SUGAR SOLUTION TREATMENT TO ATTRACT NATURAL ENEMIES AND ITS IMPACT ON FALL ARMYWORM *Spodoptera frugiperda* IN MAIZE FIELDS

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SUMMARY

This study aimed to survey the effects of supplemental foods to attract natural enemies in maize field and their impact on Spodoptera frugiperda *(JE Smith) (Lepidoptera:Noctuidae). The research was carried out in Londrina City, Parana State, Brazil, for three seasons, between 2009 and 2010. The following treatments were sprayed weekly: water only, water and white sugar, and water and molasses. Predators and fall armyworms were randomly quantified in 60 maize plants per treatment during each evaluation. To evaluate parasitism, 360 larvae were collected per treatment. The leaf injuries were surveyed randomly in 80 plants per treatment during each evaluation. Overall, spraying of sugar solution led to no increase in the predator population; however, it showed the highest number of parasitized fall armyworms (11.38%) relative to that when only water was sprayed (6.38%). The parasitoid* Campoletis flavicincta *(Ashmead) (Hymenoptera:Ichneumonidae) was the most abundant (30.48%), followed by* Winthemia trinitatis *Thompson (14.29%) and* Exorista *sp. (Meigen) (11.43%) (Diptera:Tachinidae). In addition, the species belonging to the family Tachinidae were more numorous when sugar and molasses were sprayed. Nevertheless, the higher parasitism caused by white sugar and molasses treatment reduced neither pest population nor leaf injuries. Taken together, our results show that white sugar and molasses treatment increases the parasitism on* S. frugiperda *in the field, but does not reduce the pest population.*

Introduction

The fall armyworm, *Spodoptera frugiperda* (JE Smith), is a major pest of maize (*Zea mays* L.) in Brazil. The insect causes production losses of ~34-40% (Cruz and Turpin, 1982), and sometimes up to 100%. In extreme cases, 10–14 applications of insecticides are required for pest control (Valicente and Mourão, 2008). Excessive use of chemicals not only favors selection pressures for resistant pests but also affects the biodiversity of non-target organisms such as biological control agents.

A diversity of natural predators and parasitoids of *S. frugiperda*, which facilitate natural biological control, is known to exist (Cruz *et al.*, 2002). Recently, an inventory of ~150 species of parasitoids of *S. frugiperda* was developed in the USA (Molina-Ochoa *et al.*, 2003). However, in Brazil, studies in this regard are scarce, and few studies have registered the natural enemies of fall armyworms that occur in the agro-ecosystem. Most of the recorded parasitoids of *S. frugiperda* are omnivorous, indicating that the adults feed on pollen and nectar (Winkler *et al.*, 2006). This behavior, which is also observed in many predators (Coll and Guershon, 2002) enables survival in agro-ecosystems, even in the absence of prey. In addition, the use of alternative food sources may enhance the ability of parasitoids and predators to regulate the pest population. Several studies have shown that carbohydrates are widely used as an energy source by predators and parasitoids (Stepphun and Wackers, 2004), and that this may increase their longevity, fecundity, and reproductive capacity (Casas *et al.*, 2003; Azzouz, 2004).

Although these foods are necessary for sustaining biological control agents, they are not always available. Therefore, biological control programs must provide food sources in the field (Lee and Heimpel, 2008). Environmental manipulation through the provision of artificial sugars is being used to promote the arrest or increase the population of natural predators and parasitoids (Lavandero *et al.*, 2005).

