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Survival and development of fall armyworm (Lepidoptera: Noctuidae) in weeds during the off-season

Tamara Moraes¹, Alexandre Ferreira da Silva², Natália Alves Leite³, Décio Karam², and Simone Martins Mendes^{2,*}

Abstract

The persistence and high dispersal of weeds during the off-season can favor the survival of pests and diseases that threaten cultivated crops in Brazil. The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is one of the principal polyphagous pests that takes advantage of the no-tillage system. Despite its pest status, little is known about *S. frugiperda* survival and development in alternative hosts, including those resistant to glyphosate. The purpose of this study was to investigate, in laboratory and greenhouse conditions, the adaptive capacity of *S. frugiperda* in volunteer maize and 6 weeds commonly found in Brazilian agroecosystems, including species with biotypes known for glyphosate resistance, such as fleabane, sourgrass, and goosegrass. We found that *S. frugiperda* survival and biomass were significantly higher in goosegrass, maize, and johnsongrass in both laboratory and greenhouse conditions. In contrast, fleabane, benghal dayflower, sourgrass, and smooth pigweed caused a decrease in *S. frugiperda* fitness. Along with *S. frugiperda* adaptive capacity, our results suggest that its persistence in the field can be directly related to weed control inefficiency during the off-season, increasing the demand for integrated pest and weed management.

Key Words: *Spodoptera*; glyphosate-resistance; fitness; biology; adaptive capacity

Resumo

A persistência e a alta dispersão de plantas daninhas durante a entressafra podem favorecer a sobrevivência de pragas e doenças que ameaçam culturas cultivadas no Brasil. A lagarta-do-cartucho, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), é uma das principais pragas polífagas que tira proveito do sistema de plantio direto. Apesar de sua importância, pouco se sabe sobre a sobrevivência e o desenvolvimento de *S. frugiperda* em hospedeiros alternativos, incluindo aqueles resistentes ao glifosato. O objetivo deste estudo foi investigar, em condições de laboratório e casa-de-vegetação, a capacidade adaptativa de *S. frugiperda* em milho e 6 plantas daninhas comumente encontradas em agroecossistemas brasileiros, incluindo espécies com biótipos conhecidos pela resistência ao glifosato, como buva, capim-amargoso e capim-pé-de-galinha. Descobrimos que a sobrevivência e a biomassa de *S. frugiperda* foram significativamente maiores em capim-pé-de-galinha, milho e capim-massarabá to sorgo selvagem, tanto em laboratório como em casa-de-vegetação. Por outro lado, buva, trapoeraba, capim-amargoso e amaranto causaram uma diminuição na aptidão de *S. frugiperda*. Além da capacidade adaptativa de *S. frugiperda*, nossos resultados sugerem que sua persistência no campo pode estar diretamente relacionada à ineficiência no controle de plantas daninhas durante a entressafra, sendo importante o manejo integrado de pragas e ervas daninhas.

Palavras Chave: resistência ao glifosato; aptidão; biologia; capacidade adaptativa

Weeds can serve as alternative hosts for pests in the absence of a principal host. The no-tillage system and herbicide-resistant transgenic plants have favored the increase of herbicide use to control weeds, which has generated great selection pressure on these herbicide-resistant weeds, and as a result has increased the reported cases of resistant weeds (Christoffoleti & Lopez-Ovejero 2008; Carvalho et al. 2011; Cerdeira et al. 2011; Heap & Duke 2018). This fact is alarming, because it may cause an increase in the seed bank of an area, and consequently the increase of weeds during the off-season, which then serves as a green-bridge for insect pests and diseases (Dalazen et al. 2016).

