held in 6 ventilated plastic containers labeled with collection information and maintained in 1 Bugdorm (model 2120F [MegaView Science Co., Ltd., Taiwan]). This double cage containment was implemented to reduce the likelihood of accidental escape by adult weevils. Seed containers were checked daily and the number and sex of emerged adult weevils were recorded for each collection site. Seeds from which weevils failed to emerge were opened and examined 36 days and 24 days after last recorded adult emergence for La Palapa and La Barranca, respectively. A total of 44 (23 females and 21 males) and 23 (12 females and 11 males) adult weevils emerged from seeds collected from La Palapa and La Barranca, respectively.

Adult *H. lauri* that emerged from seeds collected at each collection site were maintained in 1 of 3 Bugdorms (model 2120F [MegaView Science Co., Ltd.]) labeled by collection locality. Two cages held male and female weevils from La Palapa (cage 1 had 12 females and 12 males; cage 2 had 11 females and 9 males) and the third cage held all emerged weevils from La Barranca (i.e., 12 females and 11 males). These 3 Bugdorm rearing cages were kept individually inside a larger Bugdorm (model 2400F). This double-cage containment was implemented to reduce the likelihood of accidental escape. Adult weevils were given 4–6 fresh Hass fruit with ~10 cm pedicel and green stemmed avocado branches with immature leaves twice a week (i.e., every 3–4 days) to oviposit (i.e., fruit) or feed (i.e., fruit, pedicel, green stems, and leaves) on. All fruit and foliage were sourced from unsprayed (i.e., no insecticides or fungicides were applied to trees) Hass orchards on the University of California Riverside campus. Field harvested fruit, stems, and leaves were thoroughly washed and dried before being placed into cages. To maintain fruit in a vertical position, fruit were placed distal end down on a white plastic vial lid (25-mm diameter; 10-mm deep). Branches were kept in vials of water. To prevent weevils from drowning, the distal ~11 cm of branches were stripped of leaves and then inserted through perforated vial lids. Upon removal from cages, fruit and foliage were examined for adult weevils that were removed. Weevils were counted to confirm all adults for each cage had been collected. Fruit removed from colonies were examined for eggs (see below for details) prior to disposal and foliage was immediately discarded. All waste fruit and foliage was subjected to heat treatment and disposal protocols outlined in the conditions of USDA-APHIS permit number P526P-19-04341. Colonies were maintained in a temperature and humidity-controlled room in IQF under 12:12 (L:D), 26°C ( $\pm$  0.01), and 56% ( $\pm$  0.31) RH.

A total of 28 adult weevils collected from the La Palapa and La Barranca field sites labeled with locality data were deposited as voucher specimens in the University of California Riverside Entomology Research Museum under accession numbers UCRC ENT 598803-598830.

### Oviposition Studies

Each time fruit were removed from colony cages they were examined for oviposition holes (i.e., perforations in fruit skin) and underlying oviposition chambers. Females chew a hole into the fruit epidermis (i.e., the oviposition hole) and then use the rostrum to excavate an oviposition chamber which is a space accessed via the oviposition hole that is made in the fruit pulp immediately below the epidermis. Eggs are laid in this chamber. Oviposition holes in the epidermis and associated underlying chambers in fruit pulp were excised from fruit using a razor blade. Using a dissecting microscope at 10 $\times$  magnification, fruit pulp was cut and separated immediately below the epidermis with the razor blade to expose the oviposition chamber. The number of eggs per chamber were counted and recorded. Multiple eggs per chamber were frequently encountered (see Results), which stimulated 3 additional oviposition studies to investigate potential reuse of pre-existing oviposition chambers holding eggs by females (2 different experiments) and female use of small artificial holes that were deliberately made by puncturing fruit.

### Oviposition Hole Reuse Study 1: Eggs Left in Oviposition Chambers

To investigate reuse of oviposition chambers by different ovipositing females, 3 Hass fruit were placed into each of the La Palapa and La Barranca weevil colony cages. To maintain fruit in a vertical position,

fruit were placed distal end down on a white plastic vial lid (25-mm diameter; 10-mm deep). After 3 days, fruit were removed and labeled by cage. All oviposition holes were labeled and numbered, and the underlying oviposition chambers were then excised using a razor blade as described earlier. Eggs within chambers were then counted and recorded by colony origin, and fruit and hole number. Labeled excised oviposition chambers with and without eggs were then placed back into the original fruit and position from which they were removed. Fruit were then returned to the colony cage from which they did not originate (i.e., La Palapa fruit were placed in the La Barranca cage and vice versa) to ensure that females were encountering holes made by and eggs laid by different females. After an additional 3 days, numbered oviposition chambers were excised and numbers of eggs were recorded. This experiment with eggs left in oviposition chambers and subsequently exposed to ovipositing females in different colony cages was repeated twice.

### Oviposition Hole Reuse Study 2: Eggs Removed from Oviposition Chambers

A variation of the preceding experiment assessed hole reuse by female weevils when oviposited eggs were removed from chambers. Three Hass fruit were each placed in the La Palapa and La Barranca colony cages and left for 3 days. Following protocols described earlier, eggs found within excised oviposition chambers were counted and removed with a water-moistened 0.5-mm camel hair brush. Labeled excised chambers with eggs removed were placed back into fruit from which they originated and then fruit were placed into the colony cage from which they did not originate and left for an additional 3 days. After this second 3-day exposure period, numbered oviposition holes were excised and numbers of eggs were recorded. This experiment with eggs removed from oviposition chambers and subsequently exposed to different ovipositing females was conducted once.

### Use of Artificial Holes for Oviposition

To determine if female weevils would oviposit into holes resulting from damage to Hass fruit, artificial holes, ~2-mm wide and ~2-mm deep, were made using a small screwdriver with a Phillip's head. Artificial holes were positioned in the top, middle, and bottom third of experimental fruit. Holes in each of these 3 vertical positions were made in each of 4 quadrants of the fruit for a total of 12 holes per fruit. A total of 12 fruit, each with 12 artificial perforations, were placed into the La Palapa ( $n = 6$  fruit) and the La Barranca ( $n = 6$ ) colony cages. To maintain fruit in an upright position, fruit were placed distal end down on a white plastic vial lid (25-mm diameter; 10-mm deep) on the floor of colony cages. Following placement in cages, fruit were left for 3 days. After this 3-day exposure period, fruit were removed, artificial holes were excised, and numbers of eggs were recorded by colony cage, fruit number, and hole number and position. This experiment was repeated with 4 fruit per cage for a total of 8 fruit being exposed for 4 days to ovipositing females in the La Palapa and La Barranca colony cages.

### Comparison of Weevil Preferences for Oviposition and Feeding on Fruit and Petioles from 5 Different Avocado Cultivars

The preferences for feeding and oviposition by weevils on fruit (used for feeding and oviposition) and attached petioles ~10 cm in length (used for feeding only) of 5 avocado cultivars were assessed using 2 different "choice" tests. The first choice test consisted of 3 cultivars;

Hass, Bacon (both are commercial varieties), and G22 (a native noncommercial Guatemalan variety). The second choice test used Hass, Gem, and Lamb Hass, which are all commercial varieties. For both tests, 3 fruit of each cultivar, for a total of 9 fruit, were placed in 1 La Palapa colony cage. An additional 2 fruit of each cultivar, for a total of 6 fruit, were placed in the La Barranca colony cage. To maintain fruit in a vertical position, fruit were placed distal end down on a white plastic vial lid (25-mm diameter; 10-mm deep) on the cage floor. Fruit were left exposed to weevils for 4 days before being removed and fruit and petioles were examined for oviposition and feeding activity. The number of feeding scars (i.e., areas where weevils "grazed" on the epidermis) on individual fruit and petioles was recorded by fruit type and colony cage, as was the number of oviposition holes bored into fruit. Choice test 1, conducted with Hass, Bacon and G22, was repeated 9 times for La Palapa and La Barranca for a total of 54 fruit of each cultivar being exposed to weevils. Choice test 2, conducted with Hass, Gem, and Lamb Hass, was repeated 7 times for both colonies for a total of 42 fruit of each cultivar being exposed to weevils. All fruit used in experiments were sourced from unsprayed avocado trees on the UC Riverside campus and experiments were conducted in April 2020.

Following examination of fruit and petioles after exposure to weevils in colony cages, the dry weight of experimental fruit was measured to assess maturation equivalency across fruit types. A total of 12 each of Hass, Bacon, and G22 from choice test 1, and a total of 4 each of Hass, Gem, and Lamb Hass from choice test 2, were randomly selected for coring. Using a cork borer, a 13-mm diameter core was removed from each of 4 equidistant points around the equator of fruit. The cork borer was pushed through the fruit epidermis and underlying pulp until it hit the seed. The corer was then twisted to separate the core from the seed. Cores were then extracted from fruit, removed from the cork borer, numbered, and identified by fruit type. Freshly excised cores were weighed on a digital balance and dried in an oven at 40°C for 72 h. After drying, labeled cores were reweighed, the difference between wet and dry weight was determined, and percentage dry weight was calculated (i.e., dry weight (g)/wet weight (g) \* 100) (Lee et al. 1983).

