

Selection of resistant European Corn Borer (*Ostrinia nubilalis*) to *Bt*-corn and preliminary studies for the biochemical characterization

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Abstract: One possibility to control the European Corn Borer is the cultivation of *Bt*-corn. However, this can result in the development of resistant pest populations. To control this, larvae were collected in *Bt*-corn fields and in F₂ generation tested for resistance to the respective *Bt*-toxin. As preliminary studies for the biochemical characterization of possible mechanisms for resistance, studies on proteinase activities were conducted in the midgut of 5th instars of a susceptible German ECB population. The digestive proteinases trypsin, chymotrypsin, elastase and aminopeptidase could be identified.

Key words: European Corn Borer, *Ostrinia nubilalis*, *Bt*-corn, resistance, midgut, proteinases, trypsin, chymotrypsin, elastase, aminopeptidase

Introduction

The European Corn Borer (ECB, *Ostrinia nubilalis*) is one of the economical most important pests in corn (*Zea mays* L.) in the USA and Europe. As an effective control technology, transgenic corn expressing a truncated Cry1Ab toxin from *Bacillus thuringiensis* has been developed. The resulting *Bt*-corn produces its own protective pesticide, which is highly insecticidal to ECB larvae. However, widespread cultivation of *Bt*-corn could result in the development of resistant pest populations.

In general, the potential of insect resistance to toxins of *Bacillus thuringiensis* can be located at any step of the toxic pathway: ingestion, pH-dependent solubilization, proteolytic processing; binding to specific receptors, membrane integration; pore formation; cell lysis and finally insect death (Ferré & van Rie, 2002).

In other pest-*Bt*-toxin-systems, two main mechanisms of resistance to *Bt*-toxins, one proteinase-mediated and the other receptor-mediated, have been identified (Oppert *et al.*, 1997, McGaughey & Oppert, 1998). Proteinase-mediated resistance mechanisms includes alterations in the midgut enzymatic activity such as degradation and inactivation of the toxin. Receptor-mediated resistance mechanisms includes resistance to *Bt*-toxins correlated with altered toxin binding to midgut receptors. A change in physiological parameters e.g. pH can influence resistance as well.

Materials and methods

Photometrical studies with pure midgut sap of 5th instars larvae were conducted using chromogenic substrates and specific inhibitors [N-benzoyl-L-arg-p-nitroanilide (BApNA) and soybean-trypsin-inhibitor (SBTI) for trypsin-like proteinases, N-succinyl-ala-ala-phe-p-nitroanilide (SAAFpNA) and N-tosyl-L-phe chloromethylketone (TPCK) for chymotrypsin-

like proteinases, N-succinyl-ala-ala-pro-leu-p-nitroanilide (SAAPLpNA) and elastatinal for elastase-like proteinases as well as leu-p-nitroanilide (LpNA) and bestatin for aminopeptidase.

Results and discussion

Screening for resistance

The cultivation of *Bt*-corn could result in the development of resistant ECB pest populations. To control this, in 2001 70 larvae have been collected in about 150,000 *Bt*-corn plants (Event MON810). Biotests with 1st instars larvae in F₂ generation revealed no resistant individuals yet. The 805 larvae of 760,000 *Bt*-corn plants collected in 2002 will be tested in summer 2003.

Preliminary studies for the biochemical characterization

To establish preliminary reference systems for the characterization of potential available resistant individuals, studies on proteinase activities and on receptor binding in the midgut of susceptible 5th instars larvae were carried out.

Studies on proteinase activities

Houseman & Chin (1995) identified the digestive proteinases trypsin, chymotrypsin, elastase and aminopeptidase in the midgut of a Canadian population of *Ostrinia nubilalis*. To compare these results with German ECB and to establish a reference system for the identification of qualitative and quantitative changes in proteinase-activities (e.g. amount, molecular weight, substrate specificity) of resistant larvae, photometrical studies were carried out.