The occurrence of weeds during the off-season may vary depending on the specific weather and climate conditions for each region, besides being influenced by factors related to soil preparation, the history of herbicides sprayed, and crops cultivated in the area. The continuous

use of glyphosate herbicide in Brazilian agriculture has favored the increased frequency of resistant biotypes and herbicide tolerant species in the principal soybean producing regions of this country (Lucio et al. 2019). In Brazil, currently there are 7 species of weeds resistant to this herbicide, and among them fleabane (*Conyza* spp.; Asteraceae), sourgrass (*Digitaria insularis* [L.] Mez ex Ekman; Poaceae), and goosegrass (*Eleusine indica* [L.] Gaertn.; Poaceae) stand out as species with wide geographic distributions (Heap & Duke 2018; Lucio et al. 2019). The traffic of agricultural machines and implements, as well as seeds from the border regions with other countries, principally Argentina, Uruguay, and Paraguay, which are the main entry points for new resistant species, allow resistant species such as smooth pigweed (*Amaranthus hybridus* L.; Amaranthaceae) and johnsongrass (*Sorghum verticilliflorum* [L.] Pers.; Poaceae) to be accidentally introduced into Brazil. There is

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strong evidence that glyphosate-resistant smooth pigweed biotypes were introduced from Argentina into the southern region of Brazil in 2019 (HRAC 2019). Therefore, Brazil will have another glyphosate resistant species, a total of 8 species. All species mentioned here stand out as common plants which may be found during the off-season in Brazilian fields (Adegas et al. 2010; Concenço et al. 2012; Heap & Duke 2018). During this time, if not properly managed, weeds may increase their seed bank, becoming difficult to control, and may serve as important hosts for pests and diseases (Dalazen et al. 2016). In Brazil, these weeds often are found in regions with soybean-maize rotation in a no-tillage system, which incorporates a large production area for these crops in the country. Because of this diversity of hosts, many pests are successfully surviving not only during the crop development period, but also over the off-season.

Among the pests that attack maize, the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is considered the most important pest in Brazil (Sarmiento et al. 2002), and since 2016 has been reported as an invasive species on the African continent, spreading and threatening cultivated plants in several countries (Goergen et al. 2016). Due to the polyphagy of this species, more than 100 species of plants are registered as hosts (Pogue 1995), including cultivated and invasive plants that occur simultaneously with the susceptible crops in different regions and seasons of the yr (Sá et al. 2009; Boregas et al 2013). Due to inadequate management, weeds resistant to glyphosate tend to remain green for a longer time in the crop area, making them potential hosts for these polyphagous pests. Therefore, understanding fall armyworm fitness and biology on these invading plants helps us adjust the information on pest management practices for specific crops, because once in these cropping systems this pest can disperse from the weed to the newly planted crop and destroy the plants, reducing the crop and yield.

In this scenario where weeds are becoming more common during the off-season, which favors the survival of pests, we assessed the biological aspects of *S. frugiperda* in specific weeds that are common in Brazilian agroecosystems (volunteer maize, fleabane, sourgrass, benghal dayflower, johnsongrass, smooth pigweed, and goosegrass), and determined how the presence of these plants can influence the survival of this pest in the agricultural systems.

Materials and Methods

INSECTS

The insects used in the bioassays came from a laboratory population of *S. frugiperda* at Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais, Brazil. This population is being kept in the laboratory on the artificial diet proposed by Kasten et al. (1978), free from selection pressure by insecticides and Bt proteins.

WEED SEEDS

The weed seeds used in the present study belong to the laboratory of Weed Science of Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais, Brazil. Seeds of the species used here came from Embrapa's experimental field (19.4658333°S, 44.2469444°W).

SPODOPTERA FRUGIPERDA BIOLOGY IN THE LABORATORY

The biological bioassay was conducted using leaves of 6 weeds (Table 1) and maize (*Zea mays* L.; Poaceae) (DKB 390). The weed seedlings and maize seeds were cultivated weekly in 5 × 5 m beds in the

Table 1. Weeds used to feed *Spodoptera frugiperda* in laboratory and greenhouse experiments.

Common Name	Scientific name	Family
Goosegrass	<i>Eleusine indica</i>	Poaceae
Johnsongrass	<i>Sorghum verticilliflorum</i>	Poaceae
Smooth pigweed	<i>Amaranthus hybridus</i>	Amaranthaceae
Sourgrass	<i>Digitaria insularis</i>	Poaceae
Fleabane	<i>Conyza</i> spp.	Asteraceae
Benghal dayflower	<i>Commelina benghalensis</i>	Commelinaceae

field, then transplanted to pots containing 5 plants or seeds each. The cultivation occurred without any pesticide application.

