

Histopathology and *in situ* Hybridization of Coatomer (*CopI*) Knockdown in Western Corn Rootworm (*Diabrotica virgifera virgifera*)

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The US is the world's largest producer of maize, with nearly 100 million acres allocated to maize production annually. Western corn rootworm (WCR; *Diabrotica virgifera virgifera*) is currently the major agronomically important pest of maize in the US Corn Belt. One of the most effective methods for protecting commercial maize crops from rootworm damage is through the incorporation of insect resistance proteins. Currently, a new class of insect resistance traits based on RNAi is being developed. RNAscope *in situ* hybridization (ISH) is a single molecule sensitivity detection method for localizing mRNA in tissue sections [1], and it was recently shown to be compatible with removable resin sections [1]. Resin preserves tissue ultrastructure with greater fidelity than traditional wax, and also allows for the use of thinner sections, both of which facilitate higher quality ISH analyses.

For histopathological studies, larvae were exposed to dsRNAs targeted to *CopIa*, *CopIb*, or *YFP* (negative control). After 96 hours of feeding, larvae were fixed, infiltrated with LR White, and heat polymerized. Larvae were sectioned longitudinally at 500 nm thickness on a DiATOME histoknife, stained, and imaged. For *in situ* hybridization, larvae were collected after 48 hours on diet containing dsRNAs, fixed, infiltrated with 3:1 butyl:methyl methacrylate, and UV polymerized, all at 4°C. Larvae were sectioned at 1 μm thickness on a DiATOME histoknife, sections were deplasticized, and analyzed with the Advanced Cell Diagnostics (ACD) RNAscope VS Assay on a Ventana Discovery Ultra. Sections were imaged on a Leica DM5000B microscope and a Leica DFC 7000T camera.

The native expression patterns of *CopIa* and *CopIb* were found to be very similar, with the majority of the *CopI* mRNA in the midgut enterocytes and the cells of the Malpighian tubules (Figure 1A and 1C). Nearly complete knockdown of the mRNAs from both *CopI* genes was observed after 48 hours of feeding with gene-specific dsRNAs (Figure 1B and 1D). Furthermore, this knockdown was observed to be systemic, as evidenced by loss of *CopI* transcripts from non-alimentary system organs throughout the body of the larva. There was also loss of *CopI* transcripts from fat body adipocytes and cells of the ventral nerve cord, which are both organs distal to the alimentary system.

The most obvious histological impact of the loss of *CopI* transcripts on the morphology of WCR larvae was severe damage to the midgut enterocytes and constriction of the anterior midgut circular muscles (Figure 2C, 2D, 2E, and 2F). *CopIb* dsRNA seems to have caused slightly more damage to the AMG, and especially the MMG and PMG regions, than the *CopIa* dsRNA. More subtle changes were noted to the Malpighian tubules and hindgut, both of which were observed to be swollen following *CopI* dsRNA treatments, and the hemolymph volume appeared dramatically reduced (Figure 2C and 2E). No damage to the midgut was observed with *YFP* dsRNA feeding (Figure 2A and 2B). For both targets, the near-complete loss of the midgut enterocytes is likely lethal to WCR larvae, as the loss of midgut enterocytes and the constriction of circular muscles caused by knockdown of *CopI* is analogous to the effects seen with several types of insecticidal proteins [2].

References:

- [1] Wang et al., *Journal of Molecular Diagnostics* **14** (2012), p. 22.
 [2] Bowling et al., *Toxins* **9** (2017), p. 1.

