Crop-Livestock Integration: Attack of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on transgenic maize and *Bachiaria brizantha* (Poaceae)[1]

Paulo Henrique Baêta Nogueira de Carvalho[2], Wilson Faustino Júnior[2], Zaira Vieira Caldeira[2], Ricardo Siqueira da Silva[2], Ronnie Von dos Santos Veloso[2], Marcus Alvarenga Soares[2]*

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ABSTRACT

The Crop-Livestock Integration system has sustainable potential. But pests such as the defoliating caterpillar *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) can reduce the productivity of this system. The objective was to report transgenic maize and *Brachiaria brizantha* (A. Rich.) (Poaceae) plants attacked by *S. frugiperda* in the Crop-Livestock Integration system. Feroz VIP3® transgenic maize (SYN8A98 TLTG Viptera) and *B. brizantha* MG-5 Xaraés plants attacked by *S. frugiperda* were evaluated 20 days after planting, weekly and for 28 days. The transgenic maize plants were less attacked by *S. frugiperda* than those of *B. brizantha*. The negative impact of this pest on *B. brizantha* suggests the planning and adopting strategies for its control, such as the use of traps, resistant cultivars, and biological or chemical products, minimizing losses in animal production and, consequently, in human food.

Keywords: *Bacillus thuringiensis*; consortium; foragers; grazing; pest insect.

INTRODUCTION

The Crop-Livestock Integration (CLI) production system encourages diversification, rotation and planned to intercrop agricultural and livestock activities (Kunrath *et al*., 2015). Rational soil management in this system improves carbon accumulation and conserves fertility by forming bioactive humic substances responsible for increasing plant performance (Baldotto *et al*., 2017; Bansal *et al*., 2022). In addition, it is favorable for the establishment of crops, especially in degraded areas (Assis *et al*., 2019; Carvalho *et al*., 2020).

Livestock in Brazil is heavily dependent on pastures occupied, in the majority, by *Brachiaria* spp. Trinius (synonym *Urochloa* P. Beauv.) (Poaceae) for the production of animal protein (meat and milk), being identified as responsible for environmental impacts, such as the production of greenhouse gases related to climate change (Gléria *et al*., 2017; Lima *et al*., 2022). The intercropping of forages with annual crops, such as maize and sorghum, is an efficient and economically viable technique for forming, recovering, and renewing pastures in the CLI (Geremia *et al*., 2018). This consortium can occur with the simultaneous or sequential sowing of species in which, after harvesting the annual crop, the pasture formed is intended for animal feed (Roese *et al*., 2018; Tsufac *et al*., 2021).

The maize (*Zea mays* Linnaeus) (Poaceae) is an important cereal originating in the Americas and cultivated on a large scale in the world, with Brazil being the third largest producer with an area of 20 million hectares and production of 87 million tons (Leite *et al*., 2021; CONAB, 2021). This crop is susceptible to climatic fluctuations and

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[2] Universidade Federal dos Vales do Jequitinhonha e Mucuri, Departamento de Agronomia, Diamantina, Minas Gerais, Brazil. paulo.baeta@ufvjm.edu.br; wilson.faustino@ufvjm.edu.br; zaira.caldeira@ufvjm.edu.br; ricardo.siqueira@ufvjm.edu.br; ronnie.veloso@ufvjm.edu.br; marcus.alvarenga@ufvjm.edu.br
*Corresponding author: wilson.faustino@ufvjm.edu.br

the attack of diseases and pests, causing high economic impact (Dos Santos *et al*., 2020). *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), the main pest of maize in Brazil, feeds on a wide variety of plants, such as cotton, rice, forages, and soybeans, among others (Scoton *et al*., 2020). The control of *S. frugiperda* has been carried out mainly with the excessive use of insecticides, which select resistant individuals of this insect in the field (Omoto *et al*., 2016). The cultivation of transgenic maize expressing insecticidal proteins (Cry and Vip) derived from the bacterium *Bacillus thuringiensis* (Bt) Berliner is efficient in controlling *S. frugiperda* and is an alternative to the excessive use of chemicals (Castro *et al*., 2019; Zhao *et al*., 2020).