### Videography of Oviposition, Feeding, and Mating Behaviors

To describe and quantify oviposition, feeding, and mating behaviors of *H. lauri*, a single male and female pair were confined to a cage with a Hass avocado fruit and digitally video recorded for 24 h. Videography was conducted over the period 18 June 2020 to 14 July 2020 and a total of 12 male and female pairs were subjected to digital videography recording sessions.

The experimental cage was constructed by gluing four 15 cm highly transparent acrylic squares (~2-mm thick) (SW Plastics F2191) together to form a box. The top and 1 side of the box was covered with fiberglass fly screen material (Saint Goban ADFORS, Albion, NY, USA) for ventilation. Approximately 6 cm from the base of the cage, a hole 5 cm in diameter, was cut using a hole saw in the center of 1 acrylic panel. Around the outside perimeter of this hole museum putty (Quakehold!, Vista, CA, USA) was attached. A near mature green Hass avocado fruit, harvested from unsprayed trees on the University of California Riverside campus, was pressed into the putty to form a tight seal which allowed weevils inside the cage to access the exposed area of the fruit while eliminating gaps that would permit escape. To reduce weight strain on the putty, fruit sat on the open side of a plastic 60 ml (i.e., 2 oz) portion container (CrystalWare, Lakewood, NJ, USA) that was 3 cm in height.

Male-female weevil pairs that were introduced into the experimental cage through the 5 cm hole were digitally recorded using a Raspberry Pi Model B minicomputer with 512MB of RAM. DVR units consisted of Raspberry Pi NoIR camera, SD card, Raspberry Pi Model B, and a USB flash drive. The Raspbian operating system installed on an 8-GB SD card was used to run the camera. The motherboards, SD cards, and cameras were manufactured by Raspberry Pi (Raspberry Pi Fdn, Caldecote, UK). The camera was outfitted with infra-red LEDs to facilitate night recording. A clock with a blue digital display was placed close to the test unit enabling time stamps for recordings. Digital data were saved onto a 256-GB USB flash drive (Corsair Components Inc., Fremont, CA, USA) which could store approximately 30 h of recordings. The digital recording system was powered via a USB connection and positioned to view the experimental arena containing the avocado fruit and foraging weevils. Experiments were run in a temperature and humidity-controlled room in IQF under 12:12 (L:D), 26°C ( $\pm 0.01$ ), and 56% ( $\pm 0.31$ ) RH.

Digital video was reviewed and used to construct behavioral time and percent activity budgets. A total of 16 behaviors were observed and mean time and percent time engaged in behaviors were determined for male and female weevils for the following activities: (i) female drilling into fruit while copulating with male (i.e., in copulata), (ii) female drilling oviposition hole without a copulating male, (iii) female grazing on skin of fruit while copulating, (iv) female or male grazing on fruit alone, (v) female ovipositing into fruit while copulating, (vi) female ovipositing into fruit alone, (vii) female closing over oviposition hole while copulating, (viii) female closing oviposition hole alone, (ix) female resting on cage wall while copulating, (x) female resting alone on fruit, (xi) female or male resting on cage wall alone, (xii) female or male resting on fruit alone, (xiii) female walking on cage wall while copulating, (xiv) female walking on fruit while copulating with male, (xv) female or male walking alone on cage wall, and (xvi) female or male walking alone on fruit. Assigned behaviors were partitioned further by diurnal or nocturnal occurrence. After 24-h exposure, weevils and fruit were removed from cages. Oviposition holes were labeled and numbered and the exposed area of experimental fruit with labels was digitally photographed. Labeled oviposition chambers were excised and the number of eggs in holes was recorded. Numbered oviposition holes on each fruit in digital photos were cross referenced back to observed behaviors associated with numbered holes that were digitally video recorded for females and males associated with that specific fruit.

## Statistical Analyses

### Analysis of the Positions of *H. lauri* Oviposition Holes on Hass Fruit in Commercial Orchards

Generalized Linear Mixed Models (GLMM) and the PROC GLIMMIX procedure in SAS (SAS 2013) were employed to assess whether ovipositing female weevils exhibited a location preference on fruit for oviposition. Fruit strata (i.e., top, middle, and bottom) were treated as fixed effects in the model with hole counts per strata following a Poisson distribution. To address potential statistical dependencies, a nested structure was incorporated into the model, including sample hole replicate within sample orchard (i.e., La Palapa or La Barranca), sample fruit replicate, and fruit strata (i.e., top, middle, bottom) (see Hoddle et al., 2023, for additional details on this type of nested analysis). Significant main effects were verified through pairwise comparisons using the least-squared means option (i.e., the GLIMMIX procedure and LSMEANS statement [SAS

2013]), adjusting for multiple comparisons using the Tukey-Kramer method at a significance level below 0.05.

### Analysis of Artificial Hole Use for Oviposition

GLMMs and the PROC GLIMMIX procedure in SAS (2013) were used to analyze female weevil oviposition preferences for artificial holes located within 3 strata in each quadrant of experimental fruit. Separate analyses were conducted for each of the 2 response variables: the number of eggs laid in premade holes in each stratum (i.e., top, medium, and bottom), and the percentage of holes used for oviposition within each stratum. Strata were considered a fixed effect in both models. Egg counts followed a negative binomial distribution, whereas the percentage of holes used within strata was modeled using a binomial distribution. A nested structure was integrated into both models to address potential statistical dependencies, including sample hole replicate within trial replicate time blocks, sample fruit replicate, and fruit strata (see Hoddle et al., 2023, for additional details on nested structure analyses). Significant main effects in both models were determined through pairwise comparisons using the least-squares means option, implemented with the SAS GLIMMIX procedure and LSMEANS statement (SAS 2013). Multiple comparisons were adjusted using the Tukey-Kramer method at a significance level below 0.05.

### Analysis of Numbers of Oviposition Holes and Feeding Scars on Fruit and Petioles of 5 Different Avocado Cultivars

GLMMs and the PROC GLIMMIX procedure in SAS (SAS 2013) were used to analyze how avocado cultivar and weevil colony origin influenced the number of oviposition holes drilled into fruit, the number of feeding scars on the fruit surface, and the number of feeding scars on attached fruit petioles. Separate analyses were conducted for each of the 2 choice trials: the first-choice test included cultivars Hass (as a positive control) Bacon and G22. The second-choice test used Hass (a positive control), Gem, and Lamb Hass. Fixed effects such as avocado cultivar (i.e., Hass, Bacon, and G22, for choice trial 1, and Hass, Gem, and Lamb Hass for choice test 2), weevil source colony cage (i.e., La Palapa and La Barranca), and their interaction were included in both analyses. Separate models were developed for each of the 3 response variables investigated for each choice test: (1) number of oviposition holes drilled into fruit, (ii) number of feeding scars on fruit surface, and (iii) number of feeding scars on attached fruit petioles. A nested structure was incorporated into all models to address potential statistical dependencies, including sample fruit replicate within avocado cultivar, colony cage, and trial replicate. Based on the variances of the response variables, Poisson distributions were applied across all models.

### Analysis of Dry Weights of Fruit Used in Preference Experiments

GLMMs and the PROC GLIMMIX procedure in SAS (2013) were used to evaluate maturation equivalency across avocado cultivars. Separate analyses were conducted for each of the 2 choice trials. Avocado cultivar was a fixed effect in both models, and percentage dry weight of cored samples was modeled using a binomial distribution. A nested structure was integrated into all models to address potential statistical dependencies, including sample core replicate within avocado cultivar and sample fruit replicate. Significant main effects in all models were identified through pairwise comparisons using least-squares means using the SAS GLIMMIX procedure and LSMEANS statement (SAS 2013). Multiple comparisons were

adjusted with the Tukey-Kramer method, thereby maintaining a significance level below 0.05.

