

ANTENNAL SENSORY STRUCTURES OF *LISSORHOPTRUS ORYZOPHILUS*
(COLEOPTERA: CURCULIONIDAE) WITH NOTES ON AQUATIC ADAPTATIONS

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Abstract

A scanning electron micrograph study was conducted of adult *Lissorhoptrus oryzophilus* Kuschel to determine the types and locations of sensory structures on the antennae. The antenna consists of a scape, six-segmented funicle, and club. Females had significantly longer antennal segments than males, but no other sexually dimorphic characteristics were found. Trichoid sensilla, two types of squamaform sensilla, tuft hairs, and hydrofuge scales were found on the antennae. The distal third of the club was densely covered by trichoid sensilla that likely function in gustation and olfaction. Hydrofuge scales were on the antennal club, and plumose hydrofuge scales were on the rostrum. Squamiform sensilla were among the plumose hydrofuge scales on the rostrum. The information gained from this study may be used to design electrophysiological studies to provide information about the attractiveness of volatile compounds from rice plants and potential weevil pheromones. Aspects of the plastron of the weevil are also discussed. The antennae of rice water weevils have sensilla and hydrofuge adaptations for an aquatic environment and were found to be unique when compared to five species of terrestrial weevils.

The rice water weevil *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae) is native to North America (O'Brien and Wibmer 1982). The aquatic adults are good swimmers (Hix *et al.* 2000a, 2001), and respiration is aided by an efficient plastron (Hinton 1976). Rice water weevil adults can survive about 96 h underwater in aquatic traps (Hix *et al.* 2000b). It is the main pest of rice, *Oryza sativa* L., in the United States (Way 1990), and it is a pest in Japan, Korea, Taiwan, and China (Nagata 1990) in which it has been introduced. Weevil populations in California and countries where introduced are parthenogenetic (Takenouchi 1978).

How *L. oryzophilus* adults locate and recognize host plants and mates currently is unknown. Host and mate finding apparently depends at least in part on olfaction and gustation. Adults commonly are found feeding and mating on the foliage of partly submerged rice and other host plants. Although it is assumed that chemo-, hygro-, and mechanoreceptors common to most insects provide orientation to water, mates and host plants (Dethier 1955; Schneider 1964; Altner and Prillinger 1980), sensilla on the

antennae have not been described or classified for *L. oryzaophilus*. The positive identification of gustatory and olfactory sensilla on the antennae may allow insight into chemoreception and chemical ecology of this weevil (Dethier 1955). The presence and identification of pheromones certainly would aid in the monitoring of adults. However, previous electrophysiological studies provided limited information due to the hydrophobic properties of the adult antennae (Hix *et al.* 2000a). Clearly, more information about the locations of sensory structures and hydrofuge structures on the adult weevil antennae is needed.

Although descriptions of the internal morphology of the antennae are not available, a description of the external morphologies and positions of insect sensilla can sometimes be used to suggest functions in nature. Sensory types and locations also may be useful in collecting electrophysiological data. The objectives of this study were to: 1) locate and classify insect sensilla on the antennae of *L. oryzaophilus* with scanning electron microscopy, and 2) identify possible hydrofuge structures.

Materials and Methods

The antennae of five male and five female *L. oryzaophilus* were examined with scanning electron microscopy. Weevils examined in this study were collected in Stuttgart, Arkansas, 14 July 1997 and stored in a freezer at -20°C . The insects were treated with a protease solution of 10 $\mu\text{g}/\text{ml}$ in Tris buffer (pH 7.4) to remove possible proteinaceous substances from antennal surfaces and pores (Dyer *et al.* 1982). Weevils were dissected between the pro- and mesothorax, and the front legs were removed. The heads were then mounted on aluminum studs with silver paint in a manner to present the antennae and were sputter coated with gold. Specimens were examined with an ISI-60 scanning electron microscope at an accelerating voltage of 20 kV and micrographs made with a 35 mm camera body.

Scanning electron micrographs (SEMs) of *L. oryzaophilus* were compared to SEMs of other species of weevils: 1) the clover head weevil *Hypera meleis* (F.) (Smith *et al.* 1976); 2) the cabbage seedpod weevil *Ceutorhynchus assimilis* Paykull (Isidoro and Solinas 1992); 3) the pecan weevil *Curculio caryae* (Horn) (Hatfield *et al.* 1976); 4) the alfalfa weevil *Hypera postica* (Gyllenhal) (Bland 1981); and 5) the plum curculio *Conotrachelus nenuphar* (Herbst) (Alm and Hall 1986). Structures on the adult rice water weevil antennae were described and named *sensu lato*. Measurements of antennal segments and structures were made from SEMs and subjected to the Student's *t*-test ($P = 0.05$).