The population dynamics of pests have been studied for monocultures, and these studies are scarce for the CLI system. The CLI with transgenic maize and *B. brizantha* can be attacked by *S. frugiperda*, and the survival and development of this pest in forage can increase the pressure of insects on transgenic maize. In this context, the objective was to report transgenic maize and *B. brizantha* plants attacked by *S. frugiperda* in the Crop-Livestock Integration system.

MATERIAL AND METHODS

The experiment was carried out at Rodeio Gaúcho Farm (22°47'42.1" S and 42°26'25.8" W, 42 m.a.l.s.), coastal lowland of Araruama, state of Rio de Janeiro, Brazil, between February and May 2022, second crop (cultivation after the main harvest) of maize. According to the Köppen-Geiger classification, the region's climate is of the Aw type, with average annual temperature and precipitation of 24°C and 1103 mm, respectively (Climate-Data, 2022).

The CLI system was established in an area with 9.6 ha, after plowing and harrowing the soil, without liming and with fertilization of 350 kg/ha of formulated (08-28-16) for planting.

Feroz VIP3® transgenic maize (SYN8A98 TLTG Viptera) and *B. brizantha* Série Gold® MG-5 Xaraés were cultivated in intercropping, in this study, for transformation into silage and subsequent cattle grazing, without the application of insecticides. Maize planting was carried out with a spacing of 0.2 m between plants and 0.65 m between rows, with five plants/m linear and a density of 76,924 plants/ha. The planting of *B. brizantha* was carried out with 13 kg/ha of seeds by broadcast.

The total area was divided into three sub-areas (3.2 ha), each considered an experimental repetition (Figure 1). Ten random points (2.16 m^2) were marked with wooden stakes (1.5 m) and identification tags in each sub-area and, in each of these, 40 plants (20 of maize and 20 of *B. brizantha*) were sampled for counting plants damaged by *S. frugiperda*. The evaluations started 20 days after planting, when the plants reached 100% germination, with five evaluations, one per week, over 28 days.

The design was completely randomized with two treatments (maize and *B. brizantha*) and three replications (subareas), making a sampling effort of 6,000 evaluated plants. The behavior of the curves of attacked plants in the subareas was similar, so the data were grouped for the total area and analyzed to compare the treatments. The data did not meet the assumptions of the ANOVA, and means were compared using the Wilcoxon nonparametric test ($P < 0.05$). Analyses were performed using RStudio software (RStudio Team, 2022).

RESULTS AND DISCUSSION

The transgenic maize plants were less attacked by *S. frugiperda* than those of *B. brizantha* during the evaluated period (Figure 2). This proves the efficiency of transgenics (VIP protein) in controlling this pest. However, injuries such as leaf scraping, caused by *S. frugiperda* caterpillars of early stages, are expected, as they must ingest insecticidal proteins for their control (Zhao *et al*., 2020). The *S. frugiperda* attacks occurred with the presence of only one caterpillar per plant. This is because *S. frugiperda* has a high degree of cannibalism, allowing the development of only one individual per plant (Scoton *et al*., 2020). Obtaining clones or forage cultivars resistant to *S. frugiperda* is important to avoid reducing the productivity of green forage or straw mass caused by this insect (Harrison *et al*., 2019). These strategies prevent the multiplication of potential pests of forages and annual crops intercropped in the CLI system.

The number of *B. brizantha* plants damaged by *S. frugiperda* stabilized in the second evaluation (Figure 2). This may have occurred due to the increased population of natural enemies of *S. frugiperda* in the area. Planting forages intercropped with maize increased soil fertility and provided a diverse environment for developing *S. frugiperda* parasitoids and predators (Harrison *et al*., 2019; Bansal *et al*., 2022). Natural enemies of *S. frugiperda* can recognize herbivory-induced volatiles from intercropped maize and bean plants, consequently resulting in the control of this

Figure 1: Delimitation of the experimental area for the intercropped cultivation of Feroz VIP3® transgenic maize and *Brachiaria brizantha* cv. Xaraés in the Crop-Livestock Integration (CLI) system..

insect (Udayakumar *et al*., 2021). Thus, it is suggested that herbivory-induced volatiles from transgenic maize and *B. brizantha* plants attracted natural enemies of *S. frugiperda* and stabilized its attack on these crops.

