

External rostral characters for differentiation of sexes in the biological control agent *Mecinus janthinus* (Coleoptera: Curculionidae)

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Abstract—The stem-boring weevil, *Mecinus janthinus* (Germar), is a promising, well established classical biological control agent for the exotic invasive weed Dalmatian toadflax (*Linaria dalmatica* (L.) Mill.) (Scrophulariaceae). In this paper we present readily apparent rostral characters that can be used for sex differentiation of live stem-boring weevils at low magnification. These characters can be used by workers with little training to accurately sex weevils and can be used to rapidly determine sex ratios of field populations to improve timing of collection for redistribution.

Résumé—*Mecinus janthinus* (Germar) est un agent bien établi contrôle biologique classique qui promet de contrôler la mauvaise herbe exotique envahissant, Dalmatian toadflax (*Linaria dalmatica* (L.) Mill.) (Scrophulariaceae). Ici nous offrons caractères de rostre qui pourrait être utile à distinguer facilement de sexe des caladres vivantes à magnification petite. Les caractères demandent instruction minime de déterminer exactement le sexe d'une calandre, et ils pourraient être utiles de déterminer les proportions des sexes de populations aux changes pour perfectionner les occasions de rassembler pour repartition.

The stem-boring weevil, *Mecinus janthinus* (Germar) (Coleoptera: Curculionidae), was introduced to North America for the biological control of Dalmatian toadflax (*Linaria dalmatica* (L.) Mill.) (Scrophulariaceae) in British Columbia, Canada, in 1991 (De Clerck-Floate and Harris 2002). Since then it has become widely distributed throughout the southern parts of the western provinces of Canada and the northwestern United States through natural dispersion and active redistribution (Wilson *et al.* 2005). In British Columbia, *M. janthinus* populations increased to outbreak levels and 100% of the Dalmatian toadflax was injured within 4 years of initial release (De Clerck-Floate and Harris 2002).

A balanced sex ratio among biological control agents destined for redistribution is an essential factor in the successful establishment of new releases (Hopper and Roush 1993; Grevstad 1999; Coombs 2004). Sex ratio can vary within a season owing to protandry, as well as across seasons as population density changes. For instance, in *M. janthinus* the sex ratio shifted from 50:50 towards a ratio with a female bias

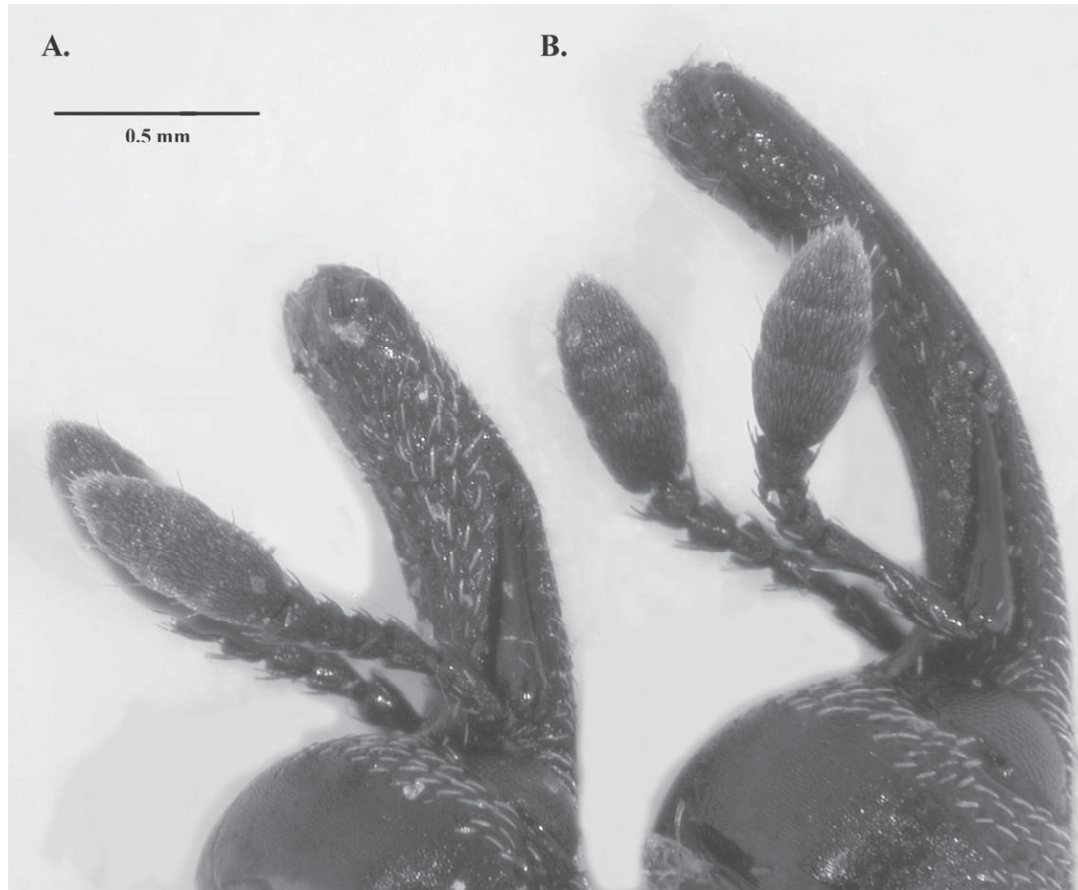
(68% female) as sites reached outbreak stages in British Columbia (Carney 2003; Nowierski 2004). Biological control agents are collected for redistribution from sites experiencing outbreak populations early in the season to ensure release prior to peak oviposition (Nowierski 2004). The ability to rapidly determine the sex ratio of field populations or of collections destined for release is therefore important for ensuring that the sex ratio of collected agents is balanced. Previously, two leg characters have been reported as useful for the rapid differentiation of fully immobilized (chilled) live stem-boring weevils under a dissecting microscope (Carney *et al.* 2004). Here, we present additional external morphological characters that can be used to differentiate male and female *M. janthinus* with minimal training and manipulation at low levels of magnification.

