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Low susceptibility of *Spodoptera cosmioides*, *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to genetically-modified soybean expressing Cry1Ac protein

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ABSTRACT

Spodoptera cosmioides (Walker), *Spodoptera eridania* (Stoll) and *Spodoptera frugiperda* (J. E. Smith) have caused significant damage on soybean *Glycine max* (L.) Merrill in Brazil. Genetically-modified MON 87701 × MON 89788 soybean that expresses the Cry1Ac protein is potentially an alternative tool for the management of these species. Purified protein bioassays were done to evaluate the susceptibility of *S. cosmioides*, *S. eridania* and *S. frugiperda* to Cry1Ac protein. The level of efficacy of the Bt soybean plants in controlling these species was measured through laboratory and greenhouse trials under high artificial insect infestations. The biology of these insects was evaluated over their development cycles to understand their life history when fed on Bt soybean. Purified Cry1Ac protein at the maximum concentration tested (100 μg Cry1Ac mL^{-1} diet) resulted in low mortality of *S. cosmioides* and *S. eridania* (<13%) and intermediate mortality of *S. frugiperda* (50%). No significant effects of the Bt soybean plants were observed in the life table parameters of *S. cosmioides* and *S. eridania*. However, *S. frugiperda* fed on Bt soybean plants had a prolonged larval stage (by 5 days), reduced larvae viability, increased mean generation time (by 8 days) and reduced intrinsic rate of increase. In general, the Bt soybean plants showed poor control of *Spodoptera* species when evaluated by leaf-disc bioassay and greenhouse trials. Consequently, other control tactics must be used in combination with MON 87701 × MON 89788 soybean in the field for the efficient management of *S. cosmioides*, *S. eridania* and *S. frugiperda*.

1. Introduction

Caterpillars from the *Spodoptera* genus have caused damage to soybean fields *Glycine max* (L.) Merrill (Fabaceae: Phaseoleae) in Brazil during recent years (Bueno et al., 2011). Within the *Spodoptera* complex, *Spodoptera eridania* (Stoll), *Spodoptera cosmioides* (Walker) and *Spodoptera frugiperda* (J. E. Smith) are prominent in causing damage. They have attacked soybeans in the Cerrado region, Central and Southern Brazil (Hoffmann-Campo et al., 2000; Santos et al., 2005). The great potential for defoliation of soybean plants (Bueno et al., 2011) and damage to flowers and pods (Hoffmann-Campo et al., 2000) by *Spodoptera* species require adoption of control tactics to prevent loss of grain yield. Control is achieved with insecticides, often indirectly as result of sprays for velvetbean caterpillar *Anticarsia gemmatalis* Hübner, soybean looper *Chrysodeixis includens* (Walker) and tobacco budworm *Heliothis virescens* (F.). The use of organophosphates, carbamates and pyrethroids has been the main control strategy for *Spodoptera* species in soybean in Brazil, but these chemicals have low efficacy and control failures are common due to high natural tolerance of these pests to insecticides (Diez-Rodríguez and Omoto, 2001; Carvalho et al., 2013). The genetically modified insect-resistant and glyphosate-tolerant soybean (event MON 87701 × MON

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89788) (CTNBio, 2010) is potentially an alternative tool to manage the population of *Spodoptera* species because of its expression of the Cry1Ac protein, but there are no data available in the literature demonstrating the impact of this protein on the life-history parameters of *Spodoptera* species. This information is important due to the threat of resistance evolution to Bt crops. The evolution of resistance to Bt crops by natural selection is dependent upon at least three conditions: (i) variation among individuals in survival on Bt crops, (ii) inheritance of survival on Bt crops, and (iii) fitness differences consistently associated with the variation in survival on Bt crops (Carrière et al., 2010). The potential risk for Bt-resistance evolution is high for *Spodoptera* species because the Brazilian crop-production system has temporal and spatial overlap of Bt-transformed host plants for *Spodoptera* species such as corn (*Zea mays* L.) (*S. frugiperda*), cotton (*Gossypium hirsutum* L.) and soybean (*S. eridania*, *S. cosmioides* and *S. frugiperda*). In the field these crops potentially expose population of *Spodoptera* species to intense selection pressure in each insect generation, increasing the risk of selecting for Bt-resistant individuals. Gene flow between source and sink habitats allows for repeated colonization of Bt fields and provides opportunities for local adaptation (Carrière et al., 2010), or it could function as a source of susceptibility and delay resistance evolution (Head et al., 2010). Additional important aspects that can favor the evolution of resistance are the natural tolerance of *Spodoptera* species to Cry1Ac protein (Luttrell et al., 1999; Sivasupramaniam et al., 2008) and the rapid ability to evolve resistance to Cry1F in the field (*S. frugiperda*) (Storer et al., 2010). This inherent tolerance of insect pest species to certain Bt proteins could impact the selection advantage of resistance by either reducing the selection coefficient (Gould, 1998) or allowing selection of incomplete resistance (Gassmann et al., 2011).

These considerations highlight the need for studies to understand the effects of MON 87701 × MON 89788 soybean on *Spodoptera* species and on the potential risk for Cry1Ac resistance evolution. This information is particularly important to support Insect Resistance Management (IRM) programs and best agricultural practices in a crop-production system where transgenic soybean and cotton events both express the Cry1Ac protein. To evaluate the susceptibility of *S. eridania*, *S. cosmioides* and *S. frugiperda* to Cry1Ac and the biological effects of MON 87701 × MON 89788 soybean against these species, Cry1Ac protein bioassays, leaf-disc and greenhouse trials were conducted and life table parameters were evaluated when those insects were fed on Bt soybean.

