Degradation of Cry1Ac protoxin can explain the decreased susceptibility in late instars of *Helicoverpa armigera*

Igor H. S. Silva[1]; Joacir do Nascimento[1]; Ariadne C. Sanches[1]; Janete A. Desiderio[1]; Fernando H. Valicente[2]; Mario Soberón[3]; Ricardo A. Polanczyk[1]; Alejandra Bravo[3]

Helicoverpa armigera (Hubner, 1805) (Lepidoptera: Noctuidae) is a polyphagous pest sensitive to Cry1Ac toxin from *Bacillus thuringiensis* (Bt). This insect is controlled by chemicals insecticides or transgenic plants expressing Cry1Ac. However, larvae from late instars are less sensitive to Cry toxins compared with early instars, reducing the efficacy of Cry toxins in the field. Among the mechanisms of insect resistance to Cry toxins it was described mutations affecting Cry toxin receptors and protoxin degradation by proteases present in the insect midgut. The aim of this work was to evaluated the susceptibility of different larval instars of *H. armigera* to Cry1Ac toxin, compare Cry1Ac binding proteins from early and late instars and to correlate the capacity of gut extracts to digest Cry1Ac. Toxicity bioassays done under controlled conditions by diet surface contamination with 6 concentrations of Cry1Ac against each larval instar, estimated the lethal concentration (LC50). The LC50 values ranged from 31-2525 ng toxin/cm² diet from 1st to 6th instar. Thus, the 6th instar larvae were 80 times less sensible to the Cry1Ac than the 1st instar. Pull down assays performed with 2nd and 5th instars and MS/MS identification of Cry1Ac-binding proteins showed that in late instars several protease proteins bind to Cry1Ac. To determine if the protease activity present in the different instars could digest Cry1Ac protoxin, proteolysis assays were performed. One hundred µg of Cry1Ac protoxin were incubated with a 5% dilution of midgut juice isolated from 2nd, 4thand 6th larval instars for 5 min a 4 ºC. These assays showed that 4th and 6th instar resulted in lower yields of the 60 kDa activated toxin, indicating that the midgut juice from these instars promote increased degradation of Cry1Ac. These results support that proteolysis of Cry1Ac protein could be one the reasons for the low toxicity of this toxin in late instar larvae of *H. armigera*.

Palavras-chave: Bacillus thuringiensis; Bt Plants; Mechanism of resistance

Apoio institucional: Fundação de Amparo às Pesquisas do Estado de São Paulo (FAPESP) (2016/21463-3 e 2015/24330-5)

Filiação institucional: Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaborticabal, SP, Brasil[1]; Departamento de Biologia Aplicada, Embrapa Milho e Sorgo, Sete Lagoas, MG, Brasil[2]; Instituto de Biotecnología, Universidad Autonóma do México, Cuernavaca, MOR, México[3].