The bioassay started in the laboratory 20 d after seed germination, when maize was in the V4 to V5 stage (Ritchie et al. 1992), while weed seedlings were close to 30 cm. It was carried out under laboratory conditions of 26 ± 3 °C, 70 ± 15% RH, and a 12:12 h (L:D) photoperiod. Plants were harvested and transversally cut into portions 5 cm long, and placed into 50 mL plastic containers. Forty-eight neonates (< 24 h old) were placed individually in the containers (6 replications of 8 neonates). Plant portions were changed every 2 d.

The response variables measured were larval survival (neonate to pupa), pre-imaginal survival (neonate to adult), larval development time (neonate to pupa), pre-imaginal development time (neonate to adult emergence), larval weight at the tenth d after emergence, and pupal weight within 24 h after pupation.

Homogeneity of variances (Bartlett test) and normality (Shapiro-Wilk test) were tested before the statistical analysis for all trials in R vers. 2.15.1 (R Development Core Team 2012). Pupal weight data were submitted to the variance analysis, and the means were compared by the Tukey test ($P \leq 0.05$; PROC ANOVA) (SAS 2002). The other data were submitted to the Kruskal-Wallis test (Breslow 1970), and means were compared with the Dunn's Kruskal-Wallis multiple comparison test from the package pggirmess (Giraudoux 2016) in R vers. 2.15.1 (R Development Core Team 2012).

SPODOPTERA FRUGIPERDA BIOLOGY IN THE GREENHOUSE

In order to evaluate the biology of *S. frugiperda* on the plants and the injury level, we conducted a greenhouse experiment. It was conducted with temperature of 27 ± 5 °C and 70 ± 15% RH. The treatments consisted of the 6 weeds (Table 1) and DKB390 maize. The plants were sown in 20 L pots filled with soil and fertilized with 50 g of nitrogen, phosphorus, and potassium (NPK) 08-28-16 and 0.3% of zinc per 100 kg of soil. Ten to 15 seeds were sown per pot, and after thinning 5 plants per pot were left. After 20 d of emergence, the plants started to be fertilized weekly with the fertilizer BIOFERT Plus® (Terral/Truemix, Contagem, Minas Gerais, Brazil). The experimental design was completely randomized, with 7 replicates (pots) per treatment, and 50 larvae per replicate (5 plants in each replicate). The plants were infested manually with *S. frugiperda* neonates (10 neonates per plant) after 20 d of germination, when maize was at the V4 to V5 stage (Ritchie et al. 1992), using a fine brush, whereas 7 replicates were not infested with neonates as control plants. All pots were covered with iron cages lined with voile fabric to avoid larvae escaping from one pot to another. Twenty-one d after infestation, the number of surviving insects, their biomass, and injury level were accessed. The injury level was proposed by Carvalho (1970) for maize, and we adapted for the other plants. It varied from 0 to 5, corresponding to: 0 = a plant with undamaged leaves; 1 = a plant presenting scraped leaves; 2 = a plant presenting perforated leaves; 3 = a plant with torn leaves; 4 = a plant with lesions in the cartridge or very destroyed; and 5 = a plant with destroyed cartridge or completely destroyed.

Homogeneity of variances (Bartlett test) and normality (Shapiro-Wilk test) were tested before the statistical analysis for all trials in R vers. 2.15.1 (R Development Core Team 2012). Insects survival, their biomass, and injury level data were submitted to the Kruskal-Wallis test (Breslow 1970), and means were compared with the Dunn's Kruskal-Wallis multiple comparison test from the package pgrmness (Giraudoux 2016) in R vers. 2.15.1 (R Development Core Team 2012).

Results

SPODOPTERA FRUGIPERDA BIOLOGY IN THE LABORATORY

There were significant differences in the larval stage survival ($\chi^2 = 32.8$; $df = 6$; $P < 0.05$), larval development time ($\chi^2 = 92.3$; $df = 5$; $P < 0.05$), pre-imaginal survival ($\chi^2 = 35.1$; $df = 6$; $P < 0.05$), and pre-imaginal development time ($\chi^2 = 78.2$; $df = 5$; $P < 0.05$), larval biomass ($\chi^2 = 175.6$; $df = 6$; $P < 0.05$), and pupal biomass ($F_{5,141} = 30.2$; $P < 0.05$) of *S. frugiperda* fed with weeds and maize in the laboratory (Fig. 1).

