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SHORT COMMUNICATION

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 Oloumi-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 Hintz (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
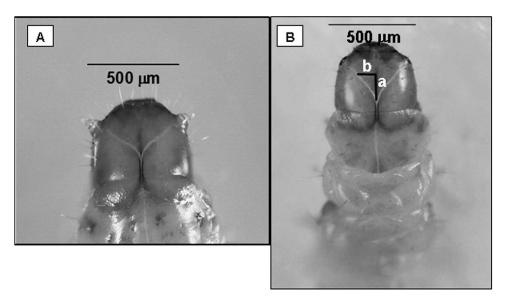
Fig. 1. Dorsal view of the larval head capsules of third instar larvae. A, western corn rootworm; B, northern corn rootworm, diagnostic distance from left frontal arm of epicranial suture to the median endocarina (b) measured at a specific distance anterad of the epicranial stem (a) for each instar.

times (i.e., each blind cohort: 20 WCR and 20 NCR; 5 replications, total n within instar and across species = 200) and then the percentage of larvae identified correctly was calculated per species and instar.

Results and Discussion

The ranges of the distance between head capsule frontal suture and the median endocarina at the appropriate distance anterad of the epicranial stem were discrete (no overlap) among instars and species (Table 1). Therefore, in combination with head capsule measurements to determine instar, epicranial suture measurements can be used to distinguish between NCR and WCR larvae.

The diagnostic accuracy in blind tests for each instar (mean percentage $\pm$ SE, $n = 200$) was 97.5 ± 0.01, first instar; 99.0 ± 0.06, second instar; and 100.0 ± 0, third instar. Within first instar, 4% of WCR and 1% of NCR larvae were misidentified; within second instar, 1% of each species was misidentified. Several factors may have contributed to some misidentification of first and second instars. Larvae of these stages were more difficult to handle than third instars. The small size and the curvature of the head capsule made it more difficult to correctly position first and second instars under the microscope prior to measurement.

Table 1. Head capsule measurements that separate western and northern corn rootworm larvae.

Species[†]	1st instar at 12× magnification*			2nd instar at 12× magnification*			3rd instar at 6× magnification*		
		b			b			b	
	a	Range	Mean ± SD[‡]	a	Range	Mean ± SD	a	Range	Mean ± SD
WCR	40	30–40	37 ± 4	80	70–80	77 ± 5	120	120–140	121 ± 3
NCR	40	20–25	21 ± 2	80	50–60	59 ± 3	120	70–90	80 ± 4

* a = distance in μm anterad of epicranial stem on the median endocarina.
b = distances in μm recorded from left frontal arm to median endocarina.
Sample size = 140 (70 WCR larvae + 70 NCR larvae).
† WCR = western corn rootworm, NCR = northern corn rootworm.
‡ Standard deviation.

Although untested, misidentification of first and second instars may possibly be avoided by making measurements under higher magnification than reported in this paper.

The head capsule has to be present to use the diagnostic technique described above, however, because the head is sclerotized, it is usually recovered when soil or root samples are processed to recover larvae. This morphological technique is a cost-effective alternative to existing electrophoretic or molecular diagnostic techniques as it requires minimal supplies and equipment, is easy to learn, and can be applied without destruction of specimens.

Acknowledgments

Literature Cited

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annual Review of Entomology 18:47–72.

Clark, T. L., L. J. Meinke, and J. E. Foster. 2001. PCR-RFLP of mitochondrial COI DNA provides diagnostic markers for selected *Diabrotica* species (Coleoptera: Chrysomelidae). Bulletin of Entomological Research 91:419–427.

George, B. W., and A. M. Hintz. 1966. Immature stages of the western corn rootworm. Journal of Economic Entomology 59:1139–1141.

Hammack, L., M. M. Ellsbury, R. L. Roehrdanz, and J. L. Pikul. 2003. Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). Journal of Economic Entomology 96:1153–1159.

Jackson, J. J. 1986. Rearing and handling of *Diabrotica virgifera* and *Diabrotica undecimpunctata howardi*. *In* J. L. Krysan and T. A. Miller (eds.), Methods for the Study of Pest Diabrotica, pp. 25–47. Springer-Verlag, New York, New York. xx + 260 pp.

Levine, E., and H. Oloumi-Sadeghi. 1991. Management of Diabroticite rootworms in corn. Annual Review of Entomology 36:229–255.

Mendoza, C. E., and D. C. Peters. 1964. Species differentiation among mature larvae of *Diabrotica undecimpunctata howardi*, *D. virgifera*, and *D. longicornis*. Journal of the Kansas Entomological Society 37:123–125.

Piedrahita, O., C. R. Ellis, and J. P. Bogart. 1985. Electrophoretic identification of the larvae of *Diabrotica barberi* and *D. virgifera virgifera* (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America 78:537–540.

Roehrdanz, R. L. 2003. Multiplex polymerase chain reaction method for differentiating western and northern corn rootworm larvae (Coleoptera: Chrysomelidae). Journal of Economic Entomology 96:669–672.