Many weevils have sexually dimorphic rostral characters, where females have a longer, more slender rostrum than males (Miles 1923). The length and width of the rostrum alone are too variable to be useful for some weevil species, such as *Anthonomus pomorum* L. (Duan *et*

Received 10 April 2006. Accepted 2 February 2007.

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Fig. 1. Male *Mecinus janthinus* weevils (A) have a short, broad rostrum with stout setae laterally along the entire length of the rostrum. Female weevils (B) have a longer, more slender rostrum than males with only a few setae on the rostrum distal to the antennal socket.



al. 1999). When combined with a secondary character, however, size of the rostrum has been used successfully in sex determination for other species (Reddy 1951; Halstead 1963; Dix and All 1985, 1986). Secondary characters used include differential punctation of the rostrum between males and females and differential antennal placement (Fowler 1891).

In *M. janthinus*, males have a short, broad rostrum with stout setae occurring laterally along the entire length (Fig. 1a). Females have a longer, more slender rostrum with a few setae at the tip (Fig. 1b). The rostrum of male stem-boring weevils appears duller because of the presence of setae, and that of females appears shinier because of the lack of setae.

We used the rostrum length and the density of setae on the rostrum to rapidly separate 2300 stem-boring weevils by gender and dissected 400 weevils to verify the efficacy of the rostral

characters. Weevils were collected on Dalmatian toadflax in Washington State and shipped to Montana State University within 48 h of collection. Weevils were retained under constant refrigeration at 4 °C until sexed. Rostral characters were assessed using a Leica MZ9.5 binocular microscope with 6.3× to 60× magnification (Leica Microsystems Inc., Bannockburn, Illinois). The gender of sexed weevils was verified using both the leg characters presented in Carney *et al.* (2004) and subsequent dissection to determine the presence or absence of a well-sclerotized aedeagus. Photographs were taken using a JVC digital camera (KY-F75U, JVC Professional Products Company, Cypress, California) mounted on a Leica MS5 stereomicroscope (Leica Microsystems Inc.) and edited using Auto-Montage Pro software (Version 5.03.0020 Beta, © 2005 Synoptics Inc., Frederick, Maryland). Additional photo editing was

performed using Adobe PhotoElements 4.0 (Adobe Systems Inc., San Jose, California).

Two thousand weevils were visually sexed by M. Schat using the rostral characters within 24 h of their arrival in the laboratory. One hundred weevils were frozen at -20°C for gender verification. An additional 300 weevils were separated into groups of 100 and stored at 4°C until three volunteers with little to no experience with *M. janthinus* were able to examine them. Volunteers were provided with a written description of the rostral characters listed above, a 35 mm petri dish containing two female and two male weevils, and a binocular microscope. Each volunteer was given as much time as needed to study the rostral characters and to sex one container of 100 weevils. Sexed weevils were separated into vials labeled with the gender of the weevils and the volunteer's name and placed in a freezer for later gender verification.

It took us approximately 16 h to sex 2000 weevils using rostral characters. Based on the 100 weevils frozen for dissection, we were 99% accurate. Each volunteer spent between 1.5 and 2 h familiarizing themselves with the characters and separating the weevils. The accuracy of the volunteers was 94%, 99%, and 86%. The overall accuracy for the 400 weevils dissected was 94% (SD 6.14), with 13 females erroneously classified as males and 9 males classified as females.

Although our overall accuracy was lower than the 99% accuracy reported by Carney *et al.* (2004) for the leg characters, the error rate we observed in this trial is also unlikely to have significant effects on sex ratio determination in release programs. The two individuals with the most experience with *M. janthinus* were as accurate using the rostral characters as Carney *et al.* (2004) were with the leg characters. Significantly less manipulation of the weevil was required to see the characters on the rostrum than characters on the leg, which may help retain maximal vigor of these biological control agents. The length and density of setae on the rostrum can be seen either laterally or dorsally by gently holding the adult weevils steady by the thorax in the microscope or hand lens field of view using a pair of featherweight forceps (BioQuip Products, Rancho Dominguez, California). It is possible to assess the rostral characters with a lower magnification (10 $\times$) than

that required to scrutinize the leg characters (40 $\times$).

We believe that the combination of length and breadth of the rostrum and the density of setae along the length of the rostrum are suitable characters for rapid field and laboratory differentiation of *M. janthinus*. The rostral characters presented here can be used by workers with little training and are sufficient for rapid and accurate evaluation of sex-ratio trends in field populations and in collections being prepared for release.

We thank J. Copeland, J. Marquez, and R. Davis for sexing weevils. We also thank M. Ivie for the use of the photographic system used to generate photographs of the weevils, R. Miller for assistance with technical terms, and D. Weaver for the use of the binocular microscope and for providing comments on earlier versions of the manuscript. We appreciate the early contributions of B. FitzGerald and K. Murray to the development of this method.

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