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SEXUAL DIMORPHISM OF *RHYSSOMATUS SUBTILIS* (COLEOPTERA: CURCULIONIDAE)

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Abstract

Examination with a binocular microscope of adults of *Rhyssomatus subtilis* Fielder (Coleoptera: Curculionidae) revealed distinct differences between the sexes in the foreleg, which permits their differentiation with complete accuracy. In the female the profemual process is weak, subacute, angulate and the protibia has an uncus and mucro. In the male the profemur process is strong, curved, subacute, tooth-like and lacks an protibia uncus.

Key Words: weevil, uncus, mucro, tibiae, femoral process

Resumen

La examinación con microscopio binocular de los adultos de *Rhyssomatus subtilis* Fielder (Coleoptera: Curculionidae) revelan diferencia entre los sexos en las patas delanteras, permitiendo diferenciarlos con completa precisión. Las hembras presentan el proceso femoral anterior débil, subagudo, angulado y la protibia con uncus y mucro presente. En los machos el proceso femoral anterior es fuerte, curvado, subagudo, con diente y la protibia no presenta uncus.

Palabras Claves: picudo, uncus, mucrones, tibia, proceso femoral

The black soybean weevil, *Rhyssomatus subtilis* Fiedler (Coleoptera: Curculionidae), is an important pest of soybean in Northwestern Argentina (NWA), which is expanding its geographical distribution and causing economic losses through the extensive reduction of yields (Cazado et al. 2013). The recent growth and spread of *R. subtilis* populations has called attention to the need for conducting more studies on the biology, behavior and ecology of the pest. As a preliminary step for these studies, it is necessary to determine the sex of adult weevils without resorting to the dissection of their genitalia. This requires finding a quick method for identifying the sex of these insects which would keep handling to a minimum (Silva- Filho et al. 2007).

Several techniques for sexing weevils have been discovered, but they differ notably from one another (Sappington et al. 2000). Internal morphology can be used to determine sex, but dissection is a tedious process which involves killing the weevils (Klassen et al. 1968; Jones et al. 1992). In some studies, sex was determined by watching weevils copulate (Mitchell 1963), but this method turns out to be impractical ordinarily. The genitalia can be partially extruded by squeezing the abdomen of some weevils, but this procedure usually injures the weevil, especially as the cuticle hardens with age (Agee 1964). It is not especially useful in most Molytinae such as *Rhyssomatus* due to the hard inflexible cuticle of the elytra and abdomen.

When dealing with the Curculionidae, there are various external sex characters that can be considered in order to determine the sex of weevils (Hoffman 1950; Silva- Filho et al. 2007). For *Cosmopolites sordidus* Germar, the angle of inclination of the last abdominal sternite and distribution of pubescence over the frons are taken into account (Longoria 1968). In the case of *Anthonomus grandis* Boheman and other species of *Anthonomus*, the focus is on differences in the tarsal claws (Agee 1964; Kovarik &

Burke 1983). The presence of a thorn directed behind the legs on each intermediate coxa helps to identify male *Anthonomus rubi* Herbst (Innocenzi et al. 2002), whereas male *Anthonomus eugenii* Cano are recognized based on their metatibial mucro, which is larger and more prominently curved than those of the female (Eller 1995).

Some species of the Molytinae possess a mucro, a secondary tooth located at the inner apical angle of the uncinate tibiae. Thus, a particular tibia can have both an uncus and a mucro (Kuschel 1952; Thompson 1992).The shapes and positions of the uncus, mucro and premucro in the genus *Tyloderma*, Cryptorhynchinae distinguishes sexes and species groups (Wibmer 1981, 1989).

This study identifies some external secondary sexual characters that are readily detectable and that form the basis for an accurate and non-disruptive technique for sexing adult *R. subtilis*.

Materials and Methods

All adult weevils were collected from an emergence cage placed in a soybean field in Rosario de la Frontera (Salta province, Argentina) (S 25° 39' 57.6" -W 64° 56' 58.1"), between Nov and Jun in 2010/2011 and 2011/2012. Adults were preserved in 70% ethanol and kept in it until they were examined in the laboratory.

In order to find secondary sexual characters of *R. subtilis* adults, the external characters of the specimens were examined, using the methodology proposed by other authors for determining sexual dimorphism of the Curculionidae (Kuschel 1952; Rosado-Neto 1987; Loja Cedeño 2011). Putative differences observed were verified by inspection of the genitalia, by means of a binocular microscope (Zeiss model Stemi DV4) with 10X magnification. For genital dissection, individuals were macerated and cleared in a KOH solution (10%) for 15 min, at 80 °C to promote dissolving of fat tissue, thus facilitating visibility of the reproductive structures as described by Castañeda-Vildózola et al. 2007.

Results

After examining 1,768 *R. subtilis* adults collected from the emergence cage, we found that the external characteristics of the apical armature of the fore tibia was useful for sexual determination (Fig. 1). Results showed a 100% concurrence with those reached by examining genitalia. The apical margin of the protibia of the female has a subcarinate ridge ending in an elongate, subacute uncus projecting posteriad, and has an acute mucro on the inner apical angle, partly concealed by 2 clusters of coarse setal brushes anterior and posterior to the mucro. The male has no uncus on the protibia, and all of its tibiae have only a mucro (Fig. 1). In addition, it was found that the difference in the profemoral process is striking between the sexes. In the female the process is weak and angulate and that of the male is strong, curved, subacute and tooth-like. By observing these differences with the aid of a 10X binocular microscope, it is possible to determine the sex of adult weevils via this quick, accurate and non-destructive technique, which keeps handling to a minimum.