Results

The antenna of *L. oryzaophilus* consisted of a: 1) scape; 2) six-segmented funicle; and 3) club (Fig. 1). Females had significantly longer antennal segments than males (Table 1). Other than body size, no apparent sexual dimorphic characteristics were observed.

For both sexes, the distal third of the club (Fig. 2A) was densely covered with trichoid sensilla, and the proximal half of the club was covered sparsely with hydrofuge scales I (hsI) (Fig. 2A–B). The trichoid sensilla on the club were sigmoid-shaped with hooked tips, and were the most abundant structures on the weevil antennae (Fig. 2C).

Squamiform sensilla I (ssI) were spatulate-shaped and occurred as a border dividing the trichoid sensilla from the proximal end of the club (Fig. 2A). Squamiform sensilla I also were arranged on the distal end of the sixth funicular segment in such a manner as to serve as possible proprioceptors (Figs. 1, 3A–B). Nine larger ssI occurred on the

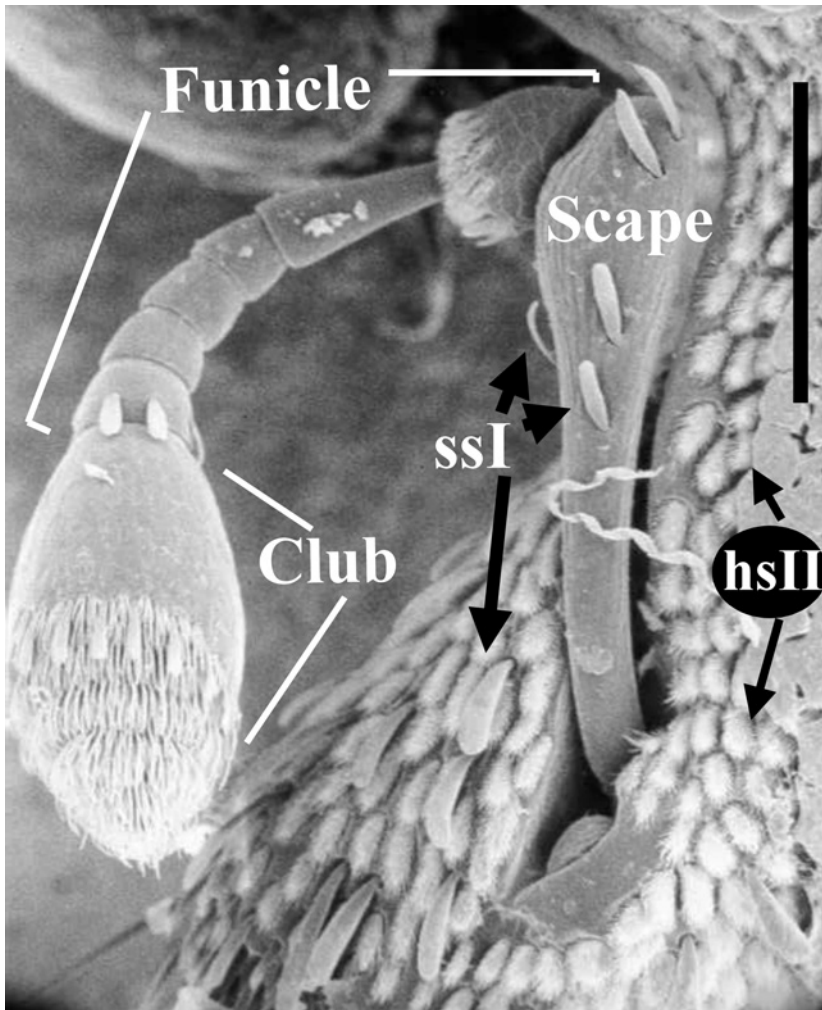


Fig. 1. Scanning electron micrograph of right antenna of female *Lissorhoptrus oryzophilus*. Bar = 100 microns. Hydrofuge scale (**hsII**), squamiform sensilla I (**ssI**). Club of antenna is pointing towards tip of rostrum.

scape (Fig. 1). Hydrofuge scales I (**hsI**) with five- to seven-branches occurred on the proximal two thirds of the club (Fig. 2A–B). Squamiform sensilla II (**ssII**) occurred on the first funicular segment near the Johnston's organ (Fig. 3C). Tuft hairs near the Johnston's organ on the first funicular segment were the second most abundant structures on the antennae (Fig. 3C). Squamiform sensilla I occurred among plumose-like hydrofuge scales II (**hsII**) on the rostrum (Figs. 1, 4A–B), and a waterproof coating covered some **hsII** in the central area of the rostrum (Fig. 4A–B). Cuticular pits were observed on the proximal two thirds of the club (Figs. 2B, 3B). Locations, sizes, and distributions of the structures are presented in Table 2.

