# Biology, Chemical Ecology, and Sexual Dimorphism of the Weevil Myllocerus undecimpustulatus undatus (Coleoptera: Curculionidae)

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# Biology, chemical ecology, and sexual dimorphism of the weevil *Myllocerus undecimpustulatus undatus* (Coleoptera: Curculionidae)

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**Abstract**

*Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae), also known as the Sri Lankan weevil, is becoming a major pest of ornamentals and tropical fruit trees in the southern USA, including Florida. Recent findings of this species in Florida citrus groves justify research into its biology and ecology. We studied morphological and sexually dimorphic characters of this species as an aid to rapid separation of sexes for studies aiming to identify semiochemicals that may be of value in management. Female weevils collected from the field in southeastern Florida were significantly larger than males in length of head, abdomen, and overall length. Females, but not males, have a characteristic black-gray marking extending from the ventral mesosternum to the second abdominal segment. Scanning electron microscopic images revealed that females had fewer ovate-to-obovate scales in this region of characteristic black-gray marking, and more plumose scales compared with males. Host choice and oviposition studies showed that Sri Lankan weevils prefer peach plants over weeds or grasses in peach orchards. Electroantennogram recordings with different peach volatiles identified peach odorants that can elicit significantly higher antennal responses. Both male and female Sri Lankan weevil antennae were highly responsive to these volatiles. Behavioral assays in olfactometers are underway to identify those semiochemicals that could be used as attractants or disruptants.

Key Words: sexual dimorphism; chemical ecology; Sri Lankan weevil; electroantennogram; peach

**Resumen**

*Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae), también conocido como el gorgojo (picudo) de Sri Lanka, se está convirtiendo en una plaga importante de ornamentales y árboles frutales tropicales en el sur de los EE. UU., incluyendo la Florida. Los hallazgos recientes de esta especie en los huertos de cítricos de Florida justifican la investigación sobre su biología y ecología. Estudiamos los caracteres morfológicos y sexualmente dimórficos de esta especie como una ayuda para la rápida separación de sexos en estudios que buscan identificar semioquímicos que puedan ser valiosos en el manejo. Los gorgojos recolectados en el campo en el sureste de Florida fueron significativamente más grandes que los machos en cuanto de la longitud de la cabeza, abdomen y cuerpo total. Las hembras, pero no los machos, tienen una marca característica de color negro grisáceo que se extiende desde el mesoesterno ventral hasta el segundo segmento abdominal. Las imágenes de microscopía electrónica de barrido revelaron que las hembras tenían menos escamas de forma ovada a obovada en esta región de esta marca negra gris característica, y más escamas plumosas en comparación con las de los machos. La selección del hospedero y los estudios de oviposición mostraron que los gorgojos de Sri Lanka prefieren las plantas de durazno (melocotón) sobre las malezas o los pastos en los huertos de durazno. Las grabaciones de los electroantenogramas con diferentes sustancias volátiles del durazno identificaron los odorantes del durazno que pueden provocar respuestas antenales significativamente más altas. Las antenas de gorgojo de Sri Lanka, tanto en los machos como en las hembras, fueran altamente sensibles a estos volátiles. Se están realizando ensayos de comportamiento en olfatómetros para identificar aquellos semioquímicos que podrían usarse como atrayentes o perturbadores.

Palabras Clave: dimorfismo sexual; ecología química; gorgojo de Sri Lanka; electroantenograma; melocotón; durazno

Citrus greening is devastating the citrus production industry in Florida. Peach (*Prunus persica*) (L.) Batsch (Rosaceae) is a specialty crop that has potential to occupy a portion of abandoned citrus acreage in Florida (Morgan & Olmstead 2013). According to the most recent USDA estimate, the number of harvested hectares for peaches increased from 1,231 in 2012 to 3,000 in 2014 in Florida (USDA-NASS 2016; Olmstead & Morgan 2013). It is estimated that annual production in Florida is approximately 4.5 million pounds, valued at US $6 million (Morgan & Olmstead 2013). Florida is the first state to produce peaches throughout the year due to favorable weather conditions. The advantage of growing peaches in Florida resides in the fact that growers can reap a premium price during the early market window, when supplies are tight, until peaches from other states enter the market. California produces approximately 75% of the nation's peach crop (713,000 tons), followed by South Carolina (95,000 tons) and Georgia (36,000 tons). These peach industries and those of other fruit crops, nurseries, and vegetables throughout the southern US are threatened by the invasive potential of Sri Lankan weevil spreading to these states. The possible