Environmental manipulation through the provision of artificial sugars can be accomplished in two ways: 1) by growing plants that are attractive to natural predators and parasitoids in areas close to the cultivation areas, and 2)

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PULVERIZACIÓN DEL AZÚCAR PARA ATRAER ENEMIGOS NATURALES Y SU IMPACTO SOBRE LA ORUGA MILITAR *Spodoptera frugiperda* EN CULTIVOS DE MAÍZ

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RESUMEN

El objetivo del presente estudio fue investigar los efectos de los azúcares para atraer a los enemigos naturales en el campo de maíz, así como estudiar su impacto en Spodoptera frugiperda *(JE Smith) (Lepidoptera:Noctuidae). El estudio se realizó en la ciudad de Londrina, estado del Paraná, Brasil, durante tres cultivos consecutivos entre 2009 y 2010. Los siguientes tratamientos fueron aplicados semanalmente: agua, agua y el azúcar blanco, la agua y melaza. En cada evaluación de la población de los predadores y las orugas se cuantificó aleatoriamente 60 plantas de maíz por tratamiento. Para determinar el parasitismo, se recogieron 360 larvas por tratamiento. Las lesiones foliares se analizaron de forma aleatoria en 80 plantas por tratamiento durante cada revisión. En general, la pulverización de la solución de azúcar no aumentó la población de de predadores; sin embargo, aumentó el parasitismo (11,38%) en comparación con el control (agua; 6,38%). Las especie de parasitoide* Campoletis flavicincta *(Ashmead) (Hymenoptera:Ichneumonidae) fue la más abundante (30,48%), seguida por* Winthemia Trinitatis *Thompson (14,29%) y* Exorista *sp. (Meigen) (11,43%) (Diptera:Tachinidae). Además, los tratamientos con el azúcar y la melaza incrementaron la abundancia de los parasitoides de la familia Tachinidae. Sin embargo, el mayor parasitismo causado por el azúcar blanco y el tratamiento de melaza no redujo la población de plagas y daños en las hojas. Por último, los resultados demuestran que los tratamientos de azúcar blanco y melaza aumentaron el parasitismo de* S. frugiperda *en el cultivo de maíz, pero no reducen la población de la plaga.*

PULVERIZAÇÃO DE AÇÚCARES PARA ATRAIR INIMIGOS NATURAIS E O SEU IMPACTO SOBRE A LAGARTA-DO-CARTUCHO *Spodoptera frugiperda* EM LAVOURAS DE MILHO

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RESUMO

Este estudo teve como objetivo investigar os efeitos de açúcares para atrair inimigos naturais em milho campo assim como o seu impacto sobre Spodoptera frugiperda *(JE Smith) (Lepidoptera:Noctuidae). A pesquisa foi realizada no município de Londrina, Estado do Paraná, Brasil, por três safras consecutivas, entre 2009 e 2010. Os seguintes tratamentos foram pulverizados semanalmente: água, água e açúcar branco e água e melaço. Em cada avaliação a população de predadores e lagartas foi quantificada de forma aleatória em 60 plantas de milho por tratamento. Para avaliar o parasitismo foram coletadas 360 larvas por tratamento. As injúrias foliares foram analisadas aleatoriamente em 80 plantas por tratamento durante cada avaliação. No geral, a pulverização da solução de açúcar não aumentou a população de predadores; no entanto, incrementou o parasitismo (11,38%) em relação à testemunha (somente água) (6,38%). A espécie de parasitoide* Campoletis flavicincta *(Ashmead) (Hymenoptera: Ichneumonidae) foi a mais abundante (30,48%), seguida por* Winthemia Trinitatis *Thompson (14,29%) e* Exorista *sp. (Meigen) (11,43%) (Diptera: Tachinidae). Além disso, os tratamentos com açúcar e melaço incrementaram os parasitoides da família Tachinidae. No entanto, o maior parasitismo causado por açúcar branco e tratamento melaço não reduziu a população de pragas nem folha nem injúrias foliares. Por fim, os resultados demonstram que o os tratamentos açúcar branco e melaço aumentam o parasitismo de* S. frugiperda *em lavoura de milho, mas não reduz a população de praga.*

by artificially spraying sugars on the leaf area (Hagen, 1986). The objective is to favor the foraging ability of natural predators and parasitoids, through increased suppression of arthropod pests.

Thus, this study aimed to evaluate the attractiveness of different sugar solutions to natural predators and parasitoids of *S. frugiperda* in corn. The hypothesis is that spraying sugars will increase the natural enemy occurrence of *S. frugiperda* and consequently reduce the pest population and leaf injuries in corn.