Table 1. Measurements of antennal segments of adult *Lissorhoptrus oryzophilus* taken from SEMs. Means are in microns (\pm SE) ($n = 5$).

	Female	Male
Scape	194.6 (\pm 14.29)a*	150.1 (\pm 28.36)b
Funicular Segment 1	43.9 (\pm 3.99)a	36.0 (\pm 5.58)a
Funicular Segment 2	49.0 (\pm 3.84)a	37.0 (\pm 6.14)b
Funicular Segment 3	18.7 (\pm 1.85)a	13.7 (\pm 2.52)b
Funicular Segment 4	19.2 (\pm 2.05)a	14.5 (\pm 2.54)b
Funicular Segment 5	17.4 (\pm 2.07)a	13.0 (\pm 2.34)a
Funicular Segment 6	22.5 (\pm 2.43)a	17.0 (\pm 3.07)a
Club	133.1 (\pm 10.12)a	98.4 (\pm 17.2)b
Whole Antenna	498.4 (\pm 40.64)a	379.7 (\pm 67.75)b

* Mean value in rows followed by different letters are significantly different ($P = 0.05$).

The antennae were unique when compared with the antennae of the five adult terrestrial weevils previously mentioned in Materials and Methods. The antennae of the terrestrial weevils lacked hydrofuge structures, and none had squamiform sensilla.

Discussion

The trichoid sensilla located apically on the club are likely to have gustatory and olfactory functions (Schneider 1964; Altner and Prillinger 1980) with mechanoreception as a secondary function. The squamiform sensilla probably function in proprioception and mechanoreception. For example, the ssI on the sixth funicular segment could determine antennal position in relation to body orientation, and the ssI located on the scape and rostrum may function as indicators of swimming speed. In addition, the membrane of the bubbles formed around the club, first funicular segment, and plastron may provide tension on the squamiform sensilla, thereby functioning as pressure receptors to determine swimming depth and orientation (Rabe 1953). Naiads of *Cordulegaster insignis* Schneider have fan-shaped squamiform sensilla on the postocular lobe (Verschuren 1989) that are similar to the ssII found on *L. oryzophilus*, and they have been suggested to function as current receptors and to detect the presence and position of prey (Corbet 1999). Furthermore, the ssII are similar to setae suggested to function as receptors of vibration in hydropsychid caddisfly larvae (Jansson and Vuoristo 1979).

The hsI (Figs. 2B, 3A–B) may function in forming a small plastron-like air bubble associated with the club. Twenty-five swimming adult weevils were observed to have thin layers of air associated with their clubs and first funicular segments (RLH). The hsI are somewhat similar to the ones on the abdominal tip of the aquatic weevil *Bagous subcarinatus* Gyllenhal (Langer and Messner 1984) and in drawings of scales from the semiaquatic weevil *Lixus paraplecticus* (L.) (Coleoptera: Curculionidae) (Thorpe and Crisp 1949). The tuft hairs were similar to tuft hairs on the gena of *Bagous binodulus* (Herbst) (Langer and Messner 1984) and scales behind the eyes of *Bagous limosus* Gyllenhal (Hinton 1976). These tuft hairs also may function when *L. oryzophilus* are out of the water as hygro- and thermoreceptors. The ssI and II could function as indicators of flight speed.

The plumose-like hsII (Fig. 1) on the rostrum are part of the weevils main plastron system and were similar to the plumose scales located ventrally between the fore-, meso-, and metacoxae. Furthermore, the hsII are located in areas around the points of articulation of the head and prothorax (Fig. 4A–B). Thorpe and Crisp (1949) published