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impact to the horticulture industry in fruit crops, nurseries, landscape services, and horticultural retailers could reach billions of dollars based on the value they generate (Khachatryan & Hodges 2012).

Sri Lankan weevil is an invasive, polyphagous weevil in Florida, with the potential to spread throughout the southern US. This pest is a native of Sri Lanka and is referred to as the Sri Lankan weevil, and also is known as the Asian grey weevil in its native range. Many subspecies of *Myllocerus undecimpustulatus* Faust (Coleoptera: Curculionidae) are considered serious pests of > 20 crops in India and Pakistan (O'Brien et al. 2006). Sri Lankan weevil was first identified in the US on *Citrus* sp. (Rutaceae) in Pompano Beach, Broward County, Florida, USA, in 2000. Sri Lankan weevil now is known to occur in 27 counties (Neal 2013) from the southern tip of the peninsula to Nassau County on the border with Georgia.

Adult Sri Lankan weevils feed on leaves, feeding inward from plant margins, causing the typical leaf notching produced by many broad-nosed weevils. Adults feed on flush and mature leaves, and eggs are laid in soil rich in organic matter. Eggs hatch in 3 to 5 d, and larvae feed on roots of plants and later pupate in soil. Under laboratory conditions, the lifecycle takes < 2 mo (Neal 2013). The adult stage attacks > 103 plant species, including native ornamentals, fruit crops, tropical fruit trees, and palms (Thomas 2005). It is the principal insect pest limiting progress in breeding peach varieties adapted to Florida's subtropical conditions, and is becoming a major pest of other stone fruits and tropical fruit trees, such as avocados, litchi, and longan, ornamentals, and palms (Neal 2013). Leaf area consumed by adult Sri Lankan weevil varies significantly between host plants, with particular preference shown for peach and avocado, and less for litchi, cocoplum, and citrus (S. Shareef, private communication). The presence of high Sri Lankan weevil populations in Florida, and the potential spread to other southern US states, led growers to request research to find appropriate and sustainable methods of control. Our current state of knowledge about the biology and ecology of this species and methods for control is inadequate for providing satisfactory pest management technologies to growers, nurseries, and landscape managers. We studied the biology, chemical ecology, and sexual dimorphism of the weevil to develop environmentally stable control strategies. Host choice, oviposition behavior, and larval development were studied on different host plants. Electroantennogram recordings were performed on Sri Lankan weevil antennae with different peach volatile odorants in order to identify semiochemicals that could be used to develop a lure for trapping adult weevils.

## Materials and Methods

### SRI LANKAN WEEVIL COLLECTION AND MAINTENANCE

Adult weevils were collected from a peach (*P. persica*) orchard in Vero Beach, Indian River County, Florida, USA. Weevils were collected by hand from peach trees into ventilated jars. The weevil adults were kept in BugDorm™ cages (BD-6630, Bioquip Inc., Rancho Dominguez, California, USA) provisioned with cocoplum leaves (*Chrysobalanus icaco* L.; Chrysobalanaceae) in a climate-controlled greenhouse at a mean temperature of 25 °C and a 12:12 h (L:D) photoperiod.

### SEXUAL DIMORPHISM OF THE WEEVIL

Identifying the sexually dimorphic characteristics is very important in separating the male and female weevils for behavioral and electrophysiological studies. Length and breadth measurements of adult weevils were done with CellSense Dimension software (Olympus Corporation, Tokyo, Japan). Adult male and female weevils were measured from the center of the ecdysial line on the head to the tip of the abdomen. Separate measurements were done for length of the head, thorax, abdomen, and the length and width of the last 4 abdominal sternites (George et al. 2015).

