

COST862: Bacterial toxins for insect control

Bt Cry toxin structure: implications for resistance
management, risk assessment and the development
of new biopesticides



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**Processing of *Bt*-corn-toxin Cry3Bb1 in the midgut
of Western Corn Rootworm**

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The Western Corn Rootworm (WCR) is one of the economical most important pests in corn (*Zea mays* L.). One possibility for its control is the cultivation of transgenic corn expressing the *Bacillus thuringiensis* toxin Cry3Bb1. However, cultivation of *B.t.*-corn may result in resistant pest populations.

The potential of insect resistance to *B.t.*-toxins can be located at any step of the toxic pathway: ingestion, solubilization, proteolytic processing, binding to specific receptors, membrane integration, pore formation, cell lysis, and insect death. However, in other *B.t.*-toxin-pest-systems, the resistance mechanisms are mainly proteinase- or receptor-mediated.

To establish reference systems for the characterization of proteinase-mediated resistance mechanisms in potential available resistant individuals, studies on proteinase activities and proteolytical processing of the *B.t.*-corn-toxin Cry3Bb1 were carried out with midgut fluid of susceptible WCR 3rd instar larvae (pH 5,75).

Studies on proteinase activities were conducted using photometrical tests. As a result, the digestive serin-endopeptidases trypsin, chymotrypsin, and elastase as well as aminopeptidase – an exopeptidase - were identified. Due to the acid midgut fluid, in *Chrysomelidae* cysteine-endopeptidases were expected. Accordingly, high activities of cathepsin L, papain, cathepsin B, and cathepsin H were found (Kaiser-Alexnat *et al.*, 2006).

By *in vitro* incubation with WCR midgut fluid, the *B.t.*-corn-toxin Cry3Bb1 was processed. To identify the midgut proteinases, which are responsible for the proteolytical processing, available model proteinases were used to simulate the midgut conditions.

In order to establish a reference system for the characterization of receptor-mediated resistance mechanisms, binding analysis were carried out with midgut epithelium of susceptible WCR 3rd instar larvae. From the gut

epithelium brush border membrane vesicles (BBMV's) were prepared. To proof the toxin binding and characterize the receptors, biotin labeled *B.t.*-corn-toxin Cry3Bb1 and the ligand-blot technique as well as streptavidin-horseradish-peroxidase-conjugat and the ECL system were used.

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- Kaiser-Alexnat, R., G.-A. Langenbruch, S. Feiertag: Untersuchungen zur Aktivierung von *B.t.*-Toxinen beim Maiszünsler (*Ostrinia nubilalis*) und Westlichen Maiswurzelbohler (*Diabrotica virgifera virgifera*). Mitt. Biol. Bundesanst. Land- Forstwirtsch. Heft 400, 360-361, 2006.

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