## Results

### Field Surveys for Hass Avocado Fruit Damaged by *H. lauri* in México

A total of 349 Hass fruit from the 2 commercial orchards located in the Municipality of Coatepec-Harinas were examined for weevil activity. A total of 693 oviposition holes were counted, the number of oviposition holes in fruit ranged 1–8, and 1 ( $n = 146$  fruit) or 2 ( $n = 124$ ) holes per fruit accounted for 77% of observed perforations in fruit (Fig. 1A). The average number of oviposition holes per fruit averaged  $1.99 \pm 0.06$  (SE). The mean number of oviposition holes on fruit sampled from La Palapa and La Barranca significantly varied across the 3 strata (i.e., top, middle, and bottom) ( $F = 92.06$ ;  $df_1 = 2$ ,  $df_2 = 696$ ;  $P < 0.0001$ ). Oviposition hole counts were highest in the middle of the fruit (55%), intermediate in the bottom third (39%), and lowest in the top third of the fruit (6%) (Fig. 1B). Consequently, hole counts were significantly lower in the top third of the fruit compared with the middle (estimate:  $-2.11$ ;  $df_1 = 1$ ,  $df_2 = 696$ ;  $t = -13.49$ ;  $P < 0.0001$ ) and bottom (estimate:  $-1.76$ ;  $df_1 = 1$ ,  $df_2 = 696$ ;  $t = -11.04$ ;  $P < 0.0001$ ) strata of fruit (Fig. 1B). Additionally, oviposition holes were significantly lower in the bottom third of fruit when compared with the numbers of holes recorded in the middle of fruit (estimate:  $-0.34$ ;  $df_1 = 1$ ,  $df_2 = 696$ ;  $t = -4.31$ ;  $P < 0.0001$ ) (Fig. 1B).

From the 121 weevil infested field collected Hass avocado seeds that were returned to IQF from México, 67 adult weevils emerged, 35 females, and 32 males, indicating a slightly female biased sex ratio (0.52). Dissection of seeds 24 (La Barranca) and 36 days (La Palapa) after last adult emergence revealed that 43 larvae (36%), 6 pupae (5%), and 5 adults (4%) (2 males and 3 females) had died inside seeds. Mortality causes were attributable to an unidentified fungal infection and death from unknown factors.

### Oviposition Studies

A total of 260 oviposition holes were excised from Hass fruit removed from weevil colony cages and examined for eggs. A total of 529 eggs were counted, the number of eggs counted in oviposition holes ranged 0–17 (Fig. 2), and for holes containing eggs (i.e., 1–17 eggs in perforations), the mean number of eggs laid was  $4.07 \pm 0.29$  (SE) per hole. Exactly 50% of holes lacked eggs and these may have resulted either from feeding by female weevils (males were not observed chewing holes into fruit [see videography results]) or failure by females to deposit eggs in holes during the 3–4 day exposure period in colony cages. Approximately 15% of holes had 1 egg, 10% had 2 eggs, and 3 or more eggs were found in 25% of perforations (Fig. 2).

### Oviposition Hole Reuse by Female Weevils with Eggs Left in Holes

In the first 3-day exposure round of Hass fruit in weevil colonies, all 6 fruit (3 fruit in each of the La Palapa and La Barranca colony cages) had oviposition holes (range 4–5 holes per fruit) and a total of 27 holes were counted. Of these 27 holes, 15 (i.e., 56%) had eggs for a total of 31 eggs oviposited (range 1–7 eggs per hole). In the second 4-day exposure round where fruit with oviposition holes and eggs were placed in different colony cages, a total of 17 additional eggs (range 1–6) were laid in 40% ( $n = 6$ ) of the 15 pre-existing holes that had eggs. Of the 12 (44%) original holes that lacked eggs in the round 1 exposure experiment, 3 (25%) were subsequently used for

oviposition in round 2 and a total of 4 eggs were laid (range 1–2) in these previously empty holes. In the round 2 exposure experiment, an additional 20 new holes were chewed into fruit of which 6 (30%) had a total of 9 eggs (range 1–3). Of the total 61 eggs counted across 2 exposure rounds, 51% were laid in holes made in the round 1 exposure, 28% were laid in round 2 into pre-existing holes with eggs that were laid by females during round 1; 6% of eggs were laid by round 2 females into empty holes chewed into fruit by females in round 1, and 15% of eggs were laid into new holes made in fruit during the second exposure round.

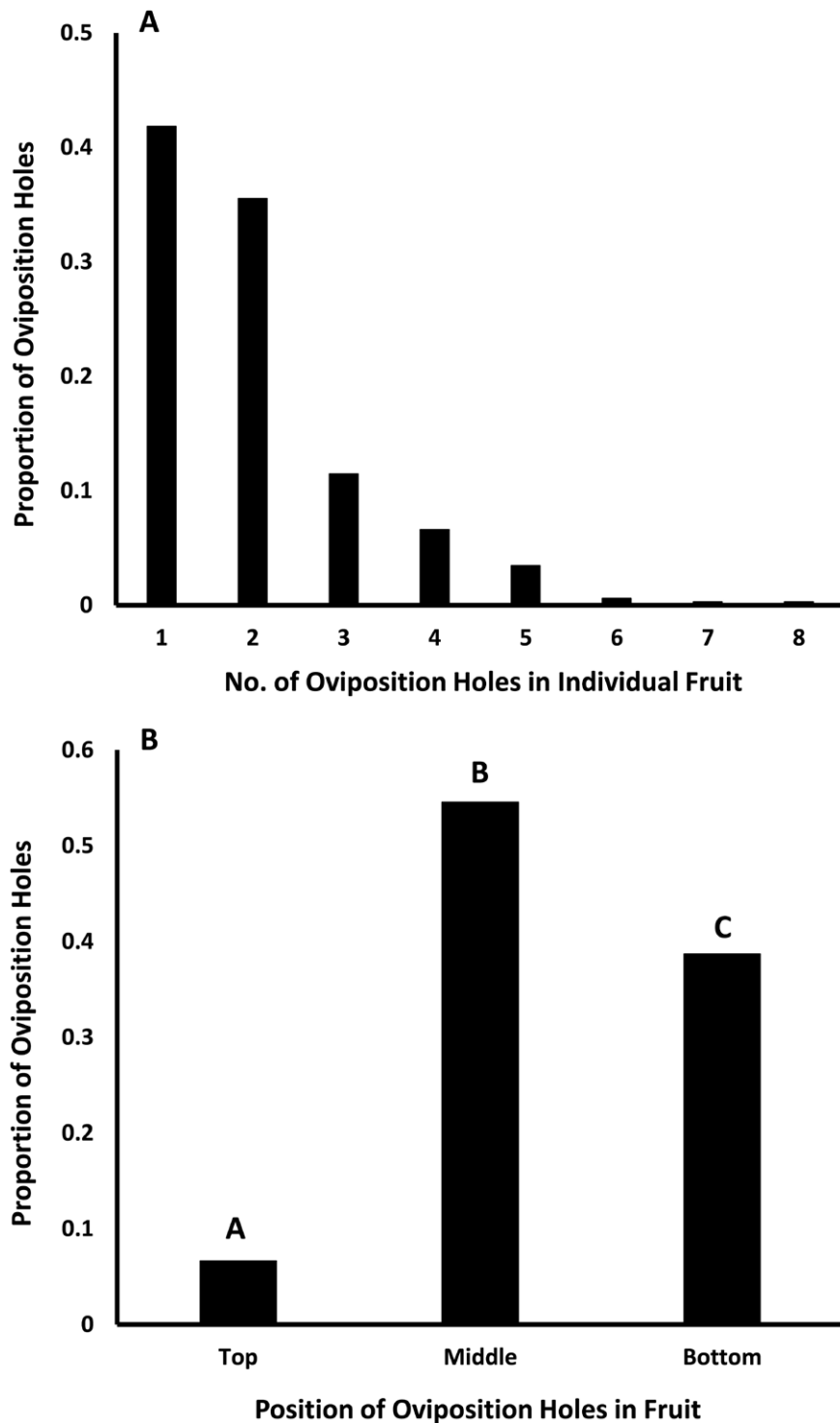
### Oviposition Hole Reuse by Female Weevils with Eggs Removed from Holes

A total of 23 holes were chewed into the original 6 Hass fruit that were placed into the La Palapa ( $n = 3$  fruit) and La Barranca ( $n = 3$ ) colony cages. Of those 23 holes, 19 holes (83%) were used for oviposition and a total of 53 eggs (mean =  $2.79 \pm 0.65$  [SE] per hole [range 1–11 eggs per occupied hole]) were oviposited into holes. Following removal of eggs from oviposition chambers and subsequent re-exposure of fruit to females in the other colony cage, 13 holes (68%) were reused and a total of 48 eggs (mean =  $3.43 \pm 0.76$  [SE] [range 1–9 eggs per occupied hole]) were laid into these previously used oviposition holes. Of the total 23 holes, 7 holes (30%) had more eggs laid in them when compared with round 1. For example, 1 hole originally had 1 egg laid in it, and in round 2, 9 eggs were deposited into this hole. Ten holes (43%) had fewer eggs laid in them in round 2. For example, 1 hole had 11 eggs laid in it in round 1, and in round 2, 3 eggs were oviposited into this hole. Six holes (26%) had no change in the number of eggs laid in them. For example, some holes had zero eggs in round 1 and 0 eggs in round 2; or holes had 1 egg in round 1, and a single egg was oviposited into the same holes in round 2.