### OVIPOSITION PREFERENCE AND LARVAL DEVELOPMENT OF SRI LANKAN WEEVIL ON DIFFERENT HOST PLANTS

Choice assays were performed to study oviposition preference and larval development of Sri Lankan weevil on host plants. Three host plants including peach (*P. persica*; Rosaceae), Bahia grass (*Paspalum notatum* Flüggé; Poaceae), and cupid's shaving brush (*Emilia sonchifolia* (L.) DC; Asteraceae) were studied based on their abundance and distribution in peach orchards. Plants were maintained in 10 L (3 gallon) pots. Choice assays were performed inside a BugDorm™ cage (BD-6630, Bioquip Inc., Rancho Dominguez, California, USA) (60 × 60 × 180 cm) with the 3 different host plants. Plants were in non-flowering phase, except cupid's shaving brush. Fifty pairs of Sri Lankan weevils were introduced into the cage (50 males + 50 females). Weevils were allowed to feed and oviposit on the plants for 8 wk. Adult weevils were removed, and the larval distribution in the root zone was measured. The soil in each treatment pot was separated into 2 equal halves (top and bottom 5 inches). Sri Lankan weevil adult oviposition choice, oviposition depth, and larval development in the soil were measured.

### ELECTROANTENNOGRAM RECORDINGS WITH PEACH VOLATILES

Volatile collections were performed on young and mature peach leaves with solid-phase microextraction fibers (polydimethylsiloxane/divinylbenzene) and analyzed by gas chromotogaphy-mass spectrometer analysis. Compounds were identified by comparing spectral data with those from commercially available standards and spectra from mass spectrometer libraries, and confirmed by retention times of authentic standards. Identified odorants were used in electroantennogram recordings ($n$ = 6) as described by Lapointe et al. (2012). Headspace volatiles of synthetic odorant were puffed into a filtered and humidified airflow upstream to the male and female antenna. Benzaldehyde was puffed at the end of each run as a positive control. Responses were adjusted by subtracting responses to clean air during the corresponding run from odorant responses. Statistical analysis was performed with JMP ver. 10 (SAS Institute 2019). One-way ANOVA was performed to analyze electroantennogram responses ($\alpha$ = 0.05) followed by Tukey's HSD for comparison of means.

## Results and Discussion

### SEXUAL DIMORPHISM OF THE WEEVIL

Adult female weevils were significantly longer than males ($F_{1, 19}$ = 38.5; $P$ < 0.0001) (Fig. 1A). The mean weight of females (35.5 mg) was significantly greater than that of males (18.2 mg) ($F_{1, 19}$ = 73.46; $P$ < 0.0001). The lengths of the head and abdomen of female weevils were greater than those of males, but there was no difference in the length of the thorax ($P$ = 0.008, < 0.0001, and 0.14, respectively) (George et al. 2015). Metathoracic inter-coxal distance was greater for females (1.5 mm) compared with males (1.3 mm) ($F_{1, 19}$ = 22.16; $P$ < 0.0001) (George et al. 2015). Female weevils have more pronounced black-gray markingd (Fig. 1B) compared to males (Fig. 1C). The distance between the metathoracic coxa was significantly greater for females, and the black-gray marking extended from the sternum of the mesothoracic