Larval stage survival varied from 0 (fleabane) to 70.8% (maize and johnsongrass) (Fig. 1a). There were no significant differences of larval survival from goosegrass to maize and johnsongrass. In addition, larval survival was similar to sourgrass and fleabane, above 18.7%. There were no significant differences of larval survival on johnsongrass and maize. Pre-imaginal survival varied from 0 (fleabane) to 66.6% (maize). Additionally, pre-imaginal survival was similar to sourgrass and fleabane, above 14.5%. Larval development time varied from 14.4 (maize) to 25.0 d (sourgrass) (Fig. 1b). There were no differences in larval development time for maize and johnsongrass (≤ 15.5 d). Although survival was similar for goosegrass, johnsongrass, and maize, larval development d was higher for goosegrass (18.3 d). Pre-imaginal development time varied from 35.1 (sourgrass) to 24.0 d (maize).

Larval biomass, measured at 10 d, varied from 5.8 (fleabane) to 341.5 mg (maize), and pupal biomass varied from 105.8 (sourgrass) to 241.9 mg (maize) (Fig. 1c). The weight gain during the larval stage on goosegrass provided similar pupal weight between this and maize. In agreement with survival results, insects that survived on sourgrass had the lowest pupal biomass.

SPODOPTERA FRUGIPERDA BIOLOGY IN THE GREENHOUSE

There were differences in the insect survival ($\chi^2 = 28.6$; $df = 6$; $P < 0.05$), insect biomass ($\chi^2 = 9.5$; $df = 6$; $P < 0.05$), and injury level ($\chi^2 = 35.3$; $df = 6$; $P < 0.05$) of *S. frugiperda* fed with 6 weeds and maize in the greenhouse after 21 d of infestation (Fig. 2).

Insect survival in the greenhouse varied from 1.1 (benghal dayflower) to 23.7% (goosegrass) (Fig. 2a). The survival of the insects on johnsongrass and maize was statistically similar to goosegrass ($\geq 21.1\%$). Lower survival was observed for smooth pigweed, sourgrass, fleabane, and benghal dayflower ($\leq 10.3\%$). Insects biomass varied from 79.6 (fleabane) to 245.0 mg (maize), and was higher for smooth pigweed, johnsongrass, maize, and goosegrass (≥ 225.6 mg) (Fig. 2b). Injury level on plants was evaluated according to Carvalho (1970) with adaptations, and varied from 0 (no damage) to 5 (plant completely destroyed). It varied from 1.1 (fleabane) to 4.3 (maize) (Fig. 2c). Injury level was higher for smooth pigweed, johnsongrass, maize, and goosegrass (≥ 3.4) and lower for benghal dayflower and fleabane (≤ 1.7).

Discussion

In general, our results showed that the survival and development of fall armyworm were higher on the off-season weeds johnsongrass