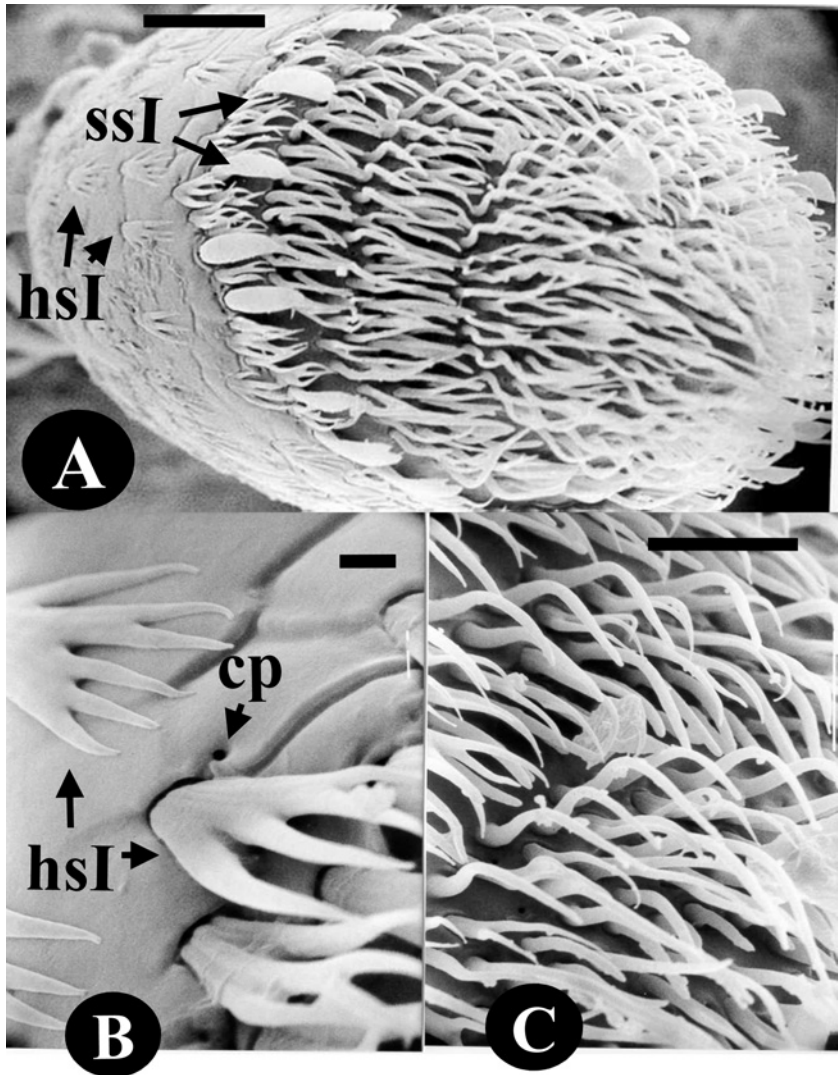


Fig. 2. Scanning electron micrographs of female *Lissorhoptrus oryzophilus* antennal club. **A)** View of distal portion of club depicting squamiform sensilla I (**ssl**) and hydrofuge scales (**hsl**), bar = 10 microns; **B)** cuticular pit (**cp**) and **hsl** on proximal area of club, bar = 1 micron; **C)** trichoid sensilla on distal portion of club, bar = 10 microns.

drawings of similar scales from *Eubrychius velatus* Beck (= *Phytobius velatus*) and *Tanysphyrus lemnae* (Paykull) (Coleoptera: Curculionidae). Hinton (1976) described the efficient plastron system of *L. oryzophilus* and provided SEMs of scales associated with the elytra. However, the plumose-like **hsl** were not depicted, although he did show similar structures behind the eyes of *Hydronomus alismatis* (Marshall), on the