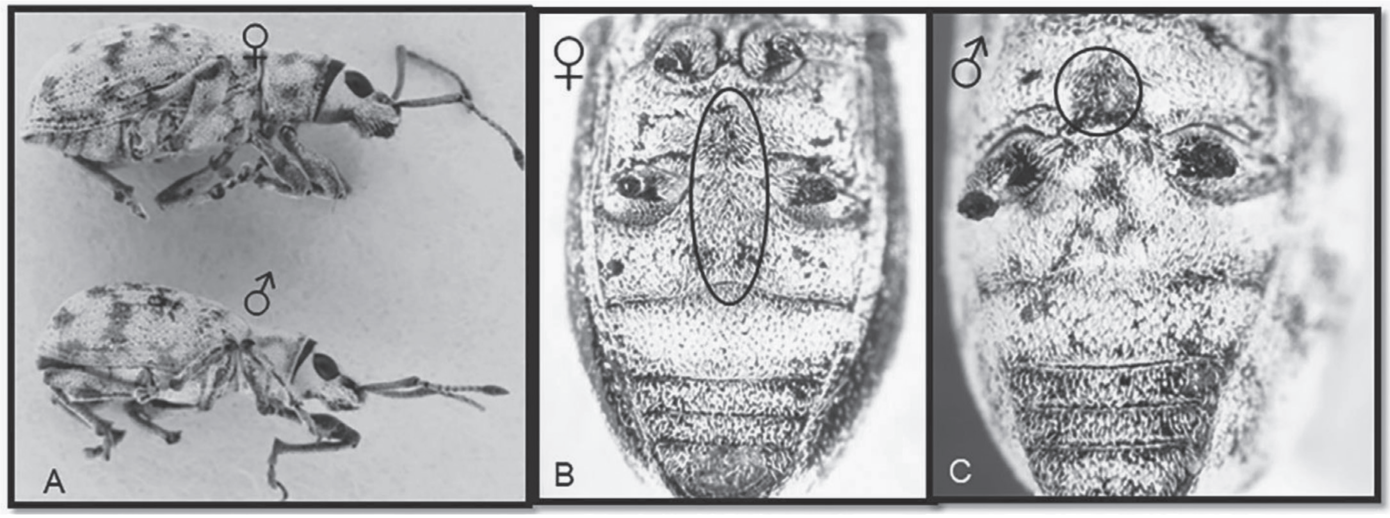


**Fig. 1.** (A) Lateral view showing the difference in size of female and male Sri Lankan weevils. Dimorphism appears as black-gray markings on the ventral mesosternum of female (B) and male weevils (C).

segment to the second abdominal segment. The males had a small black-gray marking restricted to the anterior mesosternal area (Fig. 1C). Males had more white accumulation of (presumably) cuticular hydrocarbons present on their body surface compared with females. The presence of these hydrocarbons gives the males a more whitish appearance compared with females. The dark markings of females were characterized by a dense investiture of plumose and elongate scales and no oval scales. Scanning electron microscopic images revealed that females had fewer ovate-to-obovate scales in this region of characteristic black-gray marking, and more plumose scales compared with males (George et al. 2015). These black-gray markings on female abdominal sternum are visible to the unaided eye, and can be used as a sexually dimorphic character to distinguish males from females.

## OVIPOSITION PREFERENCE AND LARVAL DEVELOPMENT OF SRI LANKAN WEEVIL ON DIFFERENT HOST PLANTS

Oviposition preference and larval development of Sri Lankan weevil were observed only in pots containing a peach plant (47.5 ± 8.2 larvae). No Sri Lankan weevil larvae were found in pots of Bahia grass and Aster weed (Asteraceae). No significant differences were observed in the distribution of first ($P$ = 0.74), second ($P$ = 0.80), third ($P$ = 0.51), fourth ($P$ = 0.12), and fifth ($P$ = 0.35) instars in the top and bottom halves of the peach soil (Fig. 2). Egg to adult development of Sri Lankan weevils took an average of 105 d. In this experiment, we showed that Sri Lankan weevil larvae can be reared successfully in association with peach roots under greenhouse conditions.