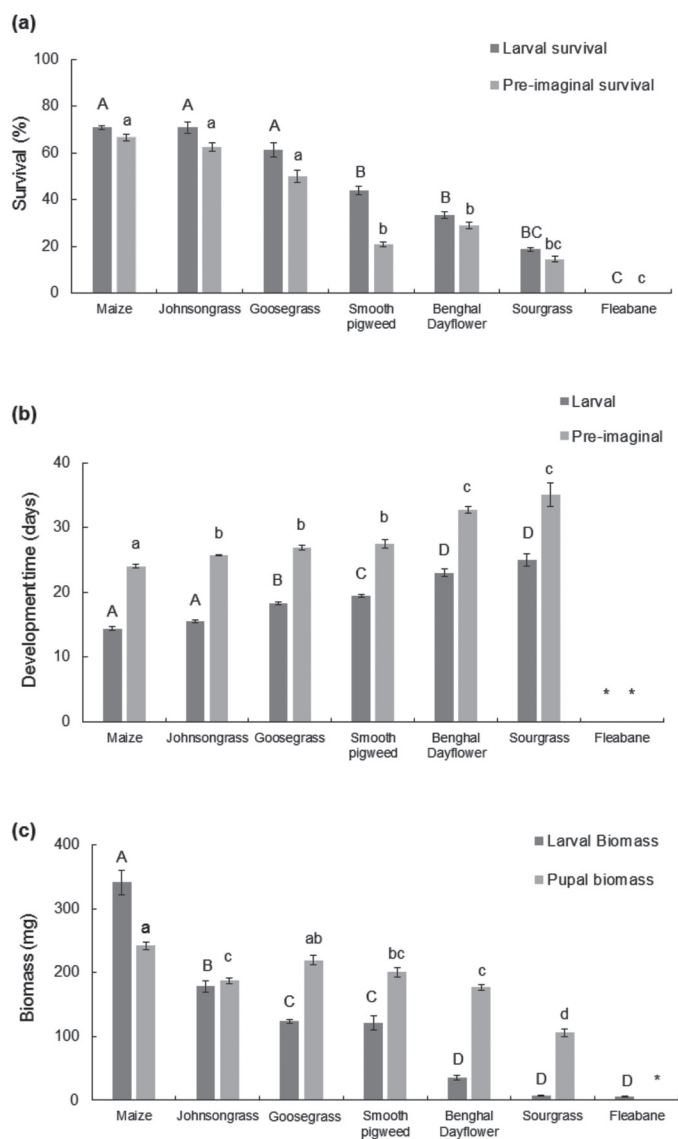


Fig. 1. Mean (\pm EP) of larval survival and pre-imaginal survival (%) (a), larval development time and pre-imaginal development time (b), and biomass of larvae and pupae of surviving individuals of *Spodoptera frugiperda* fed with 6 weeds and maize in laboratory conditions (c). Uppercase letters are used to compare larval stage, while lowercase letters are used to compare pupal stage. Means capped with the same letter do not differ significantly. Means followed by an asterisk (*) were zero (0), and were not used in the analysis.

and goosegrass, similar to maize, and that fleabane, sourgrass, and benghal dayflower were the worst hosts for this pest. Other studies, including *S. frugiperda* survival in cover crops in Brazilian conditions, were conducted and reported by Boregas et al. (2013), but the adaptive stage of this pest in glyphosate resistant weeds was never tested.

Survival in the laboratory (larval and pre-imaginal) was higher than in the greenhouse due to the cannibalism of fall armyworm in the latter (Chapman et al. 2000). In the laboratory, the larvae were reared on the plants individually, whereas in the greenhouse 10 neonates were reared per plant, simulating a more natural condition. However, the survival rates on goosegrass and johnsongrass were similar to maize in both conditions, demonstrating the potential of these weeds as hosts to this pest.

Our results showed that the pre-imaginal survival of the pest in goosegrass was similar to that which Dias et al. (2016) found in other plants used in no-tillage systems in tropical agriculture in Brazil, such as millet or signal grass, confirming the ability of the fall armyworm to