### Use of Artificial Holes for Oviposition

A total of 126 eggs were oviposited into artificial holes. The mean number of eggs laid by *H. lauri* in artificial holes made in fruit significantly differed across the 3 strata examined ( $F = 18.40$ ;  $df_1 = 2$ ,  $df_2 = 107$ ;  $P < 0.0001$ ). Egg counts were highest in the bottom third of the fruit (60% of oviposited eggs were laid in the bottom strata [mean number eggs laid in holes =  $2.59 \pm 0.37$  [SE], range = 1–7 eggs per occupied hole]), followed by the middle strata (26% [mean number eggs laid in holes =  $1.57 \pm 0.20$  [SE], range = 1–4 eggs per occupied hole]), and were lowest in the top third (14% [mean number eggs laid in holes =  $1.63 \pm 0.20$  [SE], range = 1–3 eggs per occupied hole]) (Fig. 3). Consequently, egg counts were significantly higher in the bottom third compared with the middle (estimate: 0.82;  $df_1 = 1$ ,  $df_2 = 107$ ;  $t = 3.93$ ;  $P = 0.0004$ ) and top (estimate: 1.43;  $df_1 = 1$ ,  $df_2 = 107$ ;  $t = 5.44$ ;  $P < 0.0001$ ) strata of fruit. Additionally, egg counts were significantly higher in the middle of fruit when compared with the top strata (estimate: 0.61;  $df_1 = 1$ ,  $df_2 = 107$ ;  $t = 2.07$ ;  $P = 0.04$ ) (Fig. 3).

Similarly, the percent of artificially created holes used by *H. lauri* for oviposition within each stratum varied significantly ( $F = 7.53$ ;  $df_1 = 2$ ,  $df_2 = 18$ ;  $P = 0.004$ ). The highest percentage of used holes occurred in the bottom third of the fruit (73% [ $n = 29$  holes] of total available holes [ $n = 40$  total holes available for oviposition]), followed by the middle strata (53% [ $n = 21$ ]), and was lowest in the top third (28% [ $n = 11$ ]). Consequently, percentage of holes used for oviposition was significantly lower in the top third compared with the middle (estimate:  $-1.07$ ;  $df_1 = 1$ ,  $df_2 = 18$ ;  $t = -2.25$ ;  $P = 0.03$ ) and bottom (estimate:  $-1.94$ ;  $df_1 = 1$ ,  $df_2 = 18$ ;  $t = -3.87$ ;  $P = 0.001$ ) thirds, but there was no difference between the percentage of



**Fig. 1.** A) The proportion of the number of oviposition holes per sampled fruit ( $n = 349$ ) made by female *H. lauri* in 2 commercial Hass avocado orchards located in the Municipality of Coatepec-Harinas in the state of México, México, and B) proportion of oviposition holes by strata (i.e., top, middle, and bottom). Different letters indicate significant differences at the 0.05 level.

artificial holes used in bottom and middle strata of fruit (estimate: 0.87;  $df_1 = 1$ ,  $df_2 = 18$ ;  $t = 1.83$ ;  $P = 0.08$ ).

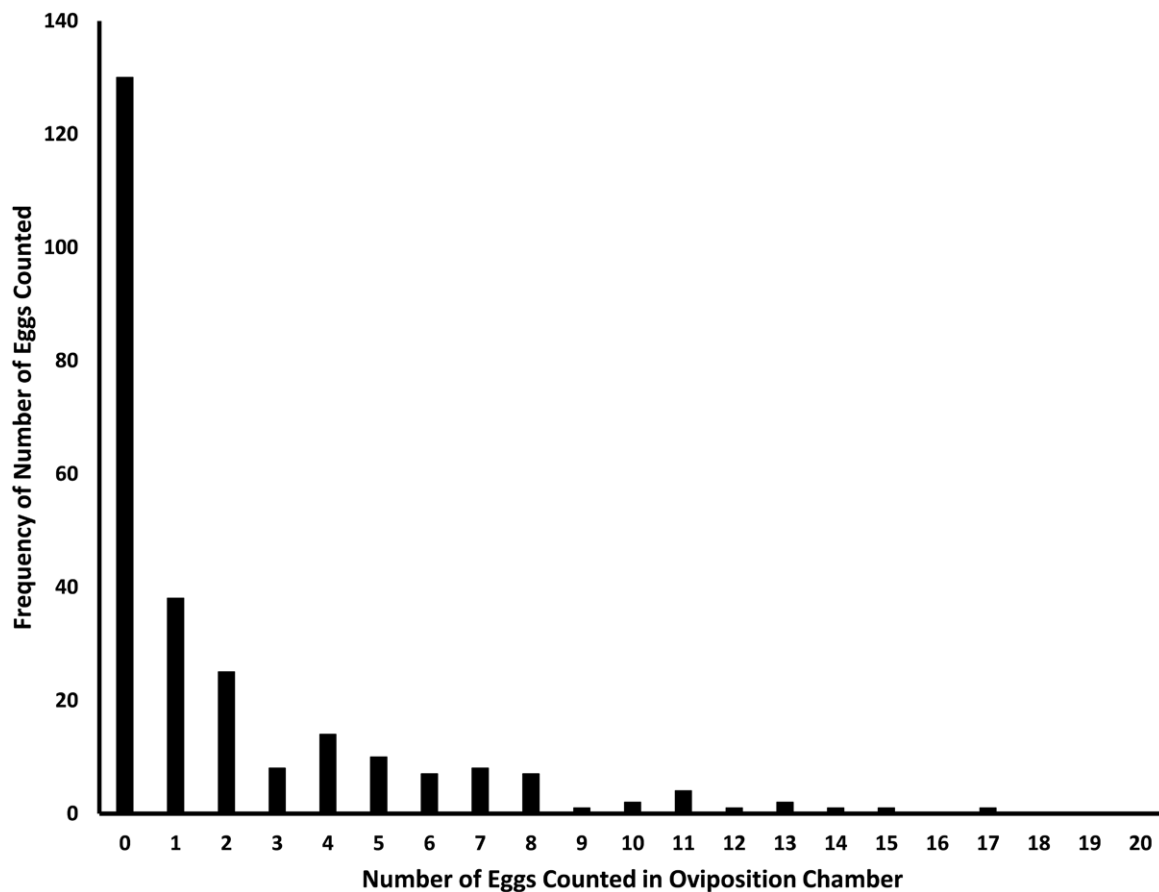
### Comparison of Weevil Preferences for Oviposition and Feeding on Fruit from 5 Different Avocado Cultivars

#### Choice Trial 1 (T1)

The mean number of oviposition holes made by *H. lauri* varied across avocado varieties ( $F = 26.28$ ;  $df_1 = 2$ ,  $df_2 = 150$ ;  $P < 0.0001$ ),

but not by weevil colony source (i.e., La Palapa vs. La Barranca) ( $F = 0.65$ ;  $df_1 = 1$ ,  $df_2 = 150$ ;  $P = 0.43$ ), and there was no significant interaction between avocado variety and weevil colony source ( $F = 1.66$ ;  $df_1 = 2$ ,  $df_2 = 150$ ;  $P = 0.19$ ) (Fig. 4A). Oviposition hole counts were lower on G-22 compared with Hass-T1 (estimate:  $-1.72$ ;  $df_1 = 1$ ,  $df_2 = 150$ ;  $t = -7.06$ ;  $P < 0.0001$ ) and Bacon (estimate:  $-1.51$ ;  $df_1 = 1$ ,  $df_2 = 150$ ;  $t = -5.83$ ;  $P < 0.0001$ ) but did not differ significantly between Hass-T1 and Bacon varieties (estimate: 0.21;  $df_1 = 1$ ,  $df_2 = 150$ ;  $t = 0.99$ ;  $P = 0.58$ ) (Fig. 4A).





**Fig. 2.** Frequency distribution of the number of eggs laid by female *H. lauri* in perforations on Hass avocado fruit exposed to male and female weevils for 3–4 days in colony cages.

The mean number of feeding scars on fruit surfaces caused by *H. lauri* did not significantly vary with avocado variety ( $F = 1.94$ ;  $df_1 = 2$ ,  $df_2 = 150$ ;  $P = 0.15$ ), weevil colony source ( $F = 2.93$ ;  $df_1 = 1$ ,  $df_2 = 150$ ;  $P = 0.09$ ), and there was no significant interaction between avocado variety and weevil colony source ( $F = 1.27$ ;  $df_1 = 2$ ,  $df_2 = 150$ ;  $P = 0.28$ ) (Fig. 4B). The mean number of feeding scars on fruit petioles caused by *H. lauri* differed significantly across avocado varieties ( $F = 5.59$ ;  $df_1 = 2$ ,  $df_2 = 143$ ;  $P = 0.005$ ), but not with weevil colony source ( $F = 2.19$ ;  $df_1 = 1$ ,  $df_2 = 143$ ;  $P = 0.15$ ), and there was no significant interaction between avocado variety and weevil colony source ( $F = 0.62$ ;  $df_1 = 2$ ,  $df_2 = 143$ ;  $P = 0.54$ ) (Fig. 4C). Counts of feeding scars on petioles were higher on G-22 compared to Hass-T1 (estimate: 0.57;  $df_1 = 1$ ,  $df_2 = 143$ ;  $t = 2.97$ ;  $P = 0.01$ ) and Bacon (estimate: 0.53;  $df_1 = 1$ ,  $df_2 = 143$ ;  $t = 2.61$ ;  $P = 0.03$ ) but did not significantly differ between Hass-T1 and Bacon varieties (estimate:  $-0.03$ ;  $df_1 = 1$ ,  $df_2 = 143$ ;  $t = -0.16$ ;  $P = 0.98$ ) (Fig. 4C).