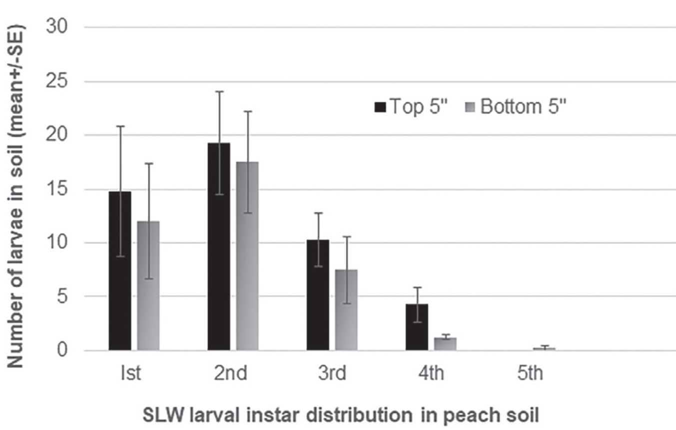


**Fig 2.** Sri Lankan weevil larval distribution in top (black columns) and bottom (gray columns) 5 inches of soil in pots containing peach seedlings. No significant differences were observed in the distribution of larval stages.

## ELECTROANTENNOGRAM RECORDINGS WITH PEACH VOLATILES

Peach volatiles identified by solid-phase microextraction and gas chromatography-mass spectrometry included alcohols, aldehydes, terpenes, esters, and other compounds. Benzaldehyde and *Z*-3-hexenyl acetate were the major odorants in peach flush, whereas mature peach leaves produced very low amounts of benzaldehyde (Fig. 3). Some of these odorants, but not benzaldehyde, also were present in the headspace of Valencia citrus (Rutaceae), also a host plant for Sri Lankan weevils.

Electroantennogram recordings were performed with excised Sri Lankan weevil antennae (Fig. 4A). The club of the antenna (Fig. 4B) was placed on a gold wire connected to the recording electrode (Fig. 4C). Gold wire for the electrode significantly improved the signal-to-noise ratio of the recordings. Many of the peach volatiles identified by solid-phase microextraction and gas chromatography-mass spectrometry elicited Sri Lankan weevil antennal responses. Benzaldehyde produced consistent antennal responses from male and female weevils, and was used as a positive control to compare electroantennogram responses from other peach odorants. Benzaldehyde has been reported as an attractant for plum curculio weevil *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae) (Akotsen-Mensah & Fadamiro 2015). *Z*-2-hexenol produced the highest antennal response from male weevils ($F_{10,55}$ = 15.8, $P$ < 0.0001), followed by Z-3-hexenol. Z-3-hexenol has been reported as a component of an attractant blend for *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) (Van Tol et al. 2012). Hexanal also produced higher electroantennogram responses from male Sri Lankan weevil antennae compared to other odorants tested (Table 1). Female weevil antennal responses were highest for Z-2-hexenol ($F_{10,55}$ = 9.15, $P$ < 0.0001), followed by Z-3-hexenol and hexanal (Table 1). Male and female Sri Lankan weevil antennae responded to peach odorants such as Z-2-hexenol, Z-3-hexenol, and hexanal and benzaldehyde. These antennaly active odorants will be tested further in olfactometer assays. Olfactometer assays will help to study the behavioral activity of