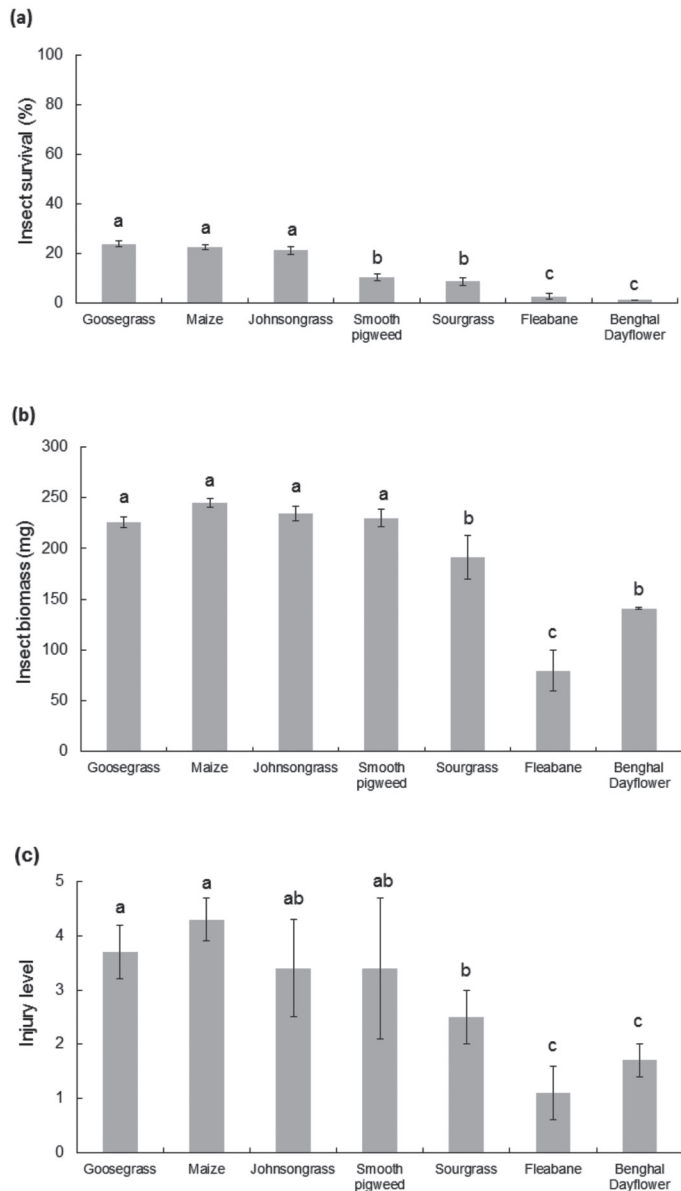


Fig. 2. Insect survival (mean \pm SE) (a), biomass (mean \pm SE) (b), and injury level (mean \pm SE) (c) of *Spodoptera frugiperda* fed with 6 weeds and maize in the greenhouse for 21 d. Means capped with the same letter do not differ significantly.

survive using these crops as a “green bridge.” Nonetheless, goosegrass with resistance to multiple herbicides (glyphosate and fenoxaprop-p-ethyl) recently was detected in the state of Mato Grosso, Brazil (Heap & Duke 2018). The lack of goosegrass control has been increasing mainly in Cerrado regions in Brazil, which is also increasing in crop production systems (Takano et al. 2016).

Injury level in the greenhouse was higher for goosegrass, smooth pigweed, and johnsongrass, similar to maize, which is concerning, since the last is the most common host to the fall armyworm. Boregas et al. (2013) found the same result for johnsongrass, reported by this author as a host that *S. frugiperda* has a very similar adaptation as maize. In addition, despite the fact that benghal dayflower and sourgrass had the lowest injury level, we still register for the first time the ability of this pest to survive in these weed species.

Clearly, plant resistance to insects may interfere within pests’ adaptation. Even though Dalazen et al. (2017) describe *Conyza* spp. as

frequent and dominant hosts for *S. frugiperda* in Brazil, in this study *Conyza* sp. was not a good host to this pest, either in the laboratory or the greenhouse assays. A similar result was also reported to sunn hemp (*Crotalaria juncea* L.; Fabaceae) weed by Dias et al. (2016), which found a small adaptation index (7%) for *S. frugiperda*. In this sense, some plants can reduce the species population growth in the field.

Furthermore, it is important to highlight that weeds in which *S. frugiperda* had a high development time, such as sourgrass and benghal dayflower, favor the presence and persistence of the pest, because the longer the pest takes to develop, the longer it stays in the field, allowing more time for its spread. The ability to diapause is not present in this species; therefore, it will feed on weeds that are available during the off-season in the field (Johnson 1987).

According to the results, the management of these weeds during the off-season is very important in Brazilian fields, with the goal to reduce fall armyworm pressure, as well as the soil bank of seeds. Additionally, many of these weeds escape control, forcing weed management strategies to be revised by farmers. The use of herbicides with different modes of action with residual effects on soil are very important to control resistant weeds, reduce emergent flows during the off-season, and provide better control of this lepidopteran pest.

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