Characterization of midgut Cadherin in Bt Cry3A-susceptible and resistant populations of the beetle Chrysomela tremulae

David Pauron, Manuella van Munster, Sylvie Augustin, Yannick Pauchet, Marcel Amichot, Marie-Paule Esposito

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Program and Abstracts

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replace old ones that perform sub-optimally and are losing efficacy. Any new therapy must be extremely cheap, be able to be produced in tremendous quantities to treat hundreds of millions of people, have a stable shelf life, and be capable of storage and delivery under adverse environmental conditions. Our research has uncovered a radical and unique new approach that solves each of these challenges: expression of vertebrate-safe, anthelmintic (anti-nematode) proteins in "probiotic-like" food-grade bacteria. Such bacteria can be produced cheaply, in great quantity, stored stably, and delivered under adverse conditions. Here we will discuss our work to develop such engineered bacterial therapy using the anthelmintic crystal protein Cry5B normally made by *Bacillus thuringiensis* (Bt). We will present data on how Cry5B can be expressed in a non-Bt bacterium related to food-grade bacteria, and the strong efficacy of such a bacterium in clearing hookworm infections in rodents. We will also update progress on engineering several food-grade bacteria to express Cry5B as a critical step towards implementation of this novel anthelmintic approach.

Contributed paper. Wednesday, 17:00. **153**

Strategies to address corn rootworm control challenges

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Corn Rootworm (CRW) *Bt* technologies containing Cry3Bb1 have been in the market for 10 years and have provided significant value to growers. However, increased damage from CRW in some fields has been observed in the last few years. Beginning in 2012, Best Management Practices (BMP's) were implemented to reduce performance inquiries in future years, as demonstrated by 2012 field results. This presentation will discuss the current status of CRW performance by Cry3Bb1 technologies in the US, and how the use of BMP's can extend the durability of current and future Cry3Bb1 technologies, including pyramiding Cry3Bb1 with Cry34/35 and dsRNA.

Contributed paper. Wednesday, 17:15. **154**

Characterization of midgut Cadherin in Bt Cry3A-susceptible and resistant populations of the beetle *Chrysomela tremulae*

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Field screening has previously allowed to detect poplar leaf beetles highly resistant to *Bacillus thuringiensis* Cry3Aa toxin. An isofemale line of *Chrysomela tremulae* (Ct) was selected on Cry3Aa-expressing poplars. Resistance to Cry3Aa was almost completely recessive and associated with a single autosomal locus. One family of putative receptor of Cry3Aa, i.e. aminopeptidases N, is apparently not affected in resistant insects : none of the 3 identified members of this family of proteases is differentially transcribed or display any amino-acid change when compared to the susceptible sequences. Pyrosequencing of *C. tremulae* larval midgut resulted in six cDNA contigs homologous to insect cadherins. These sequences were our starting point for cloning the full sequence of midgut epithelial cadherin in *C. tremulae*. Sequence analysis indicate that Ct cadherin is 1705 amino acid-long and organized in 10 predicted repeats. As expected, this protein is very similar to other coleopteran cadherins reported so far: it shares 98% similarity with *Diabrotica vergifera*'s one (another *Chrysomela*), 97% with *Tenebrio molitor* and 94% with *Tribolium castaneum* cadherins. Interestingly it also shares high similarity with several *Lepidoptera* (90-94%) and *Diptera* (90% with *Drosophila*) cadherins. Ct Cadherin is mainly expressed in the midgut of L1 larvae and adults. No qualitative or quantitative difference was detected in the cadherin transcripts of Cry3A-resistant beetles when compared to susceptible ones. However, several non silent mutations were found in the primary sequence of the resistant allele which might be related to an alteration of the binding capacity of the toxin to its target receptor.

Contributed paper. Wednesday, 17:30. **155 STU**

Inheritance of Cry1F resistance, cross-resistance and frequency of resistant alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Transgenic maize, *Zea maize* L., expressing the Cry1F protein from *Bacillus thuringiensis* (*Bt*) has been registered for *Spodoptera frugiperda* (J. E. Smith) control since 2003. Unexpected damage to Cry1F maize was reported in 2006 in Puerto Rico and Cry1F resistance in *S. frugiperda* was documented. Cry1F resistance in *S. frugiperda* represents the first instance of field failure associated with insect resistance to a *Bt* crop leading to withdrawal from the marketplace. The inheritance of Cry1F resistance was characterized in a *S. frugiperda* resistant strain originating from Puerto Rico which displayed >387-fold resistance to purified Cry1F. Inheritance experiments indicated that resistance is recessive, autosomal and conferred by a single locus. In addition, cross-resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ba, Cry2Aa and Vip3Aa was assessed in the Cry1F resistant strain. There was no significant cross-resistance to Cry1Aa, Cry1Ba and Cry2Aa, although only limited effects were observed in the susceptible strain. Vip3Aa was highly effective against susceptible and resistant insects indicating no cross-resistance with Cry1F. In contrast, significant cross-resistance (< 20-fold) was observed for both Cry1Ab and Cry1Ac. Because resistance was recessive and conferred by a single locus, an F_1 screen was used to measure the frequency of Cry1F resistant alleles from populations of Florida and Texas in 2010 and 2011. A frequency of 0.13 was found in Florida, while Texas populations had a resistant allele frequency of 0.015. Results indicate that resistance alleles exist among continental United States populations and are persistent in resistant populations (e.g. Puerto Rico).

WORKSHOP
Wednesday, 21:00-21:30

VIRUS DIVISION

Workshop. Wednesday, 21:00. **156**

Deep sequencing technology for arthropod virus discovery

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Arthropods are commonly infected with multiple viruses including sublethal, asymptomatic, and latent infections. However, conventional methods for virus isolation typically lack the sensitivity required for detection of viruses that are present in low abundance and traditional approaches have limitations for acquiring full length viral sequences. Next Generation sequencing (NGS) technologies have revolutionized virus discovery, and the study of virus prevalence. In this workshop, we will use identification of viral sequences from soybean aphid (*Aphis glycines*) and penaeid shrimp (*Litopenaeus setiferus*) to compare advantages and disadvantages of various NGS sequencing methods for virus discovery, specifically sequencing of small RNA, RNA-seq and viral RNA isolated from crudely extracted virion samples. We will also introduce bioinformatics methods used for analysis of transcriptome and small RNA data generated by high throughput sequencing technology for *de novo* assembly of viral sequences and identification of viral sequences from BLAST data. In addition, methods for generation of full-length or near full-length viral genome sequences will be discussed.