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Debora Pires Paula Brazil

P3.16 Assessing risks of GM cotton expressing Cry toxin to non-target herbivores: Evaluation of aphids on cotton

Sujii E¹, Fontes E¹, Pires C¹, Paula D¹, Bernardes T¹, Nakasu E¹, Togni P²

¹ Embrapa Cenargen, ² University of Brasilia, Brazil

The expression of Cry toxin by Bt cotton, represents an excellent tool for the control of Lepidopteran pest worms, but presents a potential risk of increased populations of the cotton aphid, *Aphis gossypii*, due to the empty niche left by the worms or due to the pleiotropic effects caused by the transgene. The aim of this work was to develop methodologies following the protocol proposed for non-target herbivores, by the GMO-ERA Project (www.gmo-guidelines.info), to test if aphids reared on Bt cotton can change their bionomics or plant choice for colonization, which results in population dynamics changes and consequently their status as a cotton pest. The bionomics of the aphid was compared in greenhouse with insects reared in individual cages containing Bt cotton plants of the variety DP 404BG (Bollgard) or its not transformed isolate DP 4049. Both seeds were donated by the company Delta & Pine. Cages with Bt and non-Bt cotton were selected as the arena for evaluation of preference by winged adults. In addition, a field experiment with 5 paired blocks of Bt and non-Bt cotton was conducted to confirm the bionomic and colonization behavior patterns of *A. gossypii*, observed in greenhouse. The monitoring of individual females and their daily production of nymphs was an appropriated methodology to evaluate the variables related to the life cycle (survivorship and fecundity) of the cotton aphid in greenhouse and field conditions. Samples of leaves from all experiments were tested by ELISA for Cry 1Ac toxin in the Bt cotton. The comparison between the variables related to the life cycle: pre-reproductive period (immature phase), reproductive period, longevity, survivorship curve and offspring production and intrinsic increase rate of the population were not significantly different. There was also no preference of colonization for any plant by winged adults resulting in equivalent initial populations on Bt and non-Bt plants in the cages. Field monitoring of natural plant colonization by aphids and their population fluctuation followed the same pattern observed in greenhouse, with no differences between the treatments. These results showed that Bt cotton plants, under the tested conditions, did not affect the population dynamics of *A. gossypii*, and consequently do not increase its potential as pest in Bt cotton crop.

P3.17 Base line information and methodologies for assessing risks of Bt cotton to pollinators in Brazil

Pires C, Ferreira C, Lima M, Pires V, Silveira F, Botelho A, Goncalves G, Paula P, Sujii E, Fontes E

Embrapa, Brazil

SP 39714c
Id 31184

The cultivation of Bt cotton raises important environmental risk assessment (ERA) issues in Brazil. Cotton is cultivated in small and large areas and in regions with distinct environmental conditions. A high diversity of arthropods is found associated with the genus *Gossypium* and there are significant variations among different regions. Wild relatives of cotton occur in the country, which raises concerns about gene flow and possible reductions of crop genetic diversity. In this context, flower visitors were considered an important ecological group for ERA because of the possible direct effects of Bt cotton on pollination services in natural and agricultural ecosystems, and the role of flower visitors on pollen transportation and gene flow.

Initially, a survey of flower visitors was conducted in *Gossypium hirsutum latifolium* (cultivated cotton) and *G. barbadense*, a wild cotton species widely distributed in the country. Bees were the main flower visitors on cultivated cotton plants. Taking into account all five studied areas, from 153 insect species collected on flowers, 47.40% (72 species) were from four families of bees and 49.78% (1,630) of the total number of individuals collected (3,274) were bees. Seventy two species of wild bees were collected on cultivated cotton, *G. hirsutum latifolium*, besides *Apis mellifera*, a domesticated species introduced in the country 200 years before for commercial purposes. *Apis mellifera* was the most abundant species on cultivated cotton in all sampling areas, while there was very low abundance on the wild species *G. barbadense* (only three specimens out of 316 total collected individuals). The bee faunas on cultivated cotton were very different among the sampling sites with only two species out of 73 being common to all cotton-producing areas. The large number of flower-visiting species and the difference on local bee fauna makes the selection of priority species on a regional scale necessary before analysis of potential impacts of Bt cotton are proceeded.

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Based on the 2003 inventory, a list of possible pollinator species was compiled for Mid-west region, the largest cotton production area. The framework and criteria outlined by the GMO ERA Project (www.gmo-guidelines.info) were used for species prioritisation: a) geographic distribution, b) abundance in the crop and the surrounding environment, c) association with the crop, d) significance as pollinator on GM crop, in other crops and in natural areas, and e) probability of direct exposure to Cry1Ac protein insecticide. Information from the literature about life history of different species was used in the process. Even though there was lack of information on their significance as pollinators on the majority of the wild bees, four species were preliminarily selected. From these, one of the wild species (*Trigona spinipes*) and *Apis mellifera* were used as model for the development of bioassay methodology for acute toxicity and sub-lethal effect evaluations on worker larvae. For the wild species, the natural food collected inside the nests was used in the bioassays. Taking into account the pollen content in the food and the toxin level expressed in the pollen grains, we defined the toxin concentrations to be tested. A protocol was developed of worker rearing from egg to adult (91% of survivorship in the control) and significant differences were not found among the tested treatments in relation to mortality, body size and weight, and development time of the larvae. The developed methodology was adequate for toxicity tests on *T. spinipes* larvae and could easily be adapted to other stingless bee species.

To fill the gaps in the base line information and proceed with the risk analysis, the foraging behaviour of the most abundant bee species on *G. hirsutum latifolium* and *G. barbadense* were studied in the field. These studies were conducted to evaluate the role of different species on pollen transference and to confirm what resource these species carry from the cultivated cotton to the nest (pollen and/or nectar). Knowledge about forage behaviour is important to access the degree of exposure of that species to the GM crop and to the toxin expressed by the plant. This information also will be useful to support the development of strategies to minimize or prevent gene flow among cotton species and to promote the co-existence between GM and conventional cotton fields.

Twelve bee species were common to *G. hirsutum latifolium* and *G. barbadense*. The majority of wild bee species, besides *A. mellifera*, preferentially collected nectar from the flowers of cultivated cotton, *G. hirsutum latifolium*. Although, *Melissodes nigroaenea* e *Melissoptila cnecomala* are very low in abundance on cultivated cotton flowers, they collected pollen in approximately 100% of the flower visits. These two wild species were more common in cotton areas without or with low insecticide application and nearby natural vegetation. The wild species *Melissoptila cnecomala*, *Melissodes nigroaenea* and *A. mellifera* are also important pollinators of cultivated cotton. *Melissodes cfr. nigroaenea* touched the stigma in approximately 100% of the flower visits on both cotton species. *Apis mellifera*, due to high abundance and wide distribution in the different cotton production areas, could be considered the major carrier of pollen among flowers of cultivated cotton, *G. hirsutum latifolium*. However because of their low presence on flowers of *G. barbadense*, the chances of *Apis mellifera* transporting pollen from cultivated cotton to this wild cotton species is very small. Additional studies to evaluate the natural cross-pollination and consequent gene flow from cultivated cotton to *G. barbadense* are necessary.

Our experience with cotton shows that, for many crops with potential to be genetically modified, information to evaluate potential risk for non-target organisms and to support the development of strategies of gene flow management it is not available for Brazilian environmental conditions. Also, we found that a significant resource and time needs to be allocated in raising basic information that forms the baseline for a solid, scientifically funded risk assessment.