#### Dry Weight Measurements for Choice Trial 1

For choice trial 1, no significant differences ( $F = 0.12$ ;  $df_1 = 2$ ,  $df_2 = 69$ ;  $P = 0.91$ ) were observed between avocado varieties with respect to fruit dry weights indicating that fruit were of comparable maturity levels having similar water and oil content levels at time of harvest and presentation to weevils (Lee et al. 1983) (Fig. 5).

#### Choice Trial 2 (T2)

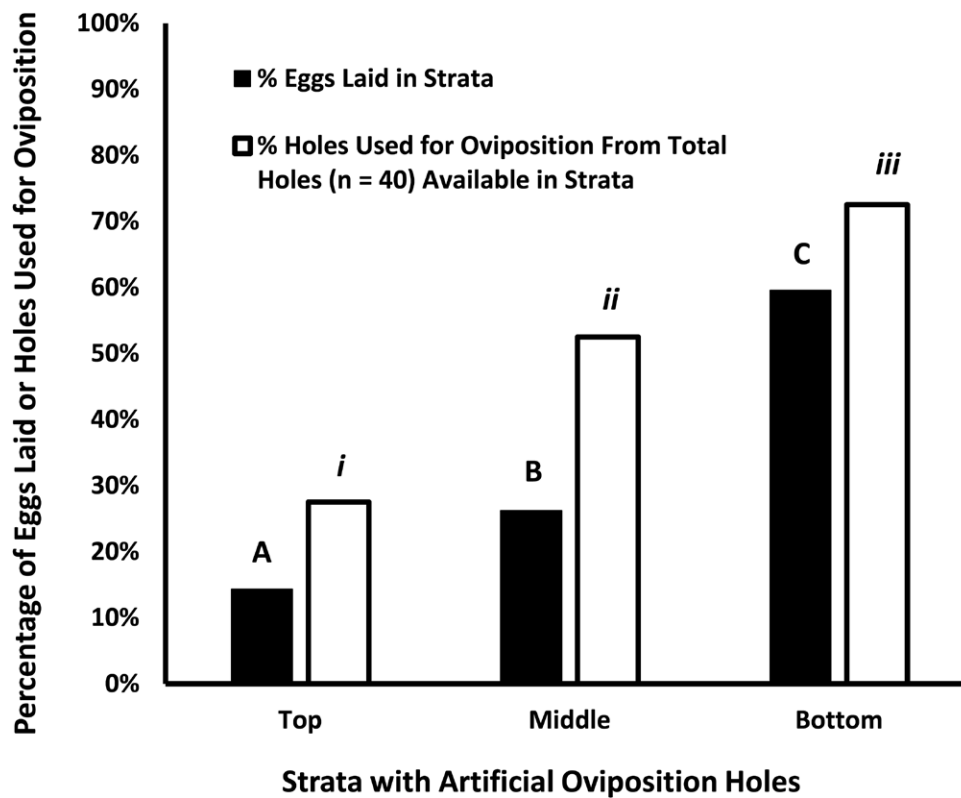
The mean number of oviposition holes made in fruit by *H. lauri* varied significantly across avocado varieties ( $F = 4.42$ ;  $df_1 = 2$ ,

$df_2 = 36$ ;  $P = 0.01$ ). There was no effect of weevil colony (i.e., La Palapa vs. La Barranca) on the numbers of holes made in fruit of different cultivars that were presented to weevils ( $F = 2.54$ ;  $df_1 = 1$ ,  $df_2 = 36$ ;  $P = 0.12$ ) (Fig. 4A). Additionally, there was no significant interaction between avocado variety and weevil colony source ( $F = 0.37$ ;  $df_1 = 2$ ,  $df_2 = 36$ ;  $P = 0.69$ ) (Fig. 4A). Oviposition hole counts were lower on Lamb Hass compared with Hass-T2 (estimate:  $-0.94$ ;  $df_1 = 1$ ,  $df_2 = 36$ ;  $t = -2.89$ ;  $P = 0.01$ ) and hole numbers did not significantly differ between Gem and Lamb Hass (estimate: 0.37;  $df_1 = 1$ ,  $df_2 = 36$ ;  $t = 1.09$ ;  $P = 0.52$ ) or Gem and Hass-T2 varieties (estimate:  $-0.57$ ;  $df_1 = 1$ ,  $df_2 = 36$ ;  $t = -1.86$ ;  $P = 0.17$ ) (Fig. 4B).

The mean number of feeding scars on fruit surfaces caused by *H. lauri* did not vary significantly with avocado variety ( $F = 1.58$ ;  $df_1 = 2$ ,  $df_2 = 36$ ;  $P = 0.22$ ), weevil colony source ( $F = 0.27$ ;  $df_1 = 1$ ,  $df_2 = 36$ ;  $P = 0.61$ ), and there was no significant interaction between avocado variety and weevil colony source ( $F = 1.48$ ;  $df_1 = 2$ ,  $df_2 = 36$ ;  $P = 0.24$ ) (Fig. 4C). Similarly, mean number of feeding scars on fruit petioles caused by *H. lauri* did not significantly vary with avocado variety ( $F = 0.34$ ;  $df_1 = 2$ ,  $df_2 = 36$ ;  $P = 0.71$ ), weevil colony source ( $F = 2.23$ ;  $df_1 = 1$ ,  $df_2 = 36$ ;  $P = 0.14$ ), and there was no significant interaction between avocado variety and weevil colony source ( $F = 1.94$ ;  $df_1 = 2$ ,  $df_2 = 36$ ;  $P = 0.16$ ) (Fig. 4C).

#### Dry Weight Measurements for Choice Trial 2

For choice trial 2, no significant differences ( $F = 0.02$ ;  $df_1 = 2$ ,  $df_2 = 45$ ;  $P = 0.98$ ) were observed between avocado varieties with respect to fruit dry weights indicating that fruit were of comparable



**Fig. 3.** Percentage of eggs oviposited by female *H. lauri* in artificial holes in Hass fruit and percentage of holes used for oviposition in 1 of 3 strata, top, middle, and bottom parts of fruit. Different upper case letters or italicized Roman numerals indicate significant differences at the 0.05 level.

maturity levels having similar water and oil content levels at time of harvest and presentation to weevils (Lee et al. 1983) (Fig. 5).