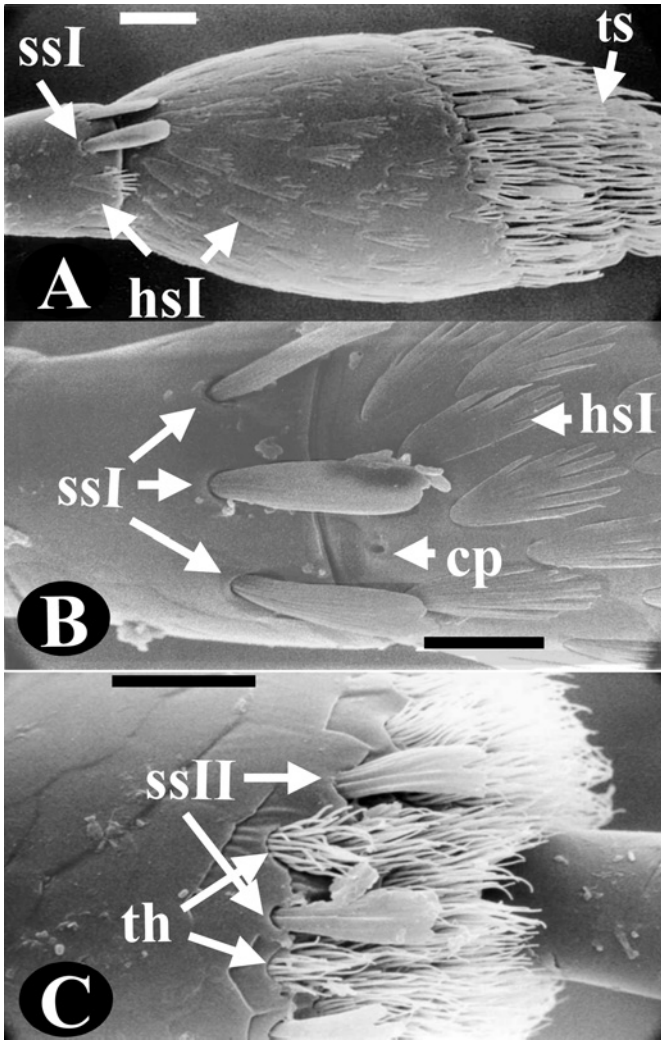


Fig. 3. Scanning electron micrographs of female *Lissorhoptrus oryzophilus* antennal segments. **A)** Lateral view of club and 6th funicular segment depicting squamiform sensilla (ssI) and trichoid sensilla (ts), bar = 5 microns; **B)** View of club-6th funicular segment junction depicting ssI, hydrofuge scales (hsI) and cuticular pit (cp), bar = 10 microns; **C)** 1st funicular segment depicting squamiform sensilla II (ssII) and tuft hairs (th) associated with Johnston's organ area, bar = 10 microns.

first abdominal sternite of *Endalus disgregus* Burke, and on the elytra of *E. disgregus*, *E. velatus*, and *Grypus equiseti* (F.) (Coleoptera: Curculionidae), and on the second abdominal sternite of *E. velatus*. Hinton (1976) showed different shapes of hydrofuge scales associated with the plastrons of *Neochitina bruchi* Hustache, *Neochitina*