Material and Methods

The study was conducted in 2009-2010, in an experimental plot of maize (*Zea mays* L.) located in Londrina, Paraná, Brazil (23°20'23''S, 51°12'32''W, elevation of 532m). The experiments were replicated three times: from June to Aug 2009 (season I), from Nov 2009 to Jan 2010 (season II), and from Apr 2009 to May 2010 (season III). Throughout the duration of the study, no other treatments (spraying of herbicides, fungicides, and insecticides) were performed.

The local climate is classified (Koppen classification, 1920) as Cfa subtropical, with hot summers and infrequent frosts, most rainfall tending to fall during the summer months, and no dry season. During the study, the average temperature and precipitation recorded during each sampling period were: season I (16.3°C and 359.1mm), season II (24.2°C and 864.7mm), and season III (19.7°C and 229.7mm).

Three treatments were compared using a randomized block design with four replications. Each plot measured 10×15m (150m^2). Spraying was carried out by means of a backpack sprayer, and the three treatments were: 1) water control; 2) water and crystal white sugar; and 3) water and molasses. The concentration of crystal white sugar was 90g·l^{-1}, while that of the molasses was 112.5g·l^{-1} (corrected concentration of 80ºBrix). The spraying of solution on corn plants was started at the V4 maize growth stage and performed no later than V16. A weekly

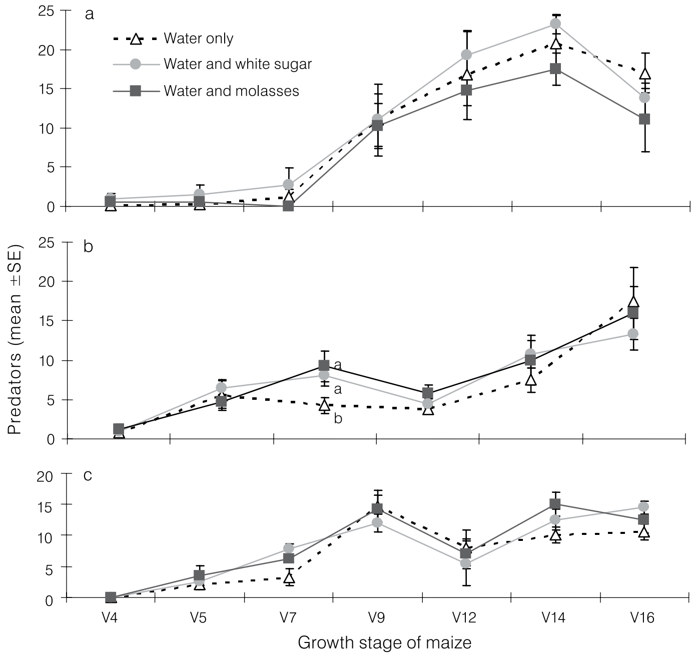

Figure 1. Seasonal population of predators (means ± SE) observed in corn plants during season I (a), season II (b), and season III (c). n= 60 plants/treatment/phonological stage. Means followed by different letters differ at the 5% significance level by Tukey test.

equivalent of 266 lit/ha was sprayed in each plot.

Assessment of predators and fall armyworms was performed between 10:00 and 14:00. For season I, assessments were performed one week after spraying started. For seasons II and III, assessments were performed on the day immediately after spraying started, to minimize interference from climatic factors. For each assessment, 15 corn plants were randomly evaluated per plot, and the numbers of fall armyworms and predators were counted.

For the evaluation of parasitism, we collected 360 larvae per treatment. The number of fall armyworms collected in each season varied according to the extent of *S. frugiperda* infestation. In seasons I, II, and III, we recorded two, three, and one collection(s), respectively. The captured larvae were transferred to Petri dishes in the laboratory and maintained in a temperature-controlled room (25 ±2°C, humidity 60 ±20%). All specimens were fed a protein diet and observed daily for the emergence of parasitoids.

