A Technique to Morphologically Differentiate Larvae of *Diabrotica virgifera virgifera* and *D. barberi* (Coleoptera: Chrysomelidae)

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte and northern corn rootworm (NCR) *Diabrotica barberi* Smith are key pests of corn in the north-central United States (Chiang, 1973; Levine and Oloymi-Sadeghi, 1991). Both species are commonly found as larvae in the same cornfields in areas where geographic distributions overlap. Attempts to distinguish larvae of each species using external characters have proven to be difficult. Mendoza and Peters (1964) reported that differences in anal plate (pygidial shield) morphology could be used to separate larvae of each species. The WCR generally has a notched, dark brown anal plate whereas the anal plate of the NCR is oval and pale. However, Piedrahita et al., (1985) found that these characteristics were not consistent in second-third instar larvae reducing the diagnostic utility of the characters (i.e., third instar NCR were misidentified as WCR 52% of the time). Electrophoretic and molecular techniques have proven to be more reliable diagnostic tools. Piedrahita et al., (1985) surveyed 20 enzyme systems and identified differences among NCR and WCR larvae using horizontal starch electrophoresis. Clark et al., (2001) and Roehrdanz (2003) utilized PCR-RFLP and multiplex PCR respectively, to identify mitochondrial differences among each species. This paper reports an alternative and reliable way to differentiate WCR and NCR larvae using morphological characters on the sclerotized head capsule of each species.

Materials and Methods

Each larval stage was collected from both species and stored in vials filled with 70% ethyl alcohol. Instars were determined by head capsule width as described by George and Hinz (1966) and Hammack et al., (2003). Larvae used to develop the diagnostic technique were offspring of NCR adults collected in southwest Minnesota in 2004 and WCR adults from the diapause colony maintained by the USDA North Central Agricultural Research Laboratory, Brookings, S.D.

A total of 70 larvae per instar were examined per species to develop the diagnostic technique. A WildM5A dissecting microscope (Wild Heerbrugg, Switzerland) capable of 50× magnification and fitted with an eyepiece reticle (20× magnification; 25 subdivisions in 1 mm increments at 6× and 50 subdivisions in 1 mm increments at 12×), calibrated with a ruler (Fine Science Tools, San Francisco, CA), was used to examine and measure larval head capsule features. The relative distance between head capsule frontal sutures and the median endocarina was used to distinguish each species. A dorsal view of the rootworm head capsule at 6× and 12× magnification reveals a Y-shaped epicranial suture which consists of the epicranial stem (coronal suture) at the base of the head capsule and the two frontal sutures (arms) which terminate at the two antennal insertions situated laterally on the frons (Fig. 1). From a dorsal perspective, the distance of the left frontal arm to the median endocarina was determined at a specific distance anterad of the epicranial stem and frontal suture juncture to differentiate between western and northern corn rootworm larvae (Fig. 1B). The distance anterad of the epicranial stem was 0.04 mm (40 microns) at 12× magnification for first instars, 0.08 mm (80 microns) at 12× magnification for second instars, and 0.12 mm (120 microns) at 6× magnification for third instars.

After diagnostic differences among species were established for each instar, a blind test was conducted to determine if the technique could be used to correctly identify larvae to species. Twenty WCR and NCR larvae of each instar were held individually in numbered 2-dram vials. Within instar, individual vial numbers were randomly chosen and the diagnostic distance between frontal suture and median endocarina was measured on each larval head capsule as described above. The blind test procedure was repeated five times.