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Biology of *Diatraea saccharalis* (Lepidoptera: Crambidae) populations and its implication for *Bt* sugarcane development in Argentina

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ABSTRACT

Diatraea saccharalis (Fabricius) is a key pest in sugarcane crop in Northwestern of Argentina and in corn and sorghum crops in the Pampas region causing damages of economic importance. The objective of this study was to examine biological parameters of populations of *D. saccharalis* collected in Tucumán and Buenos Aires provinces on sugarcane and non *Bt* corn respectively and evaluate the damage of each population. The parameters evaluated were: larva and pupa stage duration, pupa weight, adult longevity and sex ratio in corn tissue bioassay, and larval survival, numbers of holes, tunnel length and percentage of damaged plants in greenhouse assays. Significant differences in pupa weight and female longevity were found in bioassays between sugarcane and corn populations. No differences were observed in the survival larval and plants damage between populations. These results showed that the populations collected in different hosts have similar biological parameters, so corn crop could be considered an alternative refuge when the *Bt* sugarcane is released.

Key words: sugarcane borer, genetically modified crop, refuge, *Bacillus thuringiensis*.

RESUMEN

Biology of *Diatraea saccharalis* and its implication for *Bt* sugarcane

Diatraea saccharalis (Fabricius) es una plaga clave en caña de azúcar en el norte argentino y en maíz y sorgo en la región pampeana que ocasiona daños de importancia económica. El objetivo de este trabajo fue evaluar parámetros biológicos de dos poblaciones de *D. saccharalis* recolectadas en Tucumán (caña de azúcar) y Buenos Aires (maíz convencional) y el daño ocasionado por cada una de las poblaciones. Los parámetros evaluados fueron: duración de larva y pupa, peso de las pupas y longevidad de los adultos en bioensayos con tejido de maíz, y supervivencia larval, número de perforaciones, longitud de la galería y porcentaje de plantas dañadas en ensayos de invernáculo. Sólo se encontraron diferencias significativas en el peso de las pupas y longevidad de las hembras. En ensayos en invernáculo no se detectaron diferencias significativas en la supervivencia larval y daño en plantas entre las poblaciones. Estos resultados sugieren que las poblaciones provenientes de diferentes hospederos presentan parámetros biológicos similares por lo que el cultivo de maíz podría considerarse como un refugio alternativo al momento de la liberación de una caña de azúcar *Bt*.

Palabras clave: barrenador del tallo, cultivo genéticamente modificado, refugio, *Bacillus thuringiensis*.

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INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is the principal saccharine crop in the world, responsible for 78% of total sugar production (Romero *et al.*, 2015). Also is an energetic important crop for bioethanol production (Cárdenas *et al.*, 2009). Currently, it is cultivated in more than 100 countries on an area of 26.522.734 million ha, ranking Argentina in 16th place in the world production (FAO, 2020). In Argentina, Tucumán province leads 73% of the planted area (276,880 ha) (Fandos *et al.*, 2020) with 5,400 sugarcane farmers. A key problem of sugarcane is the attack of *Diatraea saccharalis* (Lepidoptera: Crambidae) (Fabricius), the sugarcane borer (SCB) which affects both its cultural and industrial yield. Sugar yield losses caused by this pest were estimated at 650 g/t of cane per 1% increase in the percentage of intensity of infestation (number of internodes bored / number of total internodes *100) (Salvatore *et al.*, 2010). During the 2020 season in Tucumán, sugar losses were more than 65,000 t by this pest (Pérez *et al.* 2020); therefore, it is necessary to develop strategies for its management, so a *Bt* sugarcane is being developed (Budeguer, 2020).

An effective tool for controlling lepidopteran pests, included *D. saccharalis*, is the use *Bt* corn, a plant genetically modified that express one or more insecticidal protein derived of the bacterium *Bacillus thuringiensis* (Hutchison *et al.*, 2010). Resistance development in target insect species is a major concern for the sustainable use of *Bt* crops (Gould, 1998; Roca, 2002). The main strategy to delay the development of *Bt* resistant insect populations is the combination of two independent concepts: high expression toxin (high doses) and the use of refuges (portion of the lot planted with conventional corn). The high expression of the insecticidal protein will make ineffective any mechanism conferring to the insect low to moderate levels of resistance and it is assumed to kill all heterozygotes. The refuge will permit a certain fraction of the population to escape selection; these susceptible insects will mate with any resistant insects having survived from the exposure to *Bt* plants and will produce susceptible (heterozygote) offspring (Ferré *et al.*, 2008). In Argentina, the adoption of the refuge use is low by the farmers (Trumper, 2014). Therefore, studying host plants that serve as natural refuge and that could act as a source of susceptible individuals is a relevant contribution to the agricultural system (Wu *et al.*, 2004; Li *et al.*, 2017). The effectiveness of natural refuge to delay the development of resistance to *Bt* crop depends on the biological characteristics of the target pest, the spatial and temporal distribution and abundance of the host plants in the agricultural system and the quality of the host plant for the pest species (Li *et al.*, 2017). The objective of this study was to compare biological parameters of two populations of *D. saccharalis* collected in Tucumán (sugarcane) and Buenos Aires (non *Bt* corn) and evaluate the damage of each population. Due both host crops (sugarcane and corn) coexist in Tucumán, this information could be useful to consider corn as an alternative host, in the absence of non *Bt* sugarcane when *Bt* sugarcane is released.