In the field, leaf injuries were evaluated for each of the three treatments. The corn whorls were evaluated weekly by visualizing at random 20 plants per plot and assigning grades on a scale of 1 to 6, adapted from Cruz and Turpin (1982), where 1: sheet without injury, 2: shaved sheet, 3: perforated sheet, 4: torn sheet, 5: destroyed maize whole, and 6: plant overthrow.

For statistical analysis, we initially evaluated the normality and homogeneity of the data. Subsequently the means (±SE) of predators and *S. frugiperda* were subjected to analysis of variance (ANOVA) and analysis by Tukey's test at the 5% probability level. For the analysis of parasitism, data for the three seasons (six samples) was pooled a χ^2 test for various proportions was conducted. The leaf injuries (grades) were analyzed by the non-parametric Friedman test, $p \leq 0.05$.

Results and Discussion

Over the three seasons, 1780 predators were quantified. There was no difference in population means (±SE) among any of the three treatments (Figure 1). Despite the diversity of predators observed throughout the experiment, the pirate bugs *Orius* sp. (Hemiptera: Anthocoridae) and *Doru lineare* (Eschscholtz, 1822) (Dermaptera: Forficulidae) represented ~80% of the total predator abundance (Table I).

A diversity of natural predators is known to exist, and their abundance and diversity is known to depend on the characteristics of the agroecosystem site. For example, in Honduras, Cañas and O'Neil (1998) observed that spraying corn with white sugar increased the occurrence of *Solenopsis geminata* (Fabricius) (Hymenoptera: Formicidae); however, this effect was not observed for any other predators (e.g., earwigs, lacewings, or spiders). In the present study, we detected omnivorous ants only in season III; moreover, this occurrence was not related to sugar treatment (Table I).

The most abundant predator (representing 49.89% of total predator abundance) was *Orius* sp., which is recognized as an important predator of the eggs and larvae of small moths (Joseph and Braman, 2009). The use of sugar supplements did not increase the

TABLE I

TOTAL NUMBER AND ABUNDANCE OF PREDATORS FOUND IN MAIZE PLANTS SPRAYED WITH WATER ONLY (T1), WATER WITH WHITE SUGAR (T2), AND WATER WITH MOLASSES (T3) DURING THE THREE SEASONS

Predators	Season I (n= 7)			Season II (n= 6)			Season III (n= 7)			Total	Abundance (%)
	T1	T2	T3	T1	T2	T3	T1	T2	T3		
Hemiptera	-	-	-	-	-	-	-	-	-	-	-
Orius sp.	181	193	183	62	82	76	26	35	50	888	49.89
Geocoris sp.	13	8	2	6	5	7	5	5	8	59	3.31
Reduviidae	1	1	-	1	2	6	4	2	-	17	0.96
Pentatomidae	-	-	-	2	-	-	-	-	-	2	0.11
Coleoptera	-	-	-	-	-	-	-	-	-	-	-
Coccinellidae	5	12	3	3	2	1	6	5	5	42	2.36
Staphylinidae	-	-	-	9	11	10	-	-	1	31	1.74
Carabidae	-	-	-	-	-	-	-	1	-	1	0.06
Calleida sp.	-	-	-	2	-	2	-	-	-	4	0.22
Lebia concinna	2	3	1	1	5	4	-	-	-	16	0.90
Dermaptera	-	-	-	-	-	-	-	-	-	-	-
Doru lineare.	2	1	2	51	49	50	100	137	134	526	29.55
Aranae	4	2	1	4	7	5	-	-	3	26	1.46
Diptera	-	-	-	-	-	-	-	-	-	-	-
Dolichopodidae	-	-	-	-	3	14	-	-	-	17	0.96
Syrphidae	2	4	2	-	-	1	5	1	4	19	1.07
Hymenoptera	-	-	-	-	-	-	-	-	-	-	-
Formicidae	-	-	-	-	-	-	46	31	21	98	5.51
Vespidae	-	-	-	-	-	-	-	3	2	5	0.28
Neuroptera	-	-	-	12	8	9	-	-	-	29	1.63
Total	210	224	194	153	174	185	192	220	228	1780	100.00

n: number of evaluations, -: no specimens collected. N= 60 plants/treatment/evaluation;

population of this predator (Table I). On the other hand, a recent study showed tripling of *Orius pumilio* predation of thrips after consumption of sugar capsules or sugar water (Shapiro *et al*., 2009), and suggested that frontloading of these supplements increases the impact of predators on prey. However, in nature, natural predators have access to various food sources and can select the most interesting and biologically advantageous supplements. This may explain why spraying of sugars had no effect on the natural enemies in the present study.