The adult weevils were placed frontally facing the microscope lens, and without exerting too much pressure with the index finger, they were held firmly so that their forelegs spread out. This procedure allowed one to observe the presence or absence of the uncus on the protibia, as well as the different profemoral process (Fig. 1).

Discussion

Based on these results, external protibial morphology provides characters for the accurate differentiation of male and female *R. subtilis* adults.

This observation is in agreement with those of other authors, who reported that the presence or absence of the uncus, mucro, and premucro is a set of characters to be considered in order to determine sexual dimorphism in various species of the Curculionidae, such as *Sternechus subsignatus* (Rosado-Neto 1987*),* and *Chalcodermus bicolor* Fiedler 1936; Loja Cedeño 2011), as well as numerous species of the genus *Bondarius* (Dos-Santos 2007).

The presence of a modified metatibial mucro is a secondary sexual character of male *Lissorrhoptrus* and its form, and shape and number of processes is species specific as well (Kuschel 1952; Everett & Newsom 1964, O'Brien & Haseeb 2014).

Determining sex of *R. subtilis* adults through external characters as described and illustrated here is as accurate as genital dissection, constituting a very quick and non-destructive technique, which involves practically no handling of the live insects.

Therefore, this information and the proposed technique should make great contributions to biological and behavioral studies, including research on aggregation pheromones in this species and studies of the economic losses caused by this soybean pest.

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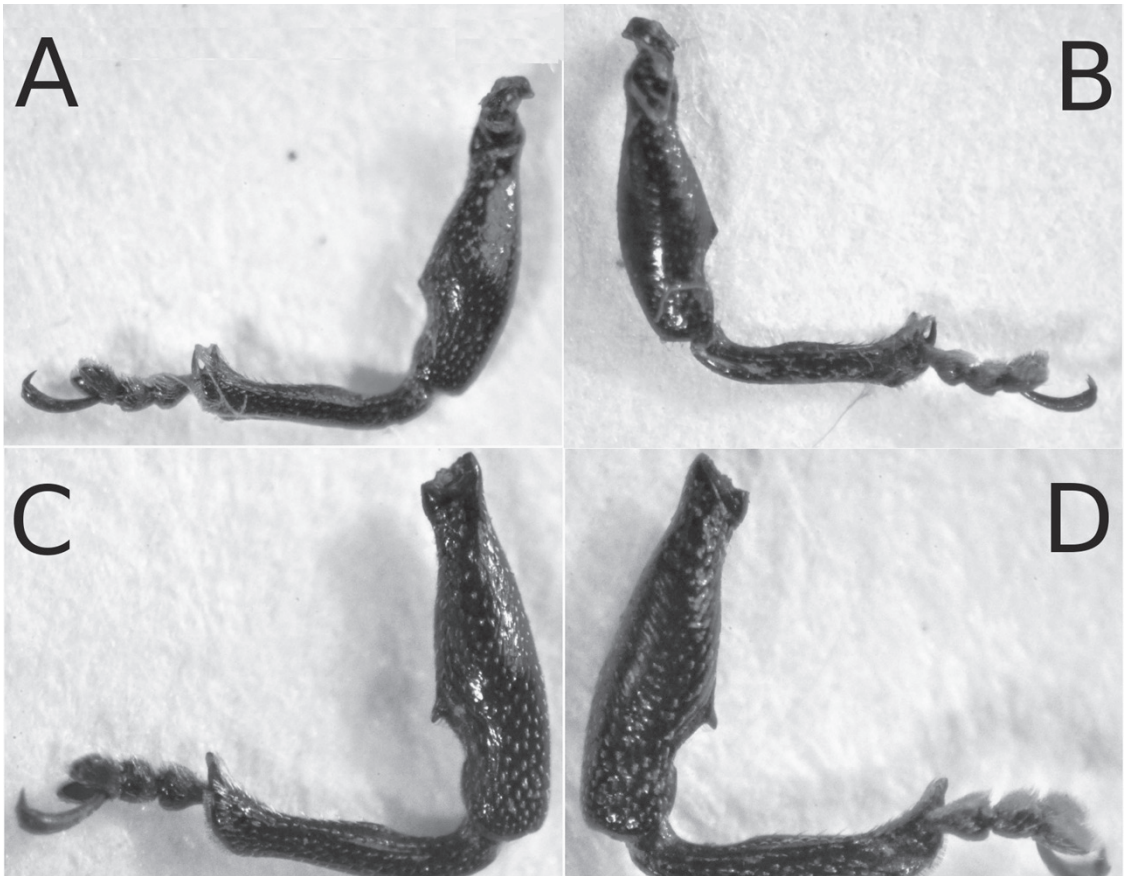

Fig. 1. Anterior view (A) and posterior view (B) female weevil: profemur with weak, subacute, angulate, internal process and protibia with uncus and mucro present; and anterior view (C) and posterior view (D) of male weevil: profemur with strong, curved, subacute, tooth-like, internal process and protibia lacking an uncus.

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