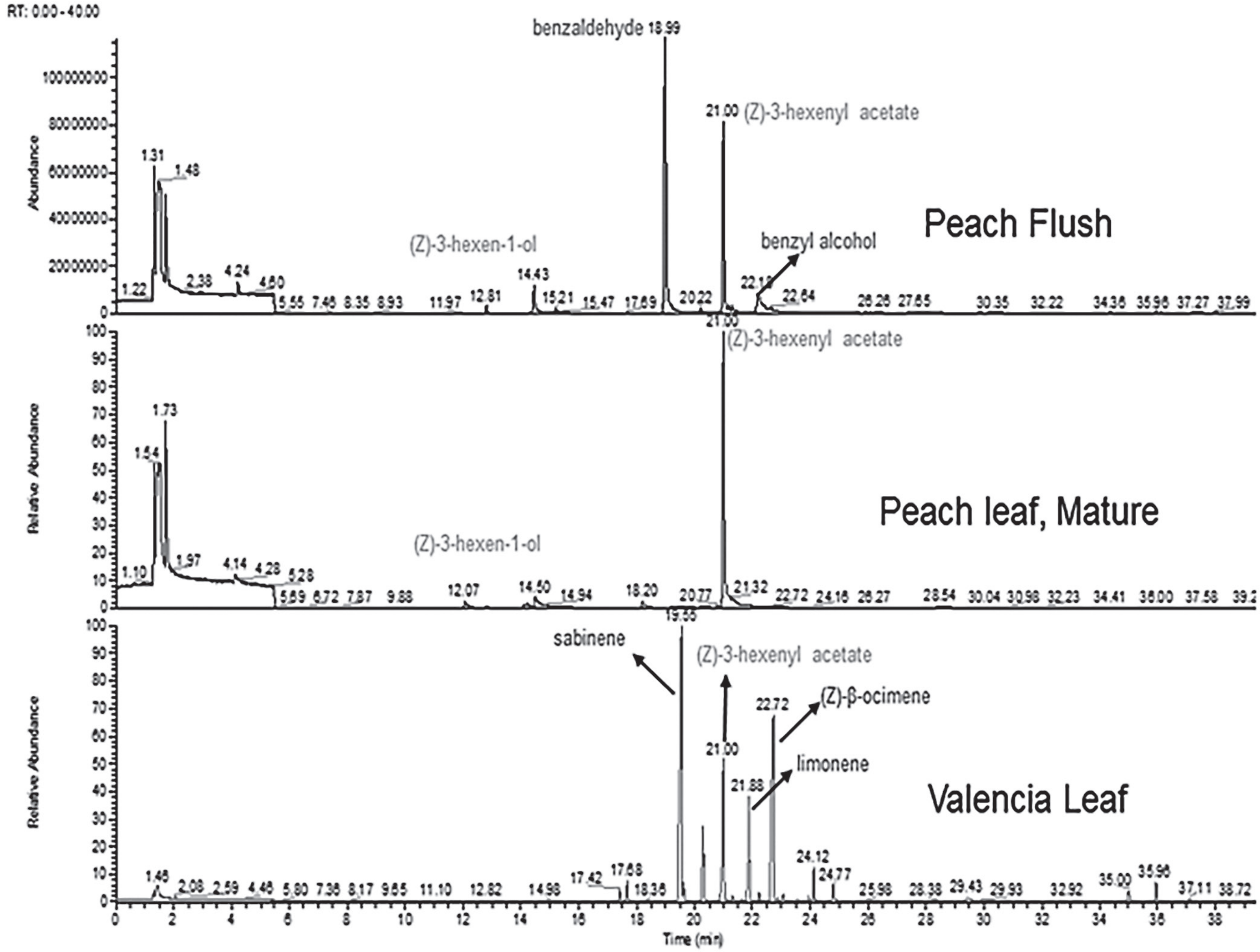


**Fig. 3.** Chromatographic detection of volatiles present in headspace of peach flush, mature peach leaves, and Valencia (sweet orange) leaves.

these odorants, and to identify the principal odorants and blends that could be developed as attractants for monitoring and trapping of these weevils in the field.

Identification of semiochemicals/odorant blends will contribute to development of monitoring tools to detect, monitor, and manage populations in peaches and other specialty crops. Analyzing the be-