TABLE II
PARASITOIDS EMERGED FROM *Spodoptera frugiperda* COLLECTED FROM MAIZE PLANTS SPRAYED WITH DIFFERENT SUPPLEMENTS DURING THE THREE EXPERIMENTAL SEASONS

Emerged parasitoids	Treatment			Parasitoids	
	Water only	Water and white sugar	Water and molasses	Total	Abundance (%)
Ichneumonidae					
Campoletis flavicincta	8	11	13	32	30.48
Eiphosoma sp.	1	3	1	5	4.76
Exasticolus sp.	2	1	3	6	5.71
Ophion flavidus	1	1	2	4	3.81
Braconidae				0	0.00
Chelonus insularis	3	3	4	10	9.52
Microgastrinae	-	3	2	5	4.76
Tachinidae				0	0.00
Archytas incertus	-	2	1	3	2.86
Archytas marmoratus	-	-	1	1	0.95
Chetogena scutellaris	-	1	1	2	1.90
Exorista sp.	2	4	6	12	11.43
Lespesia archippivora	1	5	1	7	6.67
Lespesia alletiae	-	2	1	3	2.86
Winthemia trinitatis	5	5	5	15	14.29
Total	23	41	41	105	100

-: no specimens emerged. N= 1,080 fall armyworms collected.

In contrast to the results of this study, sugars were shown to increase the attraction of others earwigs species in fennel (*Foeniculum vulgare* Mill.), particularly *Marava arachidis* (Yersin) (Dermaptera: Spongiphoridae), which was attracted not only by the density of aphids but also by the nectar of flowers (Abramsom *et al*., 2007). However, other studies have shown that responses can vary according to the studied taxa. For example, in laboratory observations, *Doru luteipes* (Scudder) (Dermaptera: Forficulidae) was attracted by a food-based protein mixture of pollen and ground dehydrated pupae of silkworm (Nonino *et al*., 2007). This probably suggests that the species found in the present study, *Doru lineare* (Eschscholtz, 1822) (Dermaptera: Forficulidae), favors others supplements, as reported previously by Nonino *et al*. (2007); however, further studies are needed to confirm this hypothesis.

In contrast to predators, parasitoids rarely feed on pollen (Jervis, 1998). To evaluate parasitism, we collected caterpillars in three experimental seasons (total= 1080), from which 105 parasitoids were obtained (Table II). Nine species, three genera and one subfamily of *S. frugiperda* parasitoids were identified. The highest abundance (33.33%) was observed for the endoparasitoid *Campoletis flavicincta* (Ashmead) (Hymenoptera: Ichneumonidae), followed by the flies *Winthemia trinitatis* Thompson (14.29%) and *Exorista* sp. (Meigen) (Diptera: Tachinidae) (11.23%).

The hymenopteran *C. flavicincta* is recognized as one of the major parasitoids of *S. frugiperda*, often occurring in different regions of Brazil (Dequech *et al*., 2004, Matos-Neto *et al*., 2004). According to Patel and Habib (2009), this species attacks fall armyworm instars at an early developmental stage, but emerges only when the fall armyworms approach the pupal stage. In contrast, the tachinid *Exorista* sp. tends to parasitize fall armyworms at a more advanced stage of development and is characterized by gregarious reproduction, laying many eggs in the same host (Mellini *et al*., 1996). Although *C. flavicincta* occurred with a relatively low abundance in the present study (11.23%), 12 parasitoids emerged, of which 10 were recorded after treatment with white sugar or molasses. The results indicate that spraying of maize with white sugar or molasses may attract this natural enemy of *S. frugiperda*.