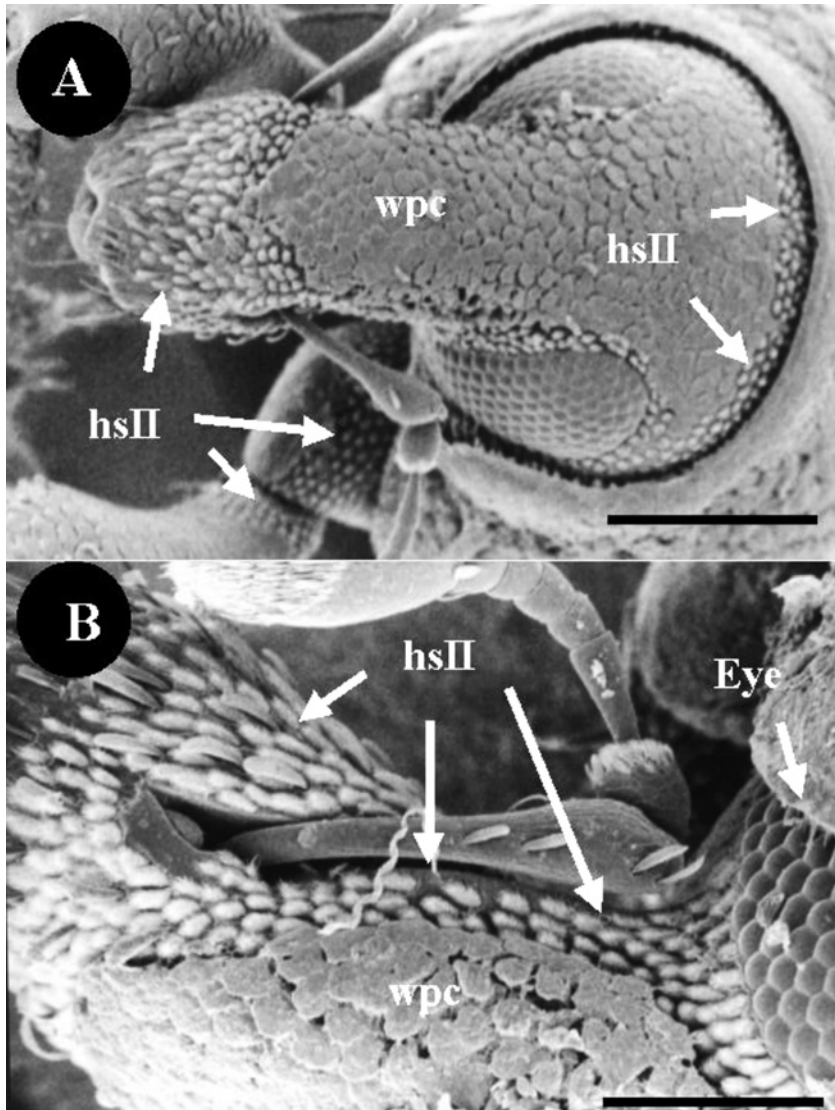


Fig. 4. Rostrum of female *Lissorhoptrus oryzophilus*. **A)** Dorsal view depicting hydrofuge scales II (**hsII**) on tip of rostrum, forecoxae, and areas of articulation of head and prothorax; waterproof coating (**wpc**) covers **hsII** in center of rostrum, bar = 200 microns; **B)** lateral view of rostrum depicting **wpc** and **hsII** on tip of rostrum and around compound eye, bar = 100 microns.

eichhorinae Warner, *Lixellus haldemani* Burke, *Bagous americanus* LeConte, *Bagous restrictus* LeConte, *Cryptobagous singularis* Hustache, and *Brachybamus electus* Germar. The **hsII** were similar to those found on the ventral surface of *T. lemnae* by Heckman (1983) and the plastron of *Dicranthus elegans* (F.) (= *Bagous elegans*) by

Table 2. Location and measurements of structures associated with *Lissorhoptrus oryzophilus* antennae and rostrum.

Structure	Location	Size Range	Mean or Density
Trichoid Sensilla	Club (distal 1/3)	13 microns	55 per 10 ² microns
Squamiform Sensilla I	Club	13–19 microns	20 per club*
	6 th Funicular Segment	16–17 microns	6 per segment*
	Scape	13–22 microns	8 per scape
	Rostrum	32–41 microns	8–9 per 100 ² microns
Squamiform Sensilla II	1 st Funicular Segment	4–5 microns	8 per segment *
Tuft Hairs	1 st Funicular Segment	10 microns	Too dense to count
Hydrofuge Scale I	Club (proximal 2/3)	10–14 microns	14 per 19 ² microns
	6 th Funicular Segment	12 microns	1 per segment
Hydrofuge Scale II	Rostrum	19 microns	Contiguous to sub-contiguous
Cuticular Pits	Club (proximal 2/3)		sparse

* Estimate.

Messner and Dieckmann (1987). Similar structures were also found on *Bagous subcarinatus* Gyllenhal, *Bagous longitarsis* Thomson, *Bagous puncticollis* Boheman, *Bagous glabriorostris* Herbst, *B. binodulus*, and *E. velatus*, (Coleoptera: Curculionidae) (Langer and Messner 1984).

The cuticular pits are very similar to the ones on the antennae of naiads of *Baetis rhodani* (Pictet) (Ephemeroptera: Baetidae) (Gaino and Rebora 1998). No putative or speculative function was suggested for the sensilla associated with the cuticular pits found on the *B. rhodani* antennae. It is unknown at this time if sensilla are associated with these pits on the antennae of *L. oryzophilus*.

Electrophysiological studies with electroantennograms (EAG) provided inconclusive information about the attractiveness of volatile compounds from rice plants to adult *L. oryzophilus* (Hix *et al.* 2000c). This was due to short life of the antennal preparation caused by hydrophobic structures on the antennae (*i.e.*, cell death) and the fact that probable chemo-sensory structures were located on the distal third of the antennal club rendering electrophysiological contact difficult to maintain with saline electrodes. A better EAG technique would be to insert tungsten electrodes into the antennae. The indifferent electrode could be inserted into the gular suture and the recording electrode into a pre-punctured hole in the tip of the antennal club.

Disruption of the plastron may be a viable strategy to manage adult weevil populations, but little work exists in the literature on strategies to disrupt the plastron system of *L. oryzophilus*. Leite *et al.* (1992) reported that soybean oil or soybean oil mixed with *Beauveria bassiana* provided up to 100% control of *Lissorhoptrus* spp. in rice in Brazil. The soybean oil may disrupt the plastron system of the weevils. Surfactants, microorganisms, etc. to be used to disrupt the plastron and hydrofuge structures on the antennae should receive more attention in future management of the weevil.

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