2. Materials and methods

2.1. Susceptibility to Cry1Ac diet-incorporation bioassay

Susceptible reference populations of *S. cosmioides*, *S. frugiperda* and *S. eridania* were collected in soybean (200 larvae per species) in Pelotas-RS, Rondonópolis-MS and Ibiporã-PR, respectively, in December 2008. In the laboratory, these populations were kept free of selection pressure by insecticides or Bt proteins for at least 3 years (>15 generations) and then they were used to evaluate the species' susceptibility to Cry1Ac protein. All populations were maintained on artificial diet based on white bean, wheat germ and yeast (adapted from Greene et al., 1976), at 25 ± 1 °C with a 14:10 h light:dark photoperiod. Purified Cry1Ac protein was provided by Monsanto of Brazil Ltda at a concentration of 1.4 mg of active Cry1Ac mL^{-1} and stored in a freezer at −80 ± 5 °C. After thawing, Cry1Ac protein was diluted in buffer consisting of 50 mM 3-(cyclohexylamine)-1-propanosulphonic acid at pH 10.25, 1 mM benzomidine-HCl, 1 mM tetraacetic diamine ethylene acid and 2.5 mM dithiothreitol. For bioassays, the insecticidal protein and buffer solution were added to artificial diet when the diet temperature reached 45–50 °C. Cry1Ac was incorporated into artificial diet using a Vortex-type tube mixer for 2 min. The diet containing the incorporated protein was kept in a water bath at ≈55 °C for later distribution into 128-well bioassay trays (BIO-BA-128, CD International, Inc., Pitman, NJ) (1 mL diet/well). A total of five concentrations, 1.8, 10, 18, 32 and 100 μg Cry1Ac mL^{-1} diet, were used. After diet solidification and cooling, one neonate larvae (<24 h old) was placed into each well using a fine brush. Bioassay trays were sealed with self-adhesive plastic sheets (BIO-CV-16, CD International Inc.) allowing gas exchange with the external environment and placed in a climatic chamber (temperature: 27 ± 1 °C; relative humidity: 60 ± 10%; photoperiod: 14:10 h light:dark). The experimental design was completely randomized with 8 replicates per concentration (16 larvae/replicate). Mortality caused by Cry1Ac was assessed by counting the number of dead larvae after seven days, including as 'dead' all larvae still in the first instar. The weight of surviving larvae was also recorded. The percentage mortality was corrected on the basis of the mortality in the control treatment, which consisted of artificial diet + buffer (Abbott, 1925). The percentage of growth inhibition was calculated as larval weight reduction relative to the control treatment. Corrected percent mortality and growth inhibition were transformed using $\sqrt{x+0.5}$ because of non-normal distributions of residuals. After transformation, mortality and growth inhibition data was subjected to two-way ANOVA with species, diet concentration, and the interaction as fixed effects. Means were compared using the Tukey test ($P \leq 0.05$) (PROC ANOVA, SAS Institute, 2000).

2.2. Leaf-disc bioassays

MON 87701 × MON 89788 soybean and near-isogenic negative checks of maturity groups 5.5 (recommended for planting in southern Brazil) and 8.3 (recommended for planting in midwestern Brazil) were sown in 12 L plastic pots (4 seeds/pot) in the greenhouse. Completely expanded leaves were removed from the upper third of the plants when they reached the phenological stages V3–V4, V5–V6 and R1–R2 (Farias et al., 2007). Leaf discs 2.4 cm in diameter were cut using a metallic cutter and placed on a non-gelled mixture of water–agar 2.5% (1 mL/well) in acrylic plates (Costar®) with 12 wells (Corning, Tewksbury, MA, USA). Leaf discs were separated from the water-agar layer by a filter paper disc. Separate bioassays were performed for *S. cosmioides*, *S. frugiperda* and *S. eridania*. In each bioassay one neonate larvae (<24 h old) was placed on each soybean leaf-disc using a fine brush. Plates were sealed with plastic film (Magipack®) and placed in a climatic chamber (temperature: 27 ± 1 °C; relative humidity: 60 ± 10%; photoperiod: 14:10 h light:dark). The experimental design was completely randomized with 8 replicates per treatment; each replicate consisted of 12 neonate larvae for a total of 96 neonate larvae tested for each species per phenological stage. Larval mortality was assessed after five days. Mortality on MON 87701 × MON 89788 soybean was corrected on the basis of the mortality in the near-isogenic negative check (Abbott, 1925). Corrected percentage mortality of each replicate was transformed using $\sqrt{x+0.5}$ because of non-normal distribution of residuals. The mortality data on MON 87701 × MON 89788 soybean and the respective near-isogenic negative check for each species and phenological stage were compared by *t*-test ($P \leq 0.05$) (PROC TTEST, SAS Institute, 2000).

2.3. Biological parameters of Spodoptera species when fed on MON 87701 × MON 89788 soybean

MON 87701 × MON 89788 soybean and near-isogenic negative checks of maturity groups 5.5 and 8.3 were sown in the greenhouse