The tachinid *W. trinitatis* was the only species for which abundance did not increase following treatment with white sugar or molasses. This parasitoid is believed to be potentially useful in biological control programs; however, in Brazil, the distribution of this species is still unknown, and little is known about its biology.

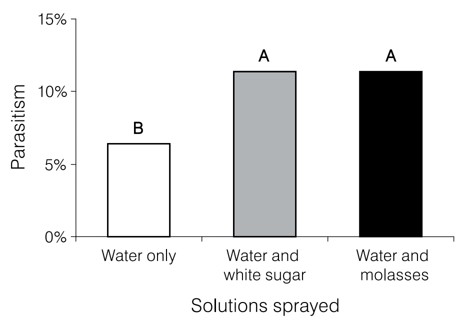

Figure 2. Percentege of *Spodoptera frugiperda* parasitized after application of different spray foods on maize crops. Columns with different letters differ at the 5% significance level by the χ^2 test for various proportions (n=360 armyworms collected in each treatment.

In comparison with the control groups, treatments with white sugar and molasses increased parasitism rates (Figure 2). In Honduras, Cañas and O'Neil (1998) observed that spraying of corn plants with white sugar increased the incidence of *Lespesia archippivora* (Riley) (Diptera: Tachinidae) on *S. frugiperda*; however, their study design, unlike the present study, used large plots (0.5×0.5ha), which probably minimized the interference effect from treatment drift. In the present study, separate statistical tests on tachinids were not carried out, because of their limited abundance; nevertheless, most specimens were found after treatment with white sugar (45%) or molasses (37%), compared with only 18% in the control. This finding suggests a preference of tachinids for sugar supplements; however, this results may be related to the prevailing taxa in the agroecosystem of the study site (Table II).

Molasses has been shown to be an efficient attractant for natural predators and parasitoids. For example, Zaki *et al*. (1998) showed that spraying of corn leaves with molasses at a concentration of 10% (similar to that used in the present study) increased parasitism of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) by *Bracon brevicornis* (Wesmael) (Hymenoptera: Braconidae). However, a similar effect was not detected when the concentration of the supplement was 5%. Thus, the concentration of sugar used is critical in determining its attractiveness to natural predators and parasitoids.

The results of the present study indicate that the treat-

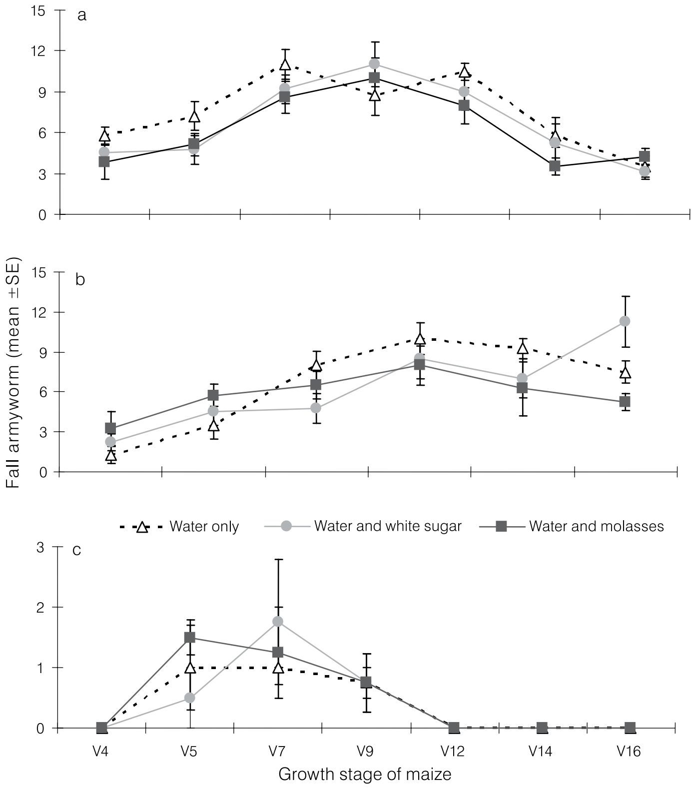

Figure 3. Seasonal population of *Spodoptera frugiperda* (mean ±SE) observed in maize plants during season I (a), season II (b), and season III (c). n= 60 plants/treatment/phonological stage.

