Method in sex discrimination in the mango pulp weevil *Sternochetus frigidus* (Fabr.) (Coleoptera: Curculionidae)

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Abstract

We describe a simple method for sexing *Sternochetus frigidus* (Fabr.) using a stereomicroscope to view the dorsum of the terminal abdominal segment. In the male, clipped wings exposed distinctly truncate penultimate segments, whereas in the female, the terminal tergite tapered posteriorly.

Key words: *Sternochetus frigidus*, Curculionidae, mango pulp weevil, sex discrimination

INTRODUCTION

The mango pulp weevil, *Sternochetus frigidus* (Fabr.) is an important quarantine pest of mango in the Philippines, where it is found only on the western island of Palawan (Basio et al., 1994) (Fig. 1). The weevil has been identified in North-east India (Assam and Tripura), Bangladesh, Burma, Thailand, Malaysia, Singapore, and Indonesia, including Irian Jaya, and has been observed to attack both cultivated and wild mango species (Kalshoven, 1981; AQIS, 1990). The immature stages of the pest feed and develop on the pulp, and even the newly developed adult remains in a pupal cell inside the fruit until it rots (De Jesus and Gabo, 2000).

The chemical basis for the weevil's preference for mango over other crop species remains obscure. Future studies in our laboratory will focus on this issue and on weevil behavior. However, an important prerequisite for these investigations is the sexing of male and female weevils. To date, the only known methods of sex discrimination are dissection of dead weevils or observation of copulating live weevils, since no apparent external morphological differences are known. The present paper presents a simple and accurate way of discriminating between live male and female mango pulp weevils.

MATERIALS AND METHODS

Pulp weevil adults were collected from infested fruits at the green ripe stage from backyard mango trees in Brooke's Point, in the southern part of Palawan in March–July 2000. The weevils were kept in the mango pulp weevil laboratory in Brooke's Point and placed in 5"×6" round plastic jars covered with nylon cloth lined with flat white napkins. Small pieces (about 2.5×1.5×1 cm) were cut from 70 to 100 days old green mango fruits were provided daily as food. After 24 h the adults were transferred to new jars with green mango substrates.

In order to determine the appropriate method for the sexing of mango pulp weevils, blind samples of 332 adults with the wings removed were examined for external morphological features. The elytra and hindwings were removed by holding the insect horizontally using blunt end forceps in the left hand and clipping the elytra and hindwings from the base with the right hand using fine tip forceps under a stereomicroscope. The visible abdominal tergites were examined and the total number was noted in all specimens. Dissections were made to verify sex using aedeagus in males and ovipositor in females as criteria.

In order to view only the terminal abdominal segments of live weevils, the tips of the wings were clipped. First the weevil was held firmly at the

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sides of the abdomen between the thumb and forefinger of the left hand under a stereomicroscope. Then, using fine tip forceps, approximately 0.5–1.0 mm was clipped from the posterior end of the elytra using the right hand. The clipped section represented the approximate length of the elytra that extended beyond the abdomen, and after clipping, the elytra and the abdomen were approximately the same length. To facilitate the viewing of the terminal tergites, the elytra could be clipped neatly by following the outline of the posterior abdomen. The elytra was clipped carefully to prevent damage to the abdomen. After it was clipped the weevil was held posterior side up in the left hand and using fine tip forceps, the right hand was used to push the dorsal terminal tergite slightly downward for a few seconds, just long enough to view it. The abdomen was pushed carefully and swiftly to prevent puncturing and tearing of intersegmental membranes. The specimens were always viewed under a stereomicroscope because the terminal tergites were too small to be visible to the naked eye.

Sexing of weevils was done within a week of their collection, before any mating occurred. After sexing, males and females were kept separately in small plastic cups measuring 7.5 cm height × 8 cm and 5 cm top and bottom diameters, respectively, and with 2 mm holes on its covers. Ten weevils of each sex were placed in a cup lined with white flat napkins; small pieces of mango fruits were also provided as food. After 24 h, the adults were transferred to new jars with green mango substrates. The weevils were reared in this manner until use in experiments.

Weevils with wings removed were fixed in formalin/acetic acid rinsed first in water, then in phosphate buffer, and were post-fixed in 1% osmium tetroxide. Samples were dehydrated using a graded ethanol series and dried in a Critical Point Dryer Hitachi 8 CP2. Dried samples were coated with gold and palladium in an ion sputter Hitachi E101 and finally viewed under a Hitachi S510 Scanning Electron Microscope (SEM) at an accelerating voltage of 25 kV and photographed. The aedeagus of the male weevils was excised using fine tip forceps and prepared for SEM in the same manner.

RESULTS

With the elytra and hindwings removed, the male

abdomen showed eight sclerotized visible tergites, the penultimate segments being strongly sclerotized. The females have seven visible tergites, with the last tergite more strongly sclerotized (Fig. 2a, b). The sexes and the number of tergites were confirmed after the 332 blind samples, which consisted of 152 male and 180 female adults were dissected. By just clipping the elytra and slightly pushing the abdomen downward with fine forceps, the male can be differentiated from the female because of the distinctly truncate penultimate tergites that contrast with the female's posteriorly tapered terminal tergite (Fig. 3a, b).

In the male, tergites 7 and 8 are distinctly separate; tergite 7 is bigger than tergite 8. Tergite 7 is broadly incised posteriorly, exhibits a pair of oblique light patches anteriorly, which reach as far as the lateral edge, and is covered with short brush-like setae from the midsection caudad. Tergite 8 is broader than it is long, and the posterior border is mostly straight to partly straight, and is entirely covered with setae (Fig. 4a). In the female, tergite 7 is broader than it is long anteriorly, tapered posteriorly, exhibits a pair of light patches basally, and is covered with short brush-like setae from the midsection caudad (Fig. 4b).

