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THURSDAY PM

CONTRIBUTED PAPERS Thursday, 16:30-18:30

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Contributed paper. Thursday, 16:30. **219**

B.t.-toxins in the midgut of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte)

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The Western corn rootworm (WCR) is one of the economical most important pests in corn. For its control, genes encoding *Bacillus thuringiensis* toxins (e.g. Cry3Bb1, Cry3A, Cry34Ab1/Cry35Ab1) were introduced into corn. The cultivation of transgenic corn expressing the respective *B.t.*-toxins may result in the development of resistant pest populations. In general, the resistance of insects to *B.t.*-toxins can be located at any step of the toxic pathway. However, in other *B.t.*-toxin-pest-systems, the resistance mechanisms are mainly proteinase- or receptor-mediated. To establish reference systems for the identification of resistance mechanisms in potential available resistant individuals, studies on proteinase activities and binding analysis were carried out with midgut fluid and midgut epithelium of WCR 3rd instar larvae. Studies on the identification and quantification of proteinase activities in the midgut fluid were conducted using photometrical tests with specific chromogenic substrates - mainly peptidyl-*p*-nitroanilid (*p*NA) - and specific inhibitors. As a result, the digestive serine endopeptidases trypsin, chymotrypsin, and elastase were identified. Besides, high digestive activities were observed for the serine endopeptidases cathepsin G, plasmin, and thrombin. 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 observed in the midgut fluid of WCR (pH 5.75). Besides, the metallo endopeptidase saccharolysin as well as the exopeptidases aminopeptidase and an omegapeptidase - acylaminoacylpeptidase - were identified. For aspartic endopeptidases no specific *p*NA substrates were available. Using the general proteinase substrate azocasein, the activity of the aspartic endopeptidase pepsin was demonstrated. Furthermore, with midgut epithelium binding analysis were carried out to study binding site competition of *B.t.*-toxins Cry3Bb1 *vs.* Cry34Ab1/Cry35Ab1. From the midgut epithelium brush border membrane vesicles (BBMV's) were prepared. To examine the toxin binding, biotin labeled *B.t.*-corn-toxins, and the ligand-blot technique as well as streptavidin-horseradish-peroxidase-conjugat and the ECL system were used.

Contributed paper. Thursday, 16:45. **220**

Mutations in the *cadherin* gene in a *O. nubilalis* strain selected for Cry1Ab resistance.

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An *Ostrinia nubilalis* colony was selected for resistance to *Bacillus thuringiensis* Cry1Ab protoxin. Previous work evidenced the implication of more than one genetic locus and the reduction of the cadherin receptor. We have now determined the contribution of the *cadherin* gene to the overall Cry1Ab resistance in this strain. Individual larval midguts from susceptible (Europe-S) and resistant (Europe-R) insects were used to prepare cDNAs from the *cadherin* gene. We found major mutations that suggested highly structural deficient proteins because they introduced premature termination codons (PTC) and/or large deletions (1383-1701 bp). In the resistant strain, these mutations were found in 13 out of 20 insects analyzed. In the susceptible strain, only one PTC was detected among the major mutations, but always in heterozygotes. To check for the contribution of the major mutations to the resistance, Europe-R insects were subjected to a high dose of Cry1Ab protoxin. The analysis of the survivors showed that major mutations were absent. These results support a polygenic inheritance of resistance in the Europe-R strain, in which mutations in the *cadherin* gene would contribute to resistance by means of an additive effect.

Contributed paper. Thursday, 17:00. **221**

Bacillus thuringiensis Cry2A toxins bind saturably to a common site in the midgut of *Helicoverpa armigera*

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For a long time, it has been assumed that the mode of action of Cry2A toxins was unique due to the apparent non-specific and non-saturable binding to a practically unlimited number of membrane receptors. However, this assumption seems to be in contrast with the highly homologous tertiary structure among the 3-domain Cry toxins, including Cry2A toxins. To verify the existing data on the particular mode of action of Cry2A toxins, binding assays were carried out with ^{125}I-Cry2Ab and ^{125}I-Cry1Ac. Saturation and competition assays showed that Cry2Ab does bind with high affinity, in a specific and saturable manner, to brush border membrane vesicles of *Helicoverpa armigera* and *H. zea*. Heterologous competition assays in *H. armigera* showed the occurrence of a common binding site for three toxins belonging to the Cry2A family (Cry2Aa, Cry2Ab, and Cry2Ae), but not for Cry1Ac. Our results question interpretations of published data of binding assays with Cry2A toxins from other authors and establish the basis of the mode of action of Cry2A toxins.

Contributed paper. Thursday, 17:15. **222**

The importance of antibiosis and inter-specific competition in the ecology of *Bacillus thuringiensis*

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Understanding that factors limiting pathogen growth and fitness can give important insight into improving their use in pest control. Here, we investigated to what extent inter-specific competition with other micro-organisms determines the biology and ecology of Bacillus thuringiensis. Firstly, we examined the distribution and expression of antibiotic genes (zwittermicin a) in pathogenic and non-pathogenic members of the Bacillus cereus group using PCR and phenotypic assays of virulence and antibiosis. Secondly, we passaged a B. thuringiensis strain derived from DiPel (Btk rifR) with low antibiotic expression through larvae of the diamondback moth, Plutella xylostella, and tested for changes in levels of antibiosis. We found that levels of expressed antibiosis and positive amplification of an antibiotic gene (zwittermicin orf7) were very good predictors of whether strains expressed bi-pyrimidal toxin crystals. These traits were better predictors of toxin expression than possession of cry genes since many strains that possessed cry genes failed to express toxins. Passage of Btk rifR through P. xylostella resulted in significant increases in levels of detectable antibiosis in three independent lineages. We conclude that antibiosis and inter-specific competition are important factors for the successful exploitation of hosts by pathogenic members of the B. cereus group.