MICROIMAGING OF *Bacillus thuringiensis* TOXIN-BINDING PROTEINS IN GYPSY MoTH LARVAL GUT USING CONFOCAL FLUORESCENCE MICROSCOPY

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**ABSTRACT**

After ingestion by susceptible insect larvae, *Bacillus thuringiensis* (Bt) insecticidal proteins bind to the brush border membranes of gut epithelial cells and disrupt the integrity of the plasma membrane by forming pores that lead to cell swelling and lysis. The presence or absence of specific Bt toxin-binding molecules on the brush border membrane of gut cells plays a critical role in determining the insecticidal activity of different Bt toxins. In gypsy moth, a membrane-anchored aminopeptidase (APN-1) and a 270 kDa glycoconjugate (BTR-270) have been identified as two major high-affinity binding proteins for Bt toxins. In other insects, cadherins (Cads) and an alkaline phosphatase (ALP) have also been characterized as Bt toxin-binding proteins. In this study, immunohistochemical localization of gypsy moth APN-1, BTR-270 and cadherin (LdCad) was compared to Bt-toxin binding sites localized using AF546 fluorescently labeled Bt toxins produced by the HD-1 Bt strain using confocal laser scanning microscopy. Microvilli on the brush border membrane were found to be exclusively decorated with the antibodies directed towards APN and BTR-270 in the midgut and hindgut regions in both 3rd and 5th instar gypsy moth larval gut sections. The fluorescently labeled Cry1A toxin binding sites were found to be co-localized with the toxin-binding receptors identified in the gypsy moth.