### Videography of Feeding, Mating, and Oviposition Behaviors

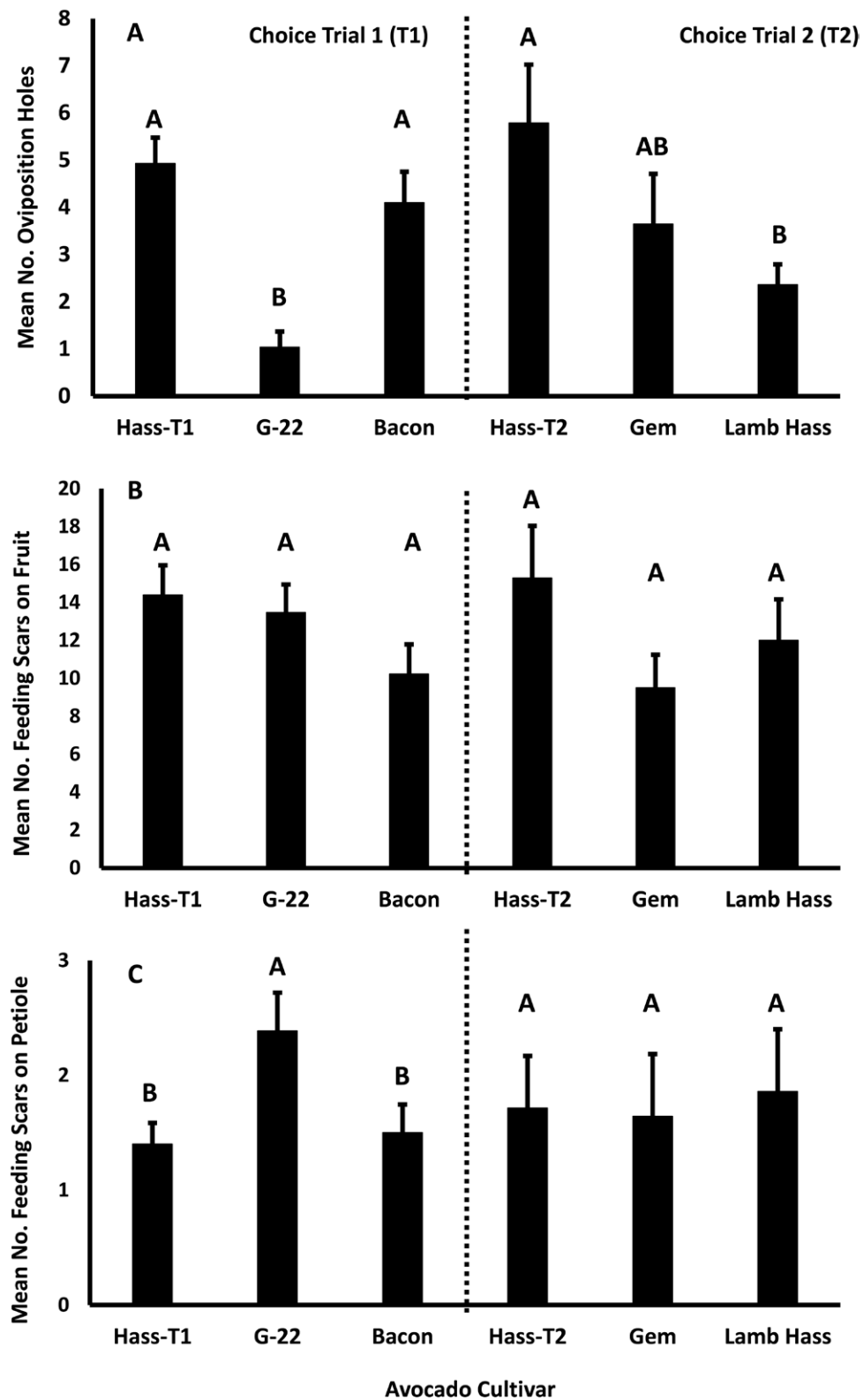
A total of 288 h of digital video of 12 male–female pairs of *H. lauri* interacting with each other and with an exposed 20 cm<sup>2</sup> area of a Hass avocado over a 24-h period was reviewed. Of the 12 females observed in digital video, 83% ( $n = 10$ ) exhibited oviposition hole excavation behavior and a total of 33 holes were drilled into fruit. Of these 33 holes, 31 (94%) were excavated during the day and 2 (6%) were made exclusively at night. Females revisited and re-excavated at night 11 (35%) of the 31 holes that were drilled during the day. The average ( $\pm$  SE) diurnal time (all times are reported as hh:mm:ss [i.e., hr:min:sec]) females took to drill oviposition holes into fruit ranged 0:18:49  $\pm$  0:03:10 when males were not in copulata with females to 0:19:24  $\pm$  0:03:24 when females were drilling holes in copulata (Fig. 6A). Females (58%;  $n = 7$ ) also engaged in nocturnal drilling of oviposition holes. The average ( $\pm$  SE) nocturnal time females took to drill or re-excavate oviposition holes into fruit ranged 0:14:10  $\pm$  0:02:39 when males were absent to 0:18:45  $\pm$  0:05:25 when females were drilling holes in copulata (Fig. 6A). Seven of the 12 females (58%) engaged in both diurnal and nocturnal oviposition hole excavation behavior. Oviposition hole excavation accounted for 12.78% of the diurnal and 6.63% of the nocturnal time budgets, respectively (Fig. 6B).

Of the 33 excavated oviposition holes, 14 (42%) had eggs laid in them and of these occupied holes, 6 (i.e., 43%), held more than 1 egg. A total of 24 eggs were deposited (mean = 1.71  $\pm$  0.35 [SE] eggs per hole; range = 1–6 eggs per hole) over the course of this study.

Of the 14 oviposition events observed, 12 (86%) occurred during the day and 2 (14%) were observed at night. Two different females revisited 1 oviposition hole each (1 diurnal and 1 nocturnal event) and probed them with the ovipositor. When this behavior occurred it was impossible to determine which probing event resulted in oviposition occurring. Oviposition time averaged 0:04:18  $\pm$  0:01:55 (range = 33 s–24 min). Oviposition activities accounted for 1.06% of the diurnal and 0.22% of the nocturnal time budgets, respectively (Fig. 6B).

Five females (42%), engaged in behavior that packed masticated fruit epidermis into the oviposition hole. Epidermis was grazed from the fruit surface immediately around the oviposition hole leaving a “bronze” colored “halo” around the perforation. A total of 9 holes (27% of the 33 holes females made in fruit) were closed over with masticated epidermis. All closed over holes had eggs (total eggs laid into closed over holes = 18; mean = 2.0  $\pm$  0.53 [SE]; range = 1–6 eggs). Of observed hole closing activities, 82% occurred during the day, and 2 females engaged in nocturnal hole closing behavior. One female returned to work on 1 hole that was excavated and packed during the day, and 1 female packed 1 hole that was exclusively excavated at night. Females packed holes either in copulata or without dorsally attached males. Average hole packing times, including mastication of epidermis, averaged 0:04:39  $\pm$  0:00:10 [SE] (range 42 s–26:44 min) (Fig. 6A). Hole packing behaviors accounted for 0.92% of the diurnal and 0.20% of the nocturnal time budgets, respectively (Fig. 6B).

During the day, resting behavior accounted for 33.84% of female time, whereas at night, resting activity accounted for 74.96% of observed behaviors (Fig. 6B). The most common diurnal behavior female weevils engaged in was walking, either solo or in copulata, which accounted for 38.96% of the time budget (Fig. 6B). Overall,

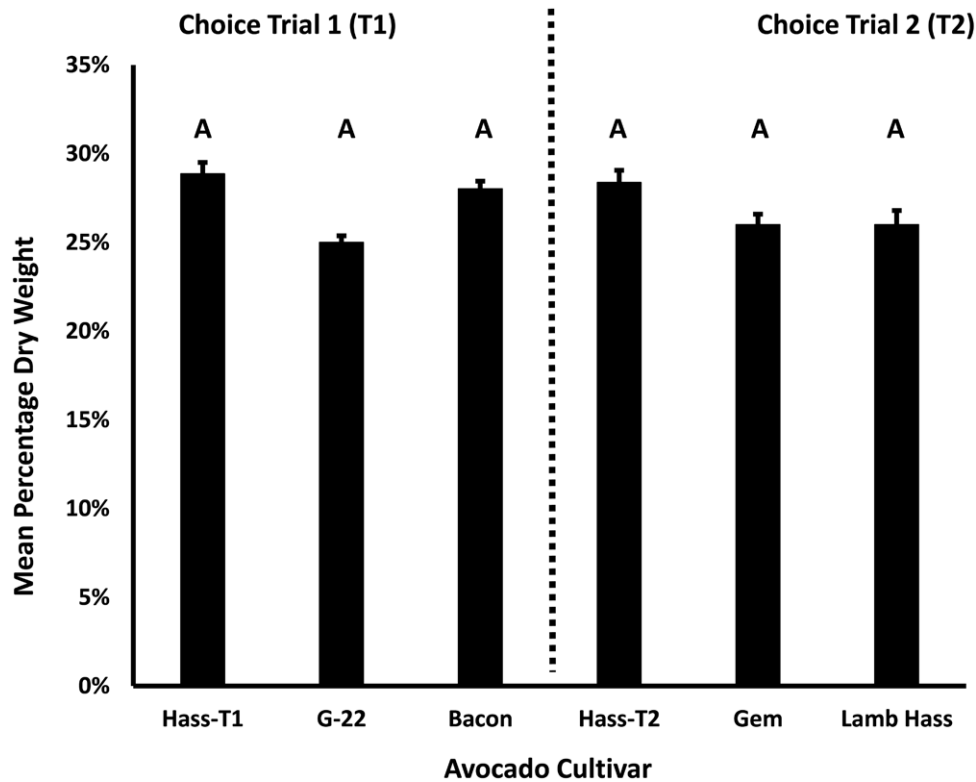


**Fig. 4.** Mean number ( $\pm$  SE) of oviposition holes, and feeding scars on avocado fruit and petioles made by adult female and male *H. lauri* in 2 different fruit choice trials, T1 and T2. Columns with the same letters are not statistically significant at the 0.05 level.

the majority of observed nonresting activities performed by female weevils were diurnal.