MATERIALS AND METHODS

- **Population source and rearing insect:** at least 400 larvae (200 of each) of two *D. saccharalis* populations from different host plants and geographical areas were collected. In the northwestern region, collections were made on sugarcane in Overo Pozo county (S 26° 50' 4,4" and O 64° 52' 8") (Tucumán province) and in the Pampas region, collections were on conventional corn (non *Bt*) in Pergamino county (S 33° 51' 51,6" and O 60° 39' 32,3") (Buenos Aires province). In the laboratory, collected larvae were placed in breeding chambers under controlled conditions (25 ± 2°C, 70 – 75%RH, 14L:10D) until adult emergence. Larvae, pupae, adults and eggs were conditioned according to the methodology described by Fogliata *et al.* (2016).

- **Vegetal material:** in greenhouse, conventional corn seeds were sown in 2 and 20L plastic pots for laboratory and greenhouse studies respectively. For laboratory studies, once the corn reached the vegetative stage V6-V8 (Ritchie *et al.* 1993) pieces of leaves (5 cm approximately) were cut. For greenhouse infestations, two seeds were placed per pot (16 plants) for each population. All the pots were provided with water regularly to ensure optimal growth.

- **Biologic parameters:** for each population, 100 neonate larvae (<24 h old) were fed with pieces of corn leaves until to reach the L2 instar. From this instar the larvae were fed with stalks pieces. Leaves and stalks were replaced every 2-3 days. Each individual was placed in a plastic tube (12 cm high and 3 cm diameter). Daily, the duration of larval instar and pupal stage, pupal weight (obtained 24 h after pupation), adult longevity and adult sex ratio were registered.

- **Larval survival and damage of *Diatraea saccharalis* populations:** in the greenhouse, this study was performed according to the methodology described by Ghimire *et al.* (2011). For each population, 10 neonates per plant (V9-V10) were inoculated into heart using a soft brush. 21 days after larval infestations, the number of surviving insects on each plant, number of holes, tunnel length each stalk and percentage of damaged plants were recorded.

- **Data analysis:** set data were tested for normality using the Shapiro-Wilk test (1965). Data were analyzed using a t-test ($P < 0.05$) to detect differences between sugarcane borer populations. All data were analyzed using Infostat program (2017).

RESULTS

Biological parameters of *D. saccharalis* populations on leaf and stalk tissue of conventional corn

Biological parameters of *D. saccharalis* populations are represented in Table 1. The parameters that presented significant differences between populations were the greater pupa weight for the Tucumán population ($t = -2.36$;

Table 1. Duration in days (mean \pm SE) of larval and pupal stages; pupal weight (mg); female and male total longevity (days); sex ratio (F:M) of *Diatraea saccharalis* populations collected in Tucumán and Buenos Aires provinces in Argentina.

Life cycle stages	Tucumán population (sugarcane)	n	Range	Buenos Aires population (non <i>Bt</i> corn)	n	Range
Overall larval stage (days)	28,04 \pm 4,34a	100	19-42	29,01 \pm 4,22a	100	19-41
Pupa (days)	4,76 \pm 0,28a	74	3-8	5,14 \pm 0,07a	77	3-8
Pupal weight (mg)	105,6 \pm 2,72b	74	50-140	93,4 \pm 4,39a	77	40-140
Sex ratio ♀:♂	1:1,2	72		1:1	71	
Female longevity (days)	4,5 \pm 0,22a	33	3-7	5,3 \pm 0,07b	35	3-7
Male longevity (days)	5,00 \pm 0,32a	39	3-8	5,00 \pm 0,36a	36	3-7

Values with the same letters within a row are not significantly different according to Student's t-test ($P > 0.05$).

df = 8; $P < 0.046$) and the longer female longevity for the Buenos Aires population ($t = 3.38$; df = 5; $P < 0.019$).

Larval survival and plant injury of two *D. saccharalis* populations on plants of conventional corn

No significant differences were observed in larval survival and plant damage of both populations after 21 days of infestations. All plants were damaged by sugarcane borer (Table 2).

These results suggest that the conventional corn (non *Bt*) could act as an alternative refuge for a *Bt* sugarcane. These studies contribute to the knowledge of *D. saccharalis* biological aspects for an insect resistance management strategy. However, additional research on quantitative assessment of corn as refuge to field for *D. saccharalis* in Tucumán province and in the regions that are going to implement the *Bt* sugarcane is necessary.

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Table 1. Larval survival (mean \pm SE); number of holes (mean \pm SE); tunnel length (mean \pm SE) and percentage of damaged plants with artificial infestations of *Diatraea saccharalis* populations after 21 days on whole plants of non *Bt* corn hybrid.

Population	% of larval survival	Number of holes per stalk	Tunnel length (cm)	% of damaged plants
Tucumán (sugarcane)	65 \pm 2.9 a	4.4 \pm 1.09 a	9.1 \pm 1.09 a	100 \pm 0.00 a
Buenos Aires (non <i>Bt</i> corn)	68,13 \pm 3,7 a	5,4 \pm 0,44 a	11,2 \pm 1,82 a	100 \pm 0,00 a

Values with the same letters within a column are not significantly different according to Student's t-test ($P > 0.05$).

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