The male genitalia, or aedeagus, is strongly sclerotized, tubular and slightly flattened, slightly curved and pointed at the apex. The ventral edge of the median orifice projects beyond the dorsal edge and is bluntly pointed (Fig. 5).

DISCUSSION

Among coleopterans, external morphological differences are usually found on the antennae, which may be more elaborate in the male than the female and used in finding a mate, courtship, or male-male combat. Differences can also be found in the fore tarsi and sternum of the male, which aids in copulation. However, in *S. frigidus* the antennae, tarsi, and sternum are morphologically similar in both sexes. In addition, body color cannot be used as a sexing characteristic because it changes with weevil age. Likewise, body shape and size are not distinctly different between the sexes. Only on the dorsal abdomen did we observe a clear external morphological difference between males and females (the number of visible tergites).

Differences in the number only of the abdominal

Fig. 1. Mango pulp weevil adult (mag. 260 $\times$).

tergites have been noted in an early work in Curculionidae, without giving a full diagnosis of the tergites (Marshall, 1916). It was found that in some subfamilies the males had the seventh tergite divided into two, while the females had only the normal seven tergites. In contrast, in several genera of Adelognathi and in the genus *Proscaphaladeres*, *Eremnus* and *Episomus*, the females had the distinct eighth tergite. As a result, the number of abdominal tergites in Coleoptera has required further investigation.

The genus *Sternochetus* was not included in the work described above. The subfamily Cryptorhynchinae, to which this genus belongs, is charac-

Fig. 2. Dorsal aspect of the abdominal segments with wing removed, a: male, tergite 7 (T7), tergite 8 (T8); b: female, tergite 7 (T7) (mag. 180 $\times$).

Fig. 3. Clipped elytra with terminal abdominal segments pushed slightly to show: a, truncate male terminal tergite (indicated by arrow); b, tapered female terminal tergite (indicated by arrow) (mag. 240 $\times$).

Fig. 4. Terminal segments of the dorsal abdomen: a, male, tergite 7 (T7), tergite 8 (T8); b: female, tergite 7 (T7).

Fig. 5. Excised male aedeagus.

terized by having a well-defined pectoral canal and developed unci on the tibiae. The genus *Sternochetus* has tibiae with the inner carina of corbels strongly laminated, with two rows of outer setose fringes; femora not or weakly sulcate beneath, the sulci covered with scales (Morimoto, 1978). Until the present study little attention had been given to the number of abdominal tergites and to descriptions of the tergites in this genus or in most Curculionidae, since taxonomic work has relied heavily on the elytra for the dorsal aspect. Importance has also been given to the front leg, hind leg, apex of the tibia, pectoral canal, mesosternal receptacle and lateral aspect of the meso- and metasternum of the weevil.

The use of the abdominal apex in sexing has been notable on the exclusively American Chrysomelid genus *Diabrotica* (White, 1977). Since the elytra of this species cover the abdomen only loosely, the tip of the abdomen is usually visi-

ble, and live beetles can be readily sexed with only a 10-power hand lens. The males possess an extra sclerite on the abdominal apex which the females do not possess. This character occurs in 78 species of Central and South American *Diabrotica* found in the United States National Museum collection. In stored-products Coleoptera, *Araecerus fasciculatus* (Deg.) and *Caryedon gonagra* (F.), the pygidium is the character used for sexing. In the males, the pygidium is not visible dorsally, but is distinctly visible in the females (Halstead, 1964).

Other significant efforts to sex live beetles using the terminal abdominal segments focused on the cacao weevil borer and ladybird beetle. In the cacao weevil borer *Pantorhytes szentivanyi* Marshall, the dorsum of the vestibulum is similar in both sexes and exhibits a single plate with bluntly conical tergites. The difference is found in the venter where the seventh sternite consists of two sclerites in the male and one sclerite in the female. In order to expose these sclerites, the last sternite must be lifted (Hassan, 1973). In the ladybird beetle, *Coleomegilla maculata* (Deg.), sexing is based upon differences in the external genitalia. Slight pressure on the abdomen forces the genitalia to protrude from the posterior end, exposing the anus and the ovipositor in the female and the aedeagus in the male (Nichols and Neel, 1970).

In *S. frigidus*, slight pressure to the abdomen does not result in protrusion of the genitalia. The elytra are slightly longer than the abdomen so they must be clipped to expose the penultimate tergites. The present paper is the first to report use of the above method for sexing live adult *S. frigidus* weevils. The use of the terminal tergite to differentiate the male from the female is also a new and signifi-

cant finding for this weevil. A previous report only described the morphological similarities of male and female weevils (De Jesus and Gabo, 2000). Even in the closely related species, *Sternochetus mangiferae*, the only established method for differentiating the sexes uses the genitalia of dead weevils after dissection. The morphology of the terminal tergites of *S. mangiferae* may also allow the present method to be adapted for sexing this equally significant quarantine pest, which fortunately is not present in the Philippines.

Figure 5 provides the first schematic of male *S. frigidus* genitalia. This weevil exhibits a typical curculionid aedeagus where the penis is divided into a ventral pedon comprising the body and the tectum, which forms a dorsal lid over the ostium (Lawrence and Britton, 1994).

Adoption of the described sexing method on a wide scale would greatly enhance further studies on the behavior and chemical ecology of this pest. The method has proven to be effective and has not affected mating or flight behavior in the laboratory.

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