Male weevils engaged in diurnal and nocturnal copulatory behavior with females, whereas females engaged in other activities

with males attached to them (i.e., in copulation) (Fig. 6C-D). For example, diurnal copulation by males with females excavating oviposition holes averaged  $0:18:47 \pm 0:03:11$  min (Fig. 6C) and accounted for 5.76% of the male time budget (Fig. 6D). At night,



**Fig. 5.** Mean percentage dry weight measures for 5 different avocados used in cultivar choice experiments with *H. lauri*. Columns with the same letters are not statistically significant at the 0.05 level.

males would rest in copulata with females on fruit for an average of  $0:19:34 \pm 0:04:09$  min (Fig. 6C) and this behavior made up 3.54% of the male time budget (Fig. 6D). Male copulatory activity with females accounted for 35.29% and 14.01% of the diurnal and nocturnal time budgets, respectively (Fig. 6D). Walking activities accounted for 31.44% and 17.04% of male diurnal and nocturnal time budgets, respectively (Fig. 6D). Resting activities accounted for 47.97% and 75.68% of male diurnal and nocturnal time budgets, respectively (Fig. 6D). These behavioral observations indicate that the majority of observed nonresting activities for male *H. lauri* were diurnal. Importantly, males were never observed chewing holes into fruit.

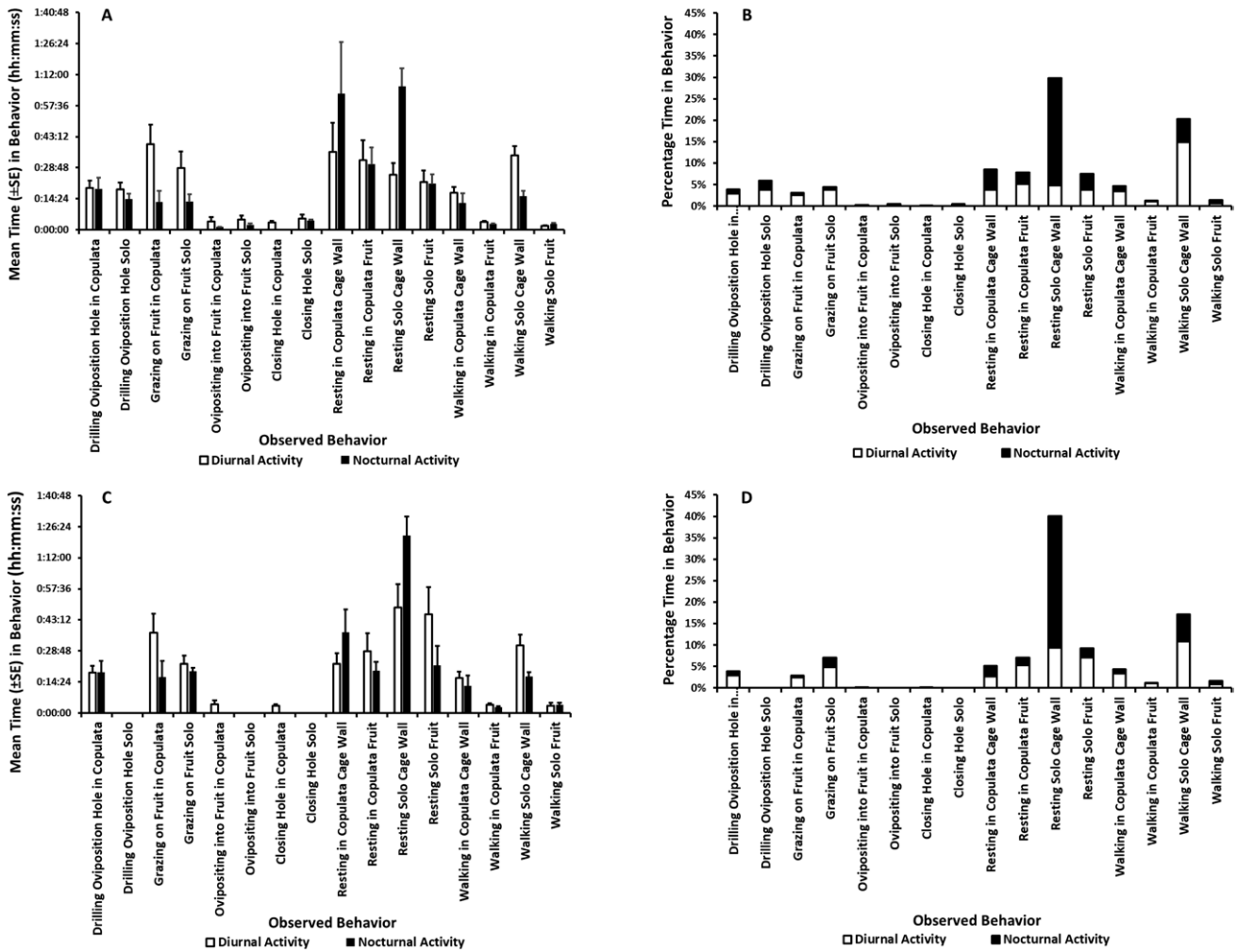
## Discussion

In 2 commercial Hass avocado orchards in the Municipality of Coatepec-Harinas in México, 38% of hanging fruit exhibited oviposition damage by *H. lauri*. Oviposition holes made by female weevils in orchards averaged 2 holes per fruit (1 hole = 42% of damaged fruit; 2 holes = 36%; range 1–8 holes per fruit). These observations are similar to findings reported by Ortega-Licon et al. (2019) for Hass fruit collected from backyards in San Juan, Acaxochitlán, Hidalgo México, where the number of perforations made by *H. lauri* averaged 2 holes per fruit (range 1–4 holes; 30% of hanging fruit were damaged by weevils).

Placement of oviposition holes in fruit hanging on trees was significantly biased toward the middle and bottom sections of fruit. Hole placement in these areas may benefit from gravity-assisted clearance of frass and drainage of perseitol residues (a white residue that sometimes forms as a response to mechanical damage), which may not easily occur when holes are located near the top of

the fruit, especially above fruit shoulders. A similar finding with respect to placement of entrances to feeding tunnels was observed for *S. catenifer* larvae, a specialist avocado seed feeding moth, infesting Hass avocados in Guatemala (Hoddle and Hoddle 2008). A comparable finding was also observed with the artificial hole experiments conducted in the laboratory. Substantially more eggs were laid in artificial holes in the middle and bottom strata of Hass fruit when compared with the top strata. However, in this instance, significantly more eggs were oviposited into holes located in the bottom strata. This may have been an artifact of the experimental set up. Fruit with artificial holes were set vertically on top of white plastic vial lids (25-mm diameter; 10-mm deep) that were placed on the cage floor. When compared with fruit hanging in trees, females ovipositing into artificial holes had relatively easy access to perforations on the bottom of fruit that they could reach directly from the cage floor. This probable ease of access to fruit from colony cage floors may have resulted in unnaturally high levels of oviposition in this stratum. Use of artificial holes for oviposition in the laboratory suggests that mechanical damage (e.g., punctures made by twigs or other insects) to fruit in the field maybe opportunistically exploited for egg laying when encountered by *H. lauri* females.

Four different oviposition studies were conducted in the laboratory to determine if females would deposit more than 1 egg per oviposition perforation. These 4 experiments included the following: (i) counts of eggs in holes made in fruit placed in colony cages for colony maintenance, (ii) oviposition into existing oviposition holes containing eggs from females from different colonies, (iii) use of artificial holes for oviposition, and (iv) videography studies that documented individual male–female behaviors over a 24-h period. Data from these 4 experiments collectively suggest that oviposition holes within which eggs are laid often hold more than 1 egg



**Fig. 6.** Average time in behavior A) and percent time budget B) for female *H. lauri* spent in diurnal and nocturnal activities. Average time in behavior C) and percent time budget D) for male *H. lauri* spent in diurnal and nocturnal activities.

and multiple eggs per oviposition hole are either laid by the same female or by conspecifics that oviposit into pre-existing holes that are holding eggs. Removal of eggs from oviposition chambers and subsequent exposure to females from different colonies also resulted in multiple eggs being laid into chambers that previously contained eggs oviposited by conspecifics.

It should be noted that oviposition holes with more than 1 egg may be the result of unnaturally high densities of female weevils in colony cages and relatively few fruit per female available for oviposition. However, female to fruit ratios may not fully account for the presence of multiple eggs in oviposition holes. In videography studies of single male–female pairs over a 24-h period, females often laid more than 1 egg per oviposition perforation as 43% of holes with eggs had more than 1 egg oviposited into them. This finding suggests that high numbers of females with relatively few fruit to oviposit in may not fully account for multiple eggs being observed in oviposition chambers.

The frequency with which multiple eggs per oviposition chamber occurs strongly suggests that *H. lauri* (Subfamily Molytinae: Tribe Hylobiini) does not use an oviposition deterring pheromone. Several species of weevil (e.g., pepper weevil, *Anthonomus eugenii* Cano, boll weevil, *Anthonomus grandis* Boheman [both Curculioninae: Anthonomini], and cabbage seed weevil, *Ceutorhynchus assimilis*

[Paykull] [Ceutorhynchinae: Ceutorhynchini]) use these types of semiochemicals to mark oviposition substrates. Such chemical signals have been demonstrated to prevent the original female that laid down the mark from visiting the same site again. These marks also deter conspecific females from ovipositing into previously exploited resources. Oviposition deterring pheromones, often applied by females to discrete resources that support complete larval development, reduce offspring competition and increase female fitness (Stansly and Cate 1984, Fergusson et al. 1999, Adesso et al. 2007, 2021).

