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P3.18 Methods for assessing risks of GM crops to natural enemies: selection of priority species and experiments to test risk hypotheses

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Entomophagous predators are an important component of agroecosystems and should be considered when assessing environmental risks of genetically modified crops (GMC). The diverse phylogenetic histories of these species suggest that they may have different susceptibilities to a GMC. In this work we illustrate the application of the GMO ERA Project methodology for species selection and prioritization for risk assessment of a GMC to non-target organisms. This methodology represents an alternative to the analysis of total biodiversity and the use of surrogate species and is a useful tool to deal with the high species diversity found in tropical agricultural fields. The case study selected was Bt cotton in the Midwest of Brazil. Starting from a field inventory and literature survey a list of more than 100 predator species associated with cotton fields in Brazil was compiled. These species were prioritized according to abundance, frequency, and functional significance in the crop using two matrixes: 1) prioritization of non-target organisms based on ecological principles; 2) evaluation of the potential direct and indirect exposure to the transgene and/or to its metabolites and consequent effects stemming from the exposure. Predator community studies were also conducted to generate information and improve the methodology. The Coccinelid species *Cycloneda sanguinea* was prioritized. Subsequently, risk hypotheses were formulated and prioritized. It has been determined that *C. sanguinea* can be directly exposed to the Bt protein by feeding on Bt cotton pollen (bitrophic exposure), or indirectly exposed by feeding on pray that had fed on Bt cotton (tritrophic exposure). To test the bitrophic hypothesis a laboratory colony of *C. sanguinea* was established to conduct a toxicology test using the Bt protein Cry1Ac expressed in commercially available Bt cotton varieties. The protoxin was produced from an *Escherichia coli* heterologous system, activated with bovine trypsin and purified. First instar larvae were individually placed in 300 ml plastic pots containing a cotton leaf infested with *A. gossypii* and exposed to two treatments: (1) negative control and (ii) Cry1Ac (500µg/mL) sprayed on the aphids. Each treatment had 50 replications. The treatments were checked every day taking notes of the mortality rate, developmental time, and predation capacity. After adult emergence, sex ratio, fecundity and longevity of the predator were evaluated. No significant difference was observed among the treatments in larval developmental time ((i)=10,25 $\pm$ 0,72, (ii)= 10,16 $\pm$ 0,87 days), with low mortality observed (<20%). After emergence the adults were coupled, placed in separated cages and fed with *A. gossypii*. Adult survival and number of eggs laid were recorded. No difference was found in the two treatments, although control females lived longer than treated females. The GMO ERA Project methodology and experimental procedures validated in this study, besides being scientifically sound and ecologically robust, proved to be useful to deal with the high species diversity found in the Brazilian agro-ecosystems.