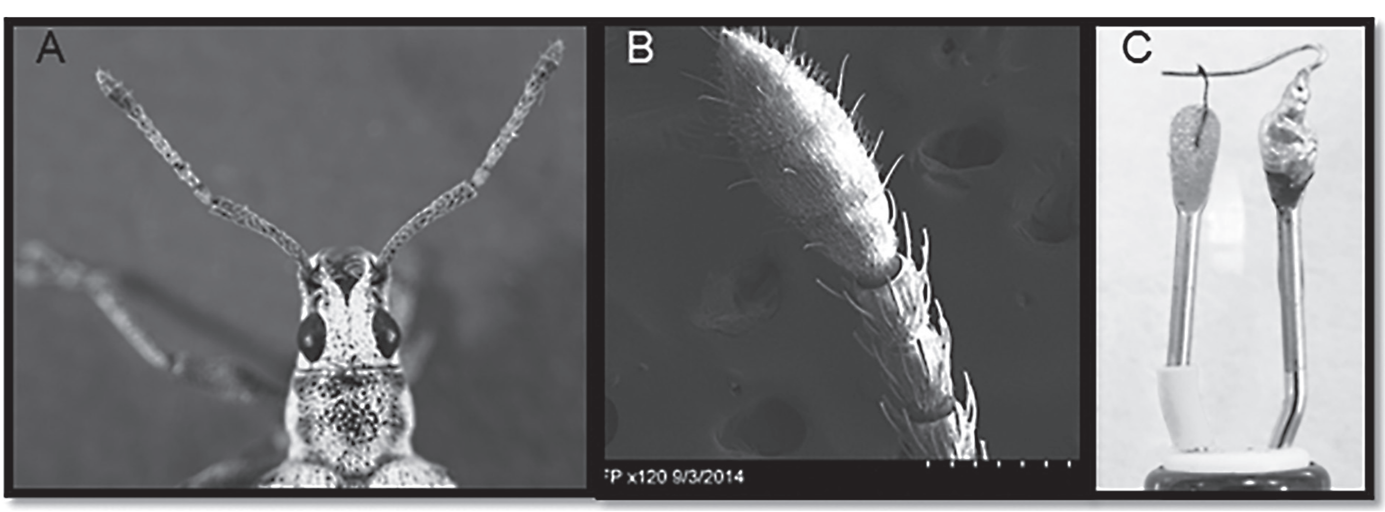


**Fig. 4.** (A) Antennae of Sri Lankan weevil; (B) scanning electron microscopy of olfactory and mechanoreceptor hairs on the club of Sri Lankan weevil antennae; (C) arrangement of antennal preparation for electroantennogram recordings.

**Table 1.** Electroantennogram responses from male and female *Myllocerus* weevils to odorants present in headspace volatile collection from peach leaf flushes (Mean ± SEM (*n* = 6). Mean electroantennogram responses of male and female Sri Lankan weevils expressed as percent of response to benzaldehyde.

| Odorant | Male response[1] mean ± SE | Female response[1] mean ± SE |
|---|---|---|
| Benzaldehyde | 100.00 ± 0.00 bc | 100.00 ± 0.00 abc |
| Benzyl alcohol | 63.86 ± 4.80 bc | 55.81 ± 12.22 bcd |
| 1,8-Cineole | 33.52 ± 11.42 c | 34.30 ± 4.01 cd |
| 2-Ethyl hexanol | 77.35 ± 5.54 bc | 75.50 ± 5.44 abcd |
| Hexanal | 143.96 ± 26.16 b | 134.43 ± 13.39 a |
| Linalool | 82.14 ± 22.37 bc | 50.14 ± 6.36 bcd |
| [*Z*]-3-Hexyl acetate | 33.27 ± 4.04 c | 34.20 ± 4.19 d |
| γ-Decalactone | 33.49 ± 7.64 c | 24.34 ± 3.36 d |
| [*Z*]-2-Hexenol | 269.46 ± 38.75 a | 137.41 ± 29.95 a |
| [*Z*]-3-Hexenol | 133.97 ± 17.06 b | 113.20 ± 25.04 ab |
| [*Z*/*E*]-β-Ocimene | 63.35 ± 10.94 bc | 40.48 ± 11.75 cd |

[1]Means within columns followed by same letter are not significantly different ($\alpha = 0.05$, Tukey's HSD following a significant ANOVA).

havior and chemical ecology of the weevil is expected to result in identification of aggregation and sex pheromones for monitoring traps and mating disruption techniques. Our findings will help reduce the use of pesticides in groves, reduce production costs, and reduce pesticide residue load on marketed fruit. In the longer term, knowledge of weevil biology and feeding behavior, and host plant resistance will help develop resistant cultivars as conventional breeding programs progress.

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