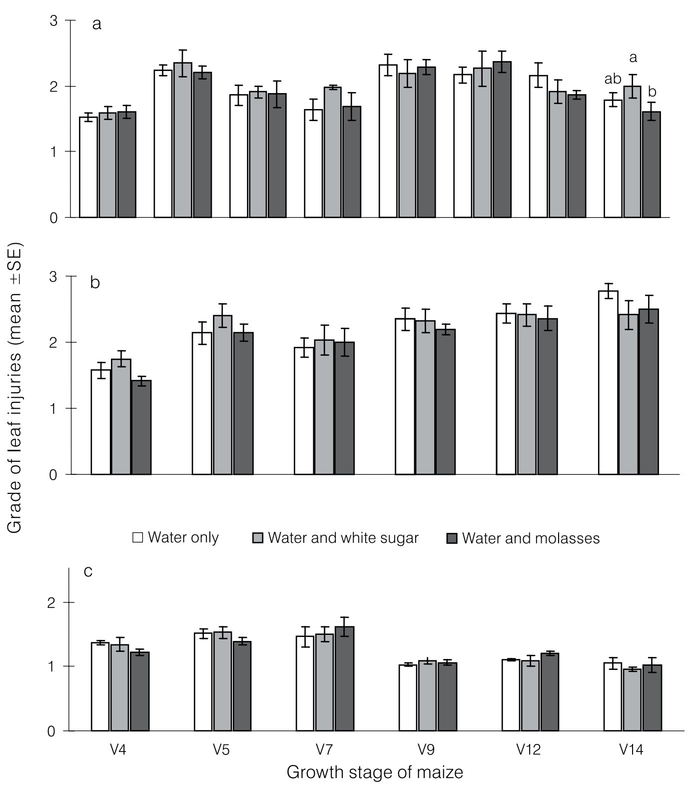

Figure 4. Leaf injuries on maize (average ±SE) observed during season I (a), season II (b), and season III (c). n= 80 plants/treatment/phonological stage. Means followed by different letters differ at the 5% significance level by Friedman test

ments with white sugar and molasses were effective in increasing parasitism. Many previous studies have shown that sugars enhance the fitness of parasitoids (Georgiev, 2005). However, the effectiveness of specific carbohydrates varies considerably according to different taxa. Thus, under field conditions, the availability of sugars may not always result in increased parasitism. In some situations, sugar, although attractive, causes adverse effects because the parasitoid may neglect the host by virtue of feeding. Another hypothesis as a limiting factor is the crystallization of sugar, which may become unavailable to natural parasitoids. Therefore, caution must be exercised when generalizing about the benefits of sugar supplements. In particular, further studies are required to evaluate the most effective supplements, and the best ways of offering these to parasitoids of specific target pests.

The observed increase in parasitism caused by the treatment with white sugar or molasses did not result in a general impact on the pest population, or a reduction in foliar damage (Figures 3-4). Surprisingly, fewer fall armyworms were found in the field during season III; this is counter to our expectations based on maize-maize succession during the study period. Our findings indicate that additional management techniques should be adopted to reduce pest populations and leaf injuries. One possibility is the release of egg parasitoids such as *Telenomus remus* Nixon (Hymenoptera: Platygastridae), which is very effective against fall armyworm (Guitiérrez-Martinez *et al*., 2012). Hence, studies on the simultaneous spraying of sugar supplements with the release of *T. remus* are required to assess the possible synergistic impacts for pest control.

Taken together, the results indicate that spraying of white sugar and molasses represents a promising strategy for increasing parasitism of the fall armyworms, but is not effective against predators. In order to reduce leaf injuries and pest populations, further research is required to test other (e.g., protein-based) supplements, which may facilitate the adoption of this technique, thereby enabling the reduction of pest damage, conservation of natural predators and parasitoids in the agroecosystem, and maintenance of high yields.

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