The attack of *S. frugiperda* on *B. brizantha* in the CLI system can be explained by the forage being considered an alternative for the pest to maintain and multiply in the area, possibly increasing its attack in this cropping system. Different species of the genus *Brachiaria* spp. were good hosts for *S. frugiperda*, allowing the complete development of this insect (Auad *et al*., 2016). Thus, selecting plants that minimize the biotic potential of this insect pest is one of the challenges in intercropped plantations in the CLI. The minor attack of transgenic maize plants by *S. frugiperda* occurred due to the Viptera technology (expression of the Vip3Aa20 protein), making these plants more resistant to the attack of this lepidopteran. Four Vip families with more than 100 toxins are known, with Vip3Aa being the only one present in commercialized transgenic crops and with no reports of resistance in the field (Tabashnik & Carrière, 2017). However, populations of *S. frugiperda* were resistant to Cry1Ab and Cry1F toxins in transgenic maize areas (Monnerat *et al*., 2015). The Vip3Aa protein can be associated with Cry in pyramidal plants (Figueiredo *et al*., 2019), delaying the possible development of lepidopteran resistance to transgenic crops.

There was no change in the number of transgenic maize and *B. brizantha* plants damaged by *S. frugiperda* in the fifth evaluation (Figure 2). The expression of Vip insecticidal proteins effectively controls this pest in maize, even when its individuals are in more advanced stages of development (Tabashnik & Carrière, 2020). Thus, studies with proteins with higher insecticidal capacity are important for managing resistant insects (MRI).

The number of *B. brizantha* plants damaged by *S. frugiperda* was higher than that of transgenic maize plants in the total cultivated area (Figure 3). The ample supply of host plants throughout the year, either through crop succession or through the use of susceptible cultivars, contributes to the occurrence of *S. frugiperda* (Omoto *et al*., 2016). Furthermore, in intercropped plantations, *S. frugiperda* can develop in one host species causing damage to the other, as reported for this insect in plants of *Pennisetum purpureum* Shum (Poaceae) (cvs. Ouma II and South Africa), *B. brizantha* (cvs. Xaraés, Piatã and Mulato II) and *Melinis minutiflora* P. Beauv. (Poaceae) cultivated in intercropping with maize (Cheruiyot *et al*., 2021). *Brachiaria brizantha*, in the present study, allowed the survival and development of *S. frugiperda*, without significant damage to the transgenic maize, with consequent loss of green mass of this forage due to the high number of attacked plants. High population densities of caterpillars in *B. brizantha* can cause more significant pest pressure on transgenic maize plants and generate a possible reduction in the efficiency of its control and, consequently, economic losses.