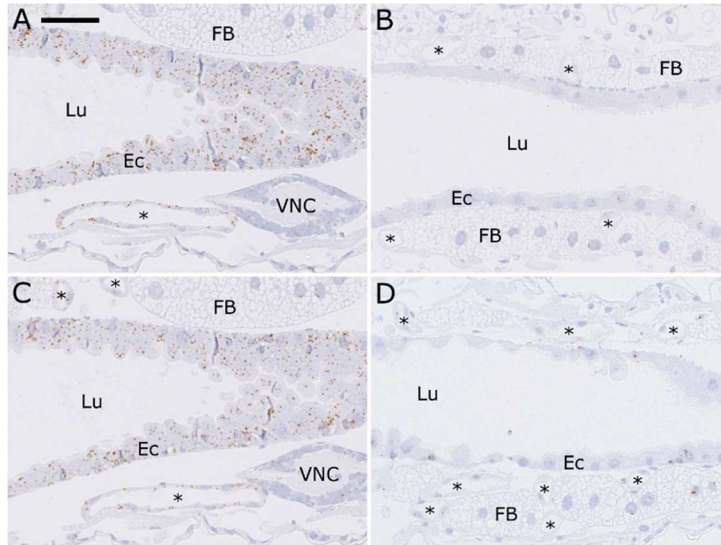


Figure 1. RNAscope ISH on longisections of WCR. Transcripts detected with DAB (brown), sections counterstained with hematoxylin. A.) Median section of a *YFP* dsRNA-fed (negative control) WCR larva showing *CopIa* transcripts most abundant in midgut enterocytes, and to a lesser extent in the Malpighian tubules. B.) Larva fed *CopIa* dsRNA, showing a dramatic reduction of *CopIa* mRNA in midgut and Malpighian tubule cells. C.) A median section of a negative control larva showing the native *CopIb* expression pattern, which is very similar to *CopIa*. D.) Larva fed *CopIb* dsRNA, showing a similar reduction of *CopIb* mRNA that was seen with *CopIa*. Ec = midgut enterocytes; VNC = ventral nerve cord ganglion; FB = fat body; Lu = midgut lumen; * = Malpighian tubule. Scale bar = 50 μ m.

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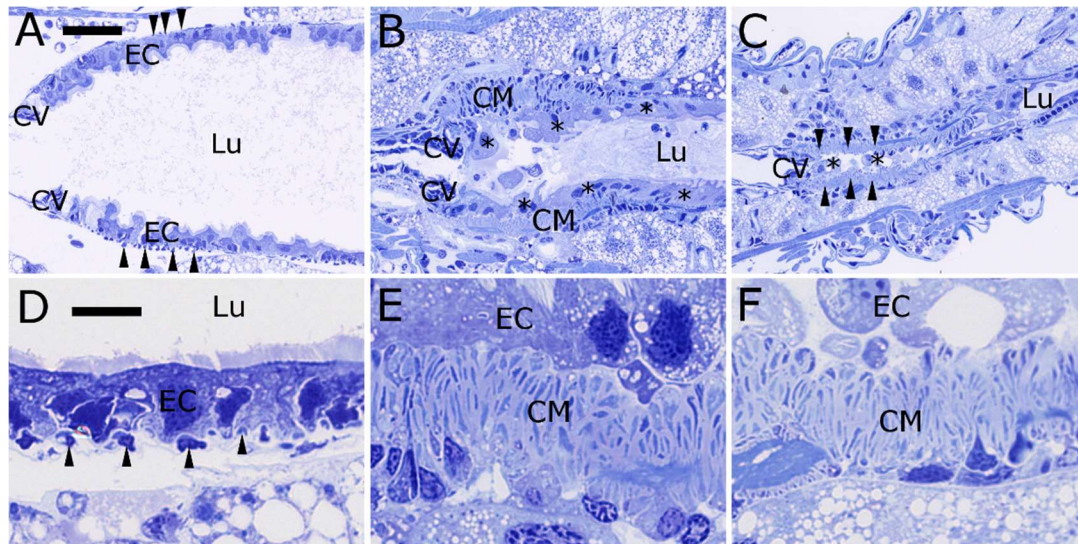


Figure 2. Histopathological impact of the loss of *CopIa* and *CopIb* mRNA. A. and D.) A larva fed *YFP* dsRNA, showing the normal appearance of the midgut. Larvae fed *CopIa* dsRNA (B and E) or *CopIb* dsRNA (C and F), showing dramatic damage to the anterior midgut cells, including lysis and shedding of the enterocytes and midgut stem cells, severe contraction of the circular muscle bands, and near-total closure of the midgut lumen. Lu = lumen; Ec = enterocytes; CM and arrowheads = circular muscles, * = enterocyte remnants. Scale bar A, B, C = 50 μ m; D, E, F = 10 μ m