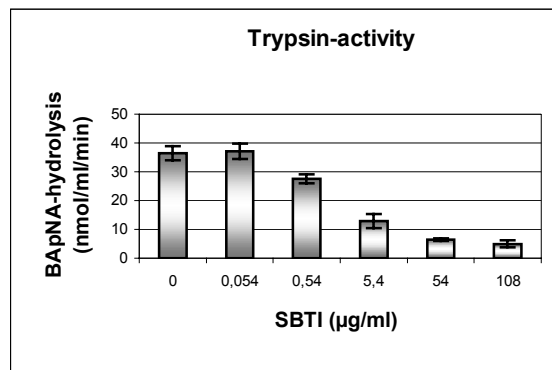


Figure 1. Trypsin-activity in midgut sap of susceptible 5th instar larvae.

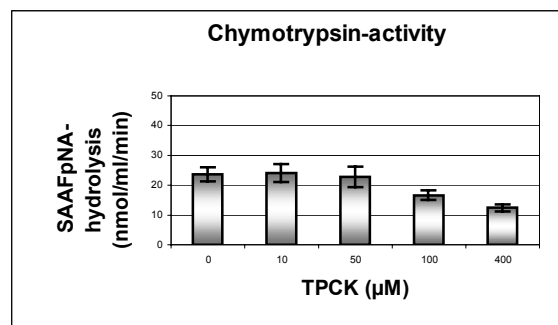


Figure 2. Chymotrypsin-activity in midgut sap of susceptible 5th instar larvae.

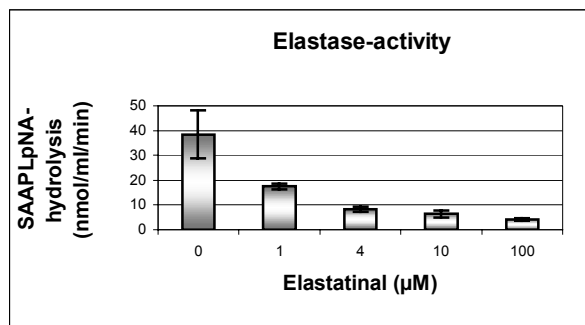


Figure 3. Elastase-activity in midgut sap of susceptible 5th instar larvae.

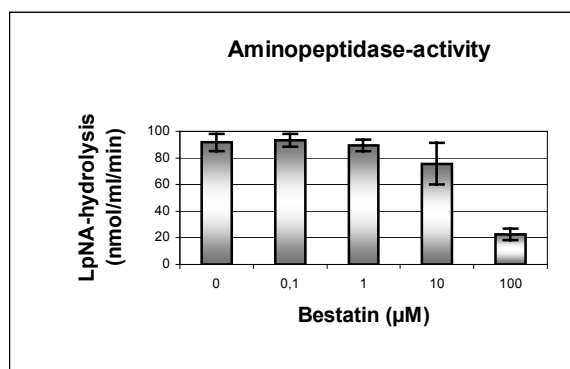


Figure 4. Aminopeptidase-activity in midgut sap of susceptible 5th instar larvae.

Similar to the above described results, in the midgut lumen of German susceptible 5th instar larvae the digestive proteinases trypsin (Fig. 1), chymotrypsin (Fig. 2), elastase (Fig. 3) and aminopeptidase (Fig. 4) could be identified. Besides, it is to point out, that the activity of the aminopeptidase was about twice higher compared to the other proteinases.

Studies on receptor binding

For binding analyses of the midgut receptors, intact brush border membrane vesicles (BBMV) have been isolated (Wolfersberger *et al.*, 1987). To proof the toxin binding and to characterize the receptor, ligand-blot was carried out. Binding was demonstrated using biotin labeled toxin that was detected with streptavidin-horseradish-peroxidase-conjugat and the ECL western-blotting system.

Studies on pH-value

The pH measurements were conducted in pure larval midgut sap of ECB 5th instar larvae. In larvae fed on corn leaves before sample preparation, a pH of 7.5 was measured, in hungry ones it was pH 7.2, and in those reared on artificial diet pH 7.3.

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