With respect to *A. eugenii* and *A. grandis*, female weevils seal oviposition holes with an “oviposition plug.” These plugs, comprised of an anal secretion and female frass, have strong female-specific deterrent effects on subsequent oviposition activities by conspecific females (Stansly and Cate 1984, Adesso et al. 2007). Videography of *H. lauri* indicated that 42% of females packed masticated fruit epidermis into oviposition holes and all “plugged” holes ( $n = 9$ ) contained eggs (range 1–6 eggs per hole). It is unknown if masticated epidermis, presumably mixed with female saliva, functions as an oviposition deterring “plug” toward conspecific females. In comparison to a cotton boll (*A. grandis*) or pepper flower (*A. eugenii*) into which female weevils oviposit, avocado fruit are large and the expansive surface area over which an oviposition deterrent would

need to act may argue against the use of masticated oviposition plugs by *H. lauri* females as an oviposition deterrent. Masticated plugs may simply provide protection for weevil eggs from desiccation or natural enemies, or plugs prevent conspecific females from opportunistically ovipositing into open oviposition chambers containing eggs. More broadly, there appear to be no reports in the published literature documenting the use of oviposition deterring pheromones by *Heilipus* spp. Lack of use of an oviposition deterring pheromone, may explain, in part, why multiple eggs are frequently found inside oviposition chambers.

Damaged Hass fruit surveyed in backyards in Hidalgo México averaged 2 oviposition perforations per fruit, which increases the likelihood of more than 1 egg being laid in fruit (Ortega-Licona et al. 2019). Results from laboratory studies conducted here (see above) supports speculation by Ortega-Licona et al. (2019) that field surveyed fruit in Hidalgo México likely contained more than 1 egg and the finding that just 1 larva dominates an avocado seed is suggestive that cannibalism among larvae occurs (Castañeda-Vildózola et al. 2013a, Ortega-Licona et al. 2019). With respect to this study, rearing in quarantine of weevil infested avocado seeds field collected from 2 commercial Hass avocado orchards in Mexico also resulted in the emergence of just 1 adult weevil per seed despite fruit often exhibiting more than 1 oviposition hole at time of seed extraction.

Regardless of findings from laboratory studies, field surveys are needed to determine if *H. lauri* females lay more than 1 egg per oviposition perforation and that multiple holes in fruit contain at least 1 egg. If multiple eggs per oviposition perforation is confirmed in the field, laboratory studies should be conducted to verify whether or not *H. lauri* larvae are cannibalistic, and which larval instars are cannibalistic (*H. lauri* larvae pass through 4 larval instars [Castañeda-Vildózola et al. 2016]). Cannibalism could occur upon larval emergence from eggs and larval or egg consumption may happen within the oviposition chamber, or cannibalism may result when larvae encounter conspecifics moving through fruit pulp toward the seed, or between larvae competing for resources within the seed.

In the field, ovipositing female *H. lauri* exhibit preferences for Hass fruit that are > 4.0 cm wide and > 5.0 cm long, and attack rates, up to 70%, are greater on non-Hass fruit of undetermined parentage, when compared with Hass (30%) (Ortega-Licona et al. 2019). In 2 laboratory choice trials, male and female *H. lauri* in colony cages were given access to fruit from 5 different cultivars; Hass, Bacon, Gem, Lamb Hass, and G22. In these choice trials, Hass fruit, the globally dominant commercial variety, acted as positive controls against which weevil responses to other fruit types were compared. Female weevils exhibited preferences for specific cultivars with respect to drilling holes into fruit (in videography studies males were not observed drilling holes into fruit). In choice trial 1, the number of holes drilled into Hass and Bacon (a commercial variety) were equivalent, and on average, greater than the number of holes made in G22, a noncommercial variety native to Guatemala. In choice trial 2, weevils made fewer holes in Lamb Hass fruit when compared with Hass, and hole numbers in Lamb Hass were similar to those observed in Gem. In comparison to Hass, these results suggest that certain commercial fruit varieties, especially Lamb Hass, and possibly to a lesser extent Gem, both of which are emerging new commercial varieties in terms of increasing production levels, may exhibit some level of antixenosis towards *H. lauri*. This possibility needs assessing under field conditions as it could be an important consideration for breeding new varieties and this may be a previously unrecognized management strategy for mitigating weevil damage to commercially produced fruit.

Videography studies indicated that adult *H. lauri* males and females are predominantly active during “daylight” hours in the laboratory. This finding supports field observations by Castañeda-Vildózola et al. (2013b). Given the range of diurnal behaviors (e.g., oviposition, walking, copulation) exhibited by male and female *H. lauri*, these activities on fruit, for example, make it more likely that adult weevils in the field will be visible and movement can aid monitoring surveys which are needed to support decision making for control programs targeting adults. Despite their diurnal activity, and in comparison to fruit, *H. lauri* adults can be difficult to detect on avocado foliage, as they are well camouflaged, and detection of movement can result in adults “freezing” or dropping from foliage. These defensive behaviors enable evasion of detection which increases the difficulty of visually observing this pest. However, visual detection of adult weevils in trees in commercial Hass orchards in Huatusco Veracruz México is used to inform control decisions. Visual searches of variable time (~1–2 min) of a visually estimated 1 × 1 m area of foliage in 1–2 randomly areas selected around the canopy of a tree of interest are employed to estimate weevil densities. The number of trees surveyed is variable and dependent on time of year (i.e., fruit presence) and intensity of pest pressure. The threshold for initiating spray applications is the cumulative visual detection of ≥ 1 weevil per sampled tree over a consecutive 3 day period (Hoddle, pers. obs. 2022).

Given the difficulty of accurately estimating adult weevil densities visually, a more effective monitoring tool is needed. Several sex-specific compounds have been identified from male *H. lauri*, including grandisol, papayanol, papayanal, and grandisal, suggesting that 1 or more of these compounds may be an attractant pheromone (Romero-Frías et al. 2019). However, this possibility remains to be confirmed by laboratory or field bioassays. Field testing of commercially available racemic grandisol, as a single component or in combination with possible minor components identified by Romero-Frías et al. (2019) at several field sites in México failed to result in weevil captures (Hoddle, Equihua-Martínez, and Millar, unpublished data). One possibility is that *H. lauri* may only be attracted to the male-produced stereoisomer, and not to the racemate. Should the pure grandisol stereoisomer become available, it needs to be field tested to determine attractiveness to *H. lauri* adults.

## Conclusion

*Heilipus lauri* is a well-recognized avocado pest in its native (i.e., México) and invaded (i.e., Colombia) ranges. Despite the pestiferousness of *H. lauri*, relatively little is known about basic aspects of its biology and behavior, especially under field situations. Surveys of damaged Hass fruit in commercial avocado orchards in México confirmed that fruit exhibited ~2 holes on average and 58% of weevil attacked fruit exhibited ≥ 2 holes. The position of oviposition holes were predominantly located in central (55% of holes) and lower strata (39%) of fruit. In weevil colonies maintained in the laboratory, 50%, 15%, and 35% of oviposition perforations in Hass fruit had 0, 1, and ≥ 2 eggs, respectively. Female weevils oviposit into holes that contain eggs of conspecifics and they also utilize oviposition perforations made by conspecific females, which originally lacked eggs or had eggs manually removed. These findings suggest that *H. lauri* does not use an oviposition deterrent pheromone to deter conspecifics from ovipositing into previously utilized resources. Opportunistic oviposition by female *H. lauri* extends to exploitation of artificial holes made in fruit. Oviposition preference trials indicated that female weevils have predilections for certain commercially grown cultivars, with Hass being preferred over Lamb Hass.

This finding may have important applications for breeding of new avocado varieties. Videography studies confirmed that *H. lauri* activity is predominantly diurnal which has important implications for monitoring and control programs.

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## Author contributions

Mark Hoddle (Conceptualization [lead], Data curation [lead], Funding acquisition [lead], Investigation [lead], Methodology [lead], Project administration [LEAD], Writing—original draft [lead], Writing—review & editing [lead]), Christina Hoddle (Data curation [supporting], Investigation [supporting], Writing—review & editing [supporting]), Ivan Milosavljevic (Formal analysis [lead], Writing—original draft [supporting], Writing—review & editing [supporting]), Edith Estrada-Venegas (Conceptualization [supporting], Investigation [equal], Resources [lead], Writing—review & editing [supporting]), and Armando Equihua Martinez (Conceptualization [equal], Investigation [equal], Project administration [equal], Writing—review & editing [supporting]).

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