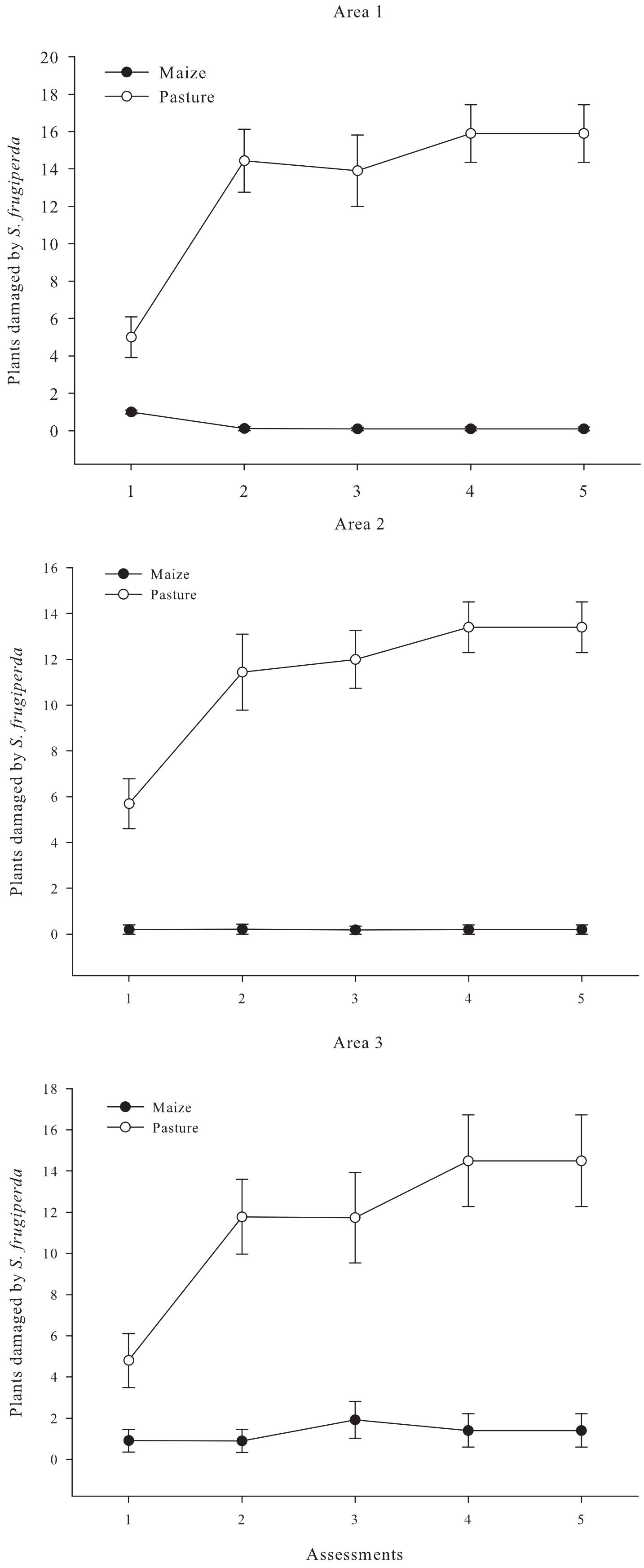

Figure 2: Number (mean ± standard deviation) of Feroz VIP3® transgenic maize plants and *Brachiaria brizantha* cv. Xaraés attacked by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) per intercropped area in the Crop-Livestock Integration (CLI) system.

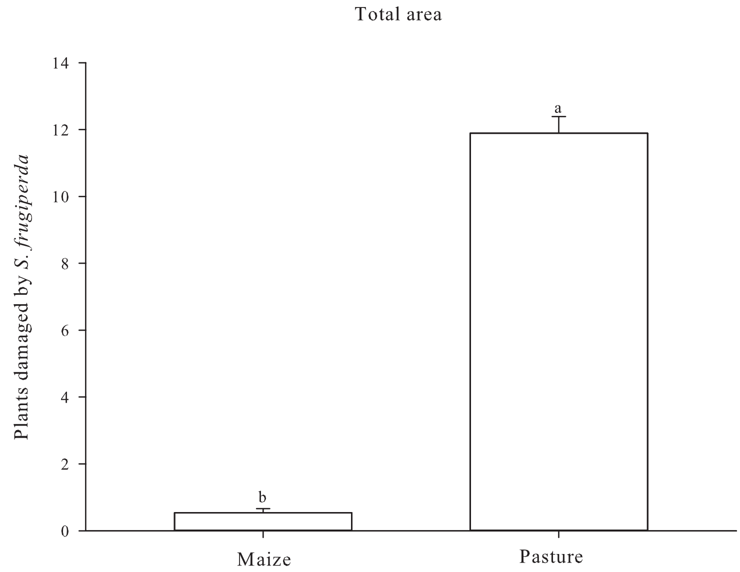

Figure 3: Number (mean ± standard deviation) of Feroz VIP3® transgenic maize plants and *Brachiaria brizantha* cv. Xaraés attacked by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the total area intercropped in the Crop-Livestock Integration (CLI) system. Means followed by different letters in the column differ by the Wilcoxon test ($P < 0.05$).

On the other hand, if the pest is controlled in the pasture, keeping populations below the economic damage level (EDL), the CLI system may be recommended to help manage insect resistance to Bt technology. In this sense, the pasture can develop susceptible adults of *S. frugiperda*, which will mate with resistant individuals and develop new individuals susceptible to insecticidal proteins, as occurs when adopting refuge areas. Therefore, studies involving the population dynamics of *S. frugiperda* are important to assess the impact of this pest on agroecosystems.

CONCLUSIONS

The infestation of pests in CLI systems with transgenic maize and *B. brizantha* demands attention, as these pests can migrate from one plant to another.

The *S. frugiperda* caterpillars developed on *B. brizantha* plants and did not cause severe damage to the transgenic maize (VIP protein). However, its negative impact on *B. brizantha* plants suggests the planning and adoption strategies for its control, such as the use of traps, resistant cultivars, and biological or chemical products, minimizing losses in animal production and, consequently, in human food.

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