The object of VipHaSe was to study the mechanism of action of different Vip3A toxins in *Helicoverpa armigera* and *Spodoptera exigua* larvae and in particular to understand which key steps in the mode of action account for the differences in susceptibility. As a first step the toxicity was studied in terms of mortality of the proteins (lysate of *E. coli* expressing Vip3Aa, Vip3Af, Vip3Ae, Vip3Ad or ISP3F) by means of surface contamination bioassays with neonate larvae. The results obtained showed that the mortality induced by the most active Vip3A proteins (Vip3Aa, Vip3Af, Vip3Ae) was much lower compared to the toxicities displayed by the Cry toxins in both species, but the toxicity in terms of growth inhibition was very high. These results led us to hypothesize that there is a different mechanism of action between the two classes of proteins, Cry and Vip. On the other hand, in *Spodoptera frugiperda*, the bioassays revealed a high toxicity in terms in both mortality and growth inhibition of Vip3Aa, Vip3Af and Vip3Ae proteins. To perform biochemical analysis of the mechanism of action, highly purified proteins were necessary. *vip3Aa*, *vip3Af* and *vip3Ae* genes have been modified to contain a His tag sequence at the N terminus of the proteins and have been cloned in pMa5-8 and expressed in *Escherichia coli* WK6. The His tag facilitated the purification because the protoxin could be separated by affinity chromatography from the mixture of proteins contained in the lysate of *E. coli* expressing Vip3A proteins. Although a protocol to obtain high yields of highly pure proteins was successfully developed, toxicity bioassays in *S. frugiperda* and *S. exigua* neonate larvae showed a partial loss of activity (about ten-fold) in *S. frugiperda* and a complete loss of activity in *S. exigua*. Thus, a different protocol of purification by anion exchange chromatography was developed, unfortunately with results similar to the previous purification. It had to be taken into account that handling these proteins during purification processes led to a decrease in activity.

A polyclonal antibody raised in rabbit against Vip3Aa protoxin was produced. The quality of the antibody has been tested by Western blot analysis, and it was suitable for the detection of Vip3Aa and also other Vip3A proteins. It was very useful to confirm the presence of the Vip3A proteins in the different fractions obtained during the purification processes. It was also useful for preliminary studies of the Vip3A receptors and Vip3A mechanism of action at cellular level in progress in the host lab.

Since in *Spodoptera frugiperda* the bioassays revealed a high toxicity in terms of mortality of Vip3Aa, Vip3Af and Vip3Ae proteins, we decided to investigate which step in the mode of action was responsible for the differences in mortality of these two closely related *Spodoptera* species. The activation with midgut juice crude extracts did not show differences in the activation profiles. The separation of serine-proteases was carried out by chromatographic techniques from the midgut juice of both species. The detailed study of the activation process by the different fractions with serine-protease activity in the two species is in progress.

Regarding the labeling of the Vip3A proteins, an initial training was made on the labeling procedures of Cry proteins with iodine (125I). This technique is already well known in the host lab, which is one of the most important reference labs in the world regarding Cry binding studies. The labeled proteins (Cry1Ac and Cry2Ab) were successfully used for binding experiments and the results are part of two different publications regarding resistance to *Bt* toxins in *Helicoverpa* spp. A number of attempts were made in labeling of Vip3A proteins with iodine (125I) after trypsin activation. Bioassays performed to check the toxicity of the protein after activation showed a decrease in the activity against *S. frugiperda* in respect to the protoxin. Although the labeling of the proteins was obtained, the iodinated proteins were not able to bind specifically to the BBMV (brush border membrane vesicles) of susceptible insects. The labeling probably affects the epitopes involved in the binding to the receptors. Alternatively, the partial loss of activity detected after purification and the additional decrease after activation could account for protein modifications critical for the binding properties of the toxins. Ligand Blot experiments with biotinylated Vip3Aa with *S. frugiperda* BBMV proteins showed that most of the toxin binds non specifically to BBMV proteins. Only a faint band of low molecular weight appeared to bind specifically Vip3Aa.

A collaborating group from the Imperial College of London has selected a colony of *Heliothis virescens* resistant to Vip3Aa protoxin and they sent us the midguts isolated from susceptible and resistant insects in order to investigate the mechanism of resistance. To that purpose, activated Vip3Aa protein was biotinylated and the binding to the BBMV from both strains was analysed. Although the mechanism has not been clearly identified yet, preliminary results suggested that the most common mechanism of resistance, lack of binding, is not involved. Due to the qualitative nature of biotin labeling, it is under study a more quantitative approach to validate this preliminary analysis of the Vip3Aa mechanism of resistance.

The fellow had the possibility to work in a multicultural scientific environment and to establish collaborations worldwide with academic groups and companies leaders in the microbial control and in Bt mode of action. Results obtained on *Bacillus thuringiensis* mode of action and resistance are already included in three publications